Sumerianz Journal of Scientific Research, 2020, Vol. 3, No. 5, pp. 45-58
ISSN(e): 2617-6955, ISSN(p): 2617-765X
Website: https://www.sumerianz.com

Original Article Open Access

Bacteriological Assessment of Fresh Crayfish (*Macro Brachium Vollenhovenii*) Handlers and River Samples from Asejire Dam Ikire Osun State Nigeria

Oludare Temitope Osuntokun*
Department of Microbiology, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria
Email: osuntokun4m@yahoo.com

Adewole Adeyemo Muniri
Department of Animal and Environmental biology , Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria

Aina Samson Oloruntoba
Department of Microbiology, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria

Article History
Received: April 15, 2020
Revised: May 10, 2020
Accepted: May 20, 2020
Published: May 23, 2020

Abstract

The study was conducted to find out the comparative analysis of microbial loads of freshwater crayfish, handlers and river samples isolated from Ikire, Osun state. The bacteriological study showed that most of the bacteria isolated were normal microflora of fresh Crayfish and microorganisms associated with contamination from handlers, faecal contaminant and waste discharge into the water body. The bacteria isolated and there percentage of occurrence in the course of these study are as follows: *Micrococcus luteus* (4.3%), *Alcaligenes latus* (4.3%), *Citrobacter diverticus* (4.3%), *Listeria grayi* (4.3%), *Bacillus cereus* (4.3%), *Citrobacter freundii* (4.3%), *Proteus vulgaris* (4.3%), *Salinicoccus roseus* (4.3%), *Marinococcus hispanicus* (4.3%), *Morganella morganii* (4.3%), *Micrococcus halobius* (4.3%), *Alteromonas espejiana* (4.3%), *Corynebacterium cystitidis* (4.3%), *Listeria mesenteroides* (4.3%), *Micrococcus varians* (4.3%), *Salmonella choleraesuis* (4.3%), *Vibrio cholera* (8.6%), *Staphylococcus aureus* (13.0%), *Marinococcus halophilus* (4.3%) and *Klebsiella planticola* (4.3%). In this research work, *Staphylococcus aureus* is most predominant among other bacteria. Statistically, freshwater crayfish samples have the highest microbial load followed by the river sample and Handlers. The exposed samples have the lowest Colony Forming Unit followed by the refrigerated samples. It is hereby recommended that fresh crayfish should be properly and effectively preserved at temperature 4°C or smoking and handled properly to prevent it from contamination. And proper hygienic measure should be carried out during and after aqua farming of fresh crayfish to avoid faecal contaminant such as Cholera and septicaemia. Crayfish should be properly cooked before consumption. For instance, proper cooking of crayfish can help to kill psychrophilic microbes such as Staphylococcus.

Keywords: Bacteriological assessment; Fresh crayfish; *Macro brachium vollenhovenii*; Handlers; River samples.

1. Introduction

Consumers want foods that are not only free from food borne pathogens but also are less processed and contain fewer added “chemical ingredients” [1]. Some of the pathogens that contaminate the fresh crayfish are as results of activities that brings about pollution in the water bodies in which they are found and this contributes to the degradation of the water qualities. With time such degradation could be temporal, that is, natural self-purification mechanism becoming enough to ultimately restore its quality, but often, either the pollutants is such that does not restore naturally or the share volume is sufficient to overload the self-purification mechanism, in which case the water is more permanently degraded.

The quality of water and the quality of life in all its infinite forms are critical parts of the overall, ongoing health of this world, not just Ikire Community, but everywhere especially Nigeria. The constant discharges of domestic and industrial waste-water and seasonal surface run-off due to the climate have strong effect on water quality. However, Rivers are the main water sources for domestic, industrial and agricultural irrigation purposes [2]. Therefore, it is imperative to have reliable information on water quality for effective pollution control and water resource management. There are needs to evaluate the River water quality. Rivers and lakes in industry and agricultural areas may be contaminated with waste, pesticide, fertilizer and other contaminants. There concentrations may vary with time and seasons. Some contaminants that enter aquatic systems are capable of influencing the population of macro-invertebrates, aquatic animals and ends up in mammals that consume them as food [3]. The increase in human population and economic activities has grown in scale; the demands for large-scale suppliers of fresh water from various competing end users have increased tremendously [4]. The decline in the quality and quantity of surface water resources can be attributed to water pollution and the improper management of the resource [5].

Crayfish is an aquatic organism that contain importantminerals such as sodium, potassium and phosphorous with adequate amount of iron, zinc, copper and manganese which are very important for the body when consumed

*Corresponding Author

[6]. Like most sea foods, crayfish contribute immensely in the nutrition of consumers due to its abundantanimal polypeptide consisting about 36 – 45% protein which is relatively cheaper than other animal protein and possesses high nutritional value [7]. Crayfish products are available to customers in the tropics as salted, smoke dried or sundried. By far, drying is the commonest processing method and the primary aim is to prolong the shelf life of the products by reducing the water content as much as possible, thus protecting the products.

Crayfish is economically valuable in many riverine countries particularly in countries where fish production account for more than 75% of the total value of their commodity trade.In the study area (Osun State, Nigeria), crayfish has provided business and economic activities for the fishermen, crayfish dealers as well as consumers of crayfish [8]. Many Nigerian riverine Delta region women source their livelihood from marketing of smoke-dried crayfish.The essence of processing is to stop bacterial action and retain quality [9]. The commodity is processed and packaged in woven polythene or hessian bags or woven baskets and transported in dugout wooden boats from processing centres in creeks to onshore markets.Crayfish is used to a large extent in local food preparation in Nigeria and due to its nutritional benefits to human health; it has been reported to be use in complementary food formulations [7]. Crayfish is increasingly becoming an important human diet component due to its nutritional value with digestive proteins and other microelements [10]. Crayfish, however, being an extremely perishable food commodity, has quite shorter shelf life in comparison to other meats [11], and sometimes, its consumption can cause food poisoning or other diseases due to infection and intoxication [12].

Figure-1. Picture showing fresh crayfish (*Macrobrachium vollenhovenii)*

Source: Asejire dam

2. Materials and Methods

2.1. Study Area for the Collected Samples

The study area was carried out in Ikire River where the samples were collected from two different fishermen in Ikire town, Osun state of Nigeria. Ikire is the closest town to Oyo state and can be called the gateway town to the state of Osun. It is within the basin of the famous River Osun. It lies on latitude 07° 30'North and longitude 04°20'East. The population is 143,599 according to the 2006 Population census. Farming is a major activity in the town and crops grown includes yam, cocoa, cassava and plantain. The town is known for production of "Dodo Ikire", a snack made with plantain and pepper. The proximity to Ibadan, a major commercial and industrial centre in the Southwest helps in facilitating smooth movement of goods and services. During harvesting of the freshwater crayfish, they hardly harvest enough crayfish because it was in dry season (28th of November, 2019) and most of them must have entered into the mud or hide under plants in the river. In Ikire, a total of 2 distinct Crayfish sample were purchased from different fishermen together with their hands sample by swapping their hands with sterile soaked swap sticks at Asejire river, Ikire, Osun State, Nigeria. The collected samples were labelled each as Fresh crayfish sample A(FCA) Fresh crayfish sample B (FCB), Fresh Handler A(FHA) and Fresh Handler B(FHB) respectively and the river sample was also collected and labelled River sample. The samples were packed aseptically into 4 sterile containers and were taken to the Department of Microbiology laboratory at Adekunke Ajasin University, Akungba Akoko, Ondo State for microbial analysis. The whole body of the fresh crayfish was swabbed with sterileswab stick soaked in sterile peptone water. Sterile sample bottle was used for the collection of pond water.

Figure-2. Map Showing the Study Area [13]

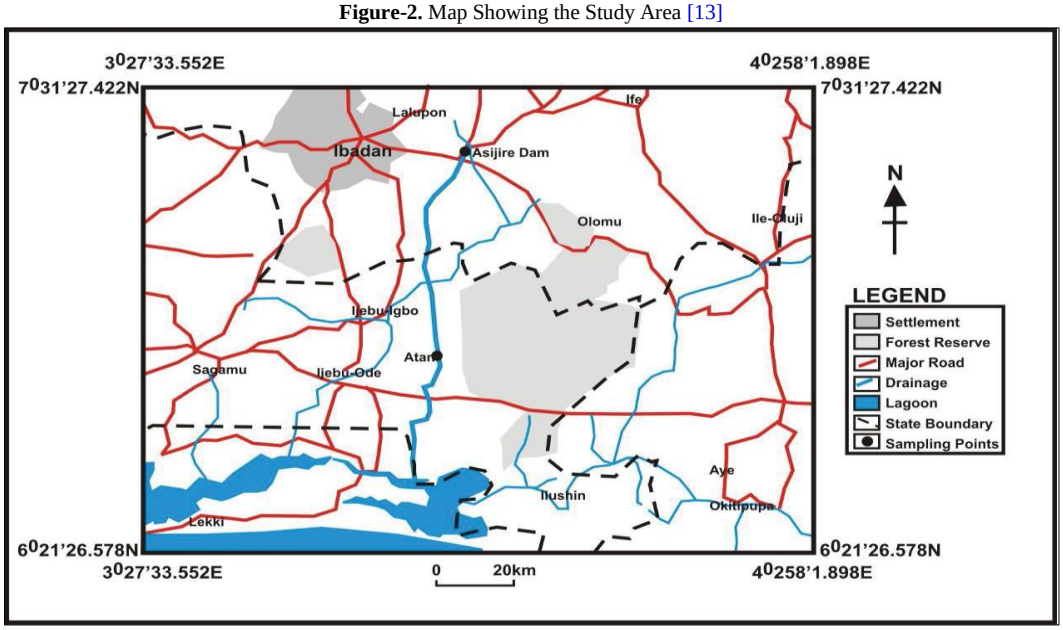
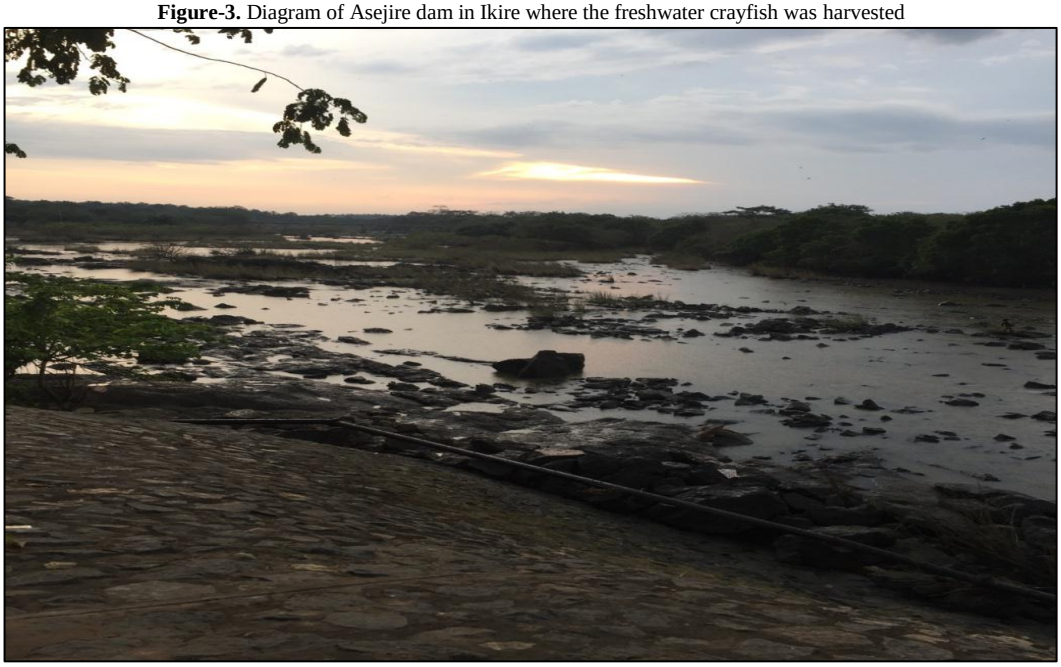


Figure-3. Diagram of Asejire dam in Ikire where the freshwater crayfish was harvested



2.2. Collection of Fresh Crayfish samples (Macrobrachium vollenhovenii)

Samples of fresh crayfishes were purchased from two fishermen at different base line. A total of two distinct fresh crayfish samples were collected, together with their hand samples(handlers) and the river sample at Asejire river in Ikire labelled as sample A and sample B. The crayfish samples collected was kept in a cold box and the hand samples were collected with the use of moistened sterile cotton swabs and immediately transferred into 5ml of peptone water.The samples were packed aseptically into 4 sterile containers and were taken to the Department of Microbiology laboratory at Adekunke Ajasin University, Akungba Akoko, Ondo State for microbial analysis.

2.3. Preservation of Fresh Crayfish (Macrobrachium vollenhovenii)

When samples are not used immediately or after daily use, samples were preserved by refrigeration at 4ºC, thereby slowing down metabolic activity of microorganisms so as to enhance good result when further used (Willey *et al.*, 2008). All the fresh samples (fresh crayfish) collected were kept in a cold box and were transported to the Microbiological laboratory of Adekunle Ajasin University, Akungba-Akoko for further evaluation.

2.4. Preparation and Inoculation of the Isolates from the fresh Crayfish (Macrobrachium vollenhovenii)

10grams of the fresh crayfish samples was weighed by taken aseptically with a sterile forceps and transferred carefully into a flask containing 9ml sterile distilled water from which 1ml was serially diluted into each of the test tubes containing 9ml of cooled sterile water, each fresh crayfish samples in different test tubes were mixed thoroughly to ensure dislodgement and even distribution of microorganisms into the suspended sterile water. A ten-

fold serial dilution of each 1ml homogenate was prepared. Exactly 0.5ml of dilution factor 10^{-1}, 10^{-3} and 10^{-5} were inoculated into the sterile petri dishes for culturing. Incubation was carried out at 37°C for 24 hours for bacteria growth. Colonies were counted in order to obtain the total viable count, discrete colonies were purified by sub-culturing into new prepared agar media and growth was observed under the microscope and then characterized using standard morphological and biochemical method, The pour plate method of Collins, *et al.* [14] was used.

2.5. Sub Culturing of Bacterial Isolates from the Fresh Crayfish (Macrobrachium vollenhovenii) Samples

Sub culturing was done using the streak method on nutrient agar surface using a sterile inoculating loop to transfer a loopful of colony from an old NA growth medium to a new medium and incubated at 27^{o}C for 24hrs [15].

2.6. Purification of the Bacterial Isolates from Macrobrachium vollenhovenii

Distinct colonies observed from the growth of mixed culture colonies after 24hrs of incubation of the isolates are sub cultured in a new agar to obtain a distinct colony; this is done by streaking plate method. After incubation and growth of the bacteria sub-cultured colonies, the pure isolates obtained were stored on slants of Nutrient Agar in the refrigerator at 4ºC. Inoculums from these sources were used for the study as desired [16].

2.7. Isolation and Identification of the Isolates from Macrobrachium vollenhovenii

Developed colonies were counted to obtain total viable colonies that appear in the plate. Isolated colonies were purified to obtain pure culture which was subsequently identified using standard methods [15]. Characterization and identification of the colony isolates was achieved by initial morphological examination of the colonies in the plate (macroscopically) for colonial appearances, shape, edge, colour, and opacity. Hence, result was recorded. Several biochemical test like catalase test, oxidase test, indole production test, urease test, starch hydrolysis test, citrate test and sugar fermentation test were also carried out on bacteria isolates. Preliminary characterization of bacterial isolates was based on Gram stain, morphological and cultural characteristics. Further characterization was carried out with various biochemical tests (catalase test, citrate test, Indole, Oxidase test, Starch hydrolysis, Urease and sugar fermentation) and Bergey's manual microbiology [16].

2.8. Gram Staining Technique of the bacteria Isolated from Macrobrachium vollenhovenii

Working solution of reagents used for the Gram staining technique was prepared according to manufacturer's instruction. Staining was carried out by emulsifying approximately one isolated 18- 24hours old colony in a drop of water placed at the centre of a clean grease free slide until a thin smear was made. The smear was air heat fixed by passing the slide through a Bunsen burner flame and then air dried. The heat fixed smear was flooded with a basic aniline dye (crystal violet) for 60 seconds. This was flooded with Lugol's iodine and allowed to remain for 60 seconds. This was then rinsed off with running tap water. The smear was decolorized with 70% ethanol which was immediately washed out to avoid total decolorization. The smear was counter stained with safranin for 60seconds, washed off with running tap water and blot-dried. The slide was then examined under oil immersion objective microscope. Organisms that retained the purple colour of crystal violet- iodine complex (CV-1 complex) were recorded as Gram- positive, while those that appeared pink were Gram- negative. Morphological characteristics of cultures were also examined and recorded as either cocci in cluster or in chains, short or long rods [17].

2.9. Biochemical Tests of the Isolated Organisms fromMacrobrachium vollenhovenii

2.9.1. Catalase Test

This test detects the presence of catalase enzyme when present in a bacterium, it catalyse the breaking down of hydrogen peroxide with the release of oxygen as bubble.
With a wire loop, a colony was picked from the pure culture and was transferred to the centre of a glass slide. 1- 2 drops of 3% hydrogen peroxide was added to the bacterial isolates. Immediate production of bubbles indicated positive result and if no bubble indicated negative (Cheesebrough 2002).

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

2.9.2. Oxidase Test

The isolated organisms were inoculated and grown in Nutrient broth for 24hrs at 37^{0}C. After 24hrs oxidase strip was dipped into the broth and colour change was observed. Microorganisms were oxidase positive when the colour changes to purple within 15 seconds to 30 seconds and oxidase negative when the colour did not change at all [15].

2.9.3. Indole Test

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole which then accumulates in the medium for indole production. Bacterial isolates were inoculated not peptone water medium contained in a sterile test .tubes then incubated at 37°C for 48 hours. After the incubation period about 3 drops of kovac's indole reagent was added to the peptone water culture. The bottles were shaken thoroughly and allowed to stand and observed for colour development. A red colour ring at the interface of the medium denotes a positive result. And if the isolate is negative, the reagent layer will remain yellow or slightly cloud [15].

2.9.4. Urease Test

The urease test is used to identify those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide . In this test each isolate was inoculated into test tubes containing sterilized urea agar medium and incubated at 37^0C. The medium was observed for a colour change at 24hrs and everyday up to 6 days. Urease production was indicated by a bright pink colour throughout the medium [16].

2.9.5. Simmon's Citrate Test

The citrate test screens bacterial isolates for the ability to utilize citrate as its carbon and energy source. Citrate agar was prepared and homogenized on a magnetic stirrer after which it was dispensed into test tubes and sterilized in the autoclave and slants were prepared. The slants were inoculated with the test organisms and incubated at 37^0C for 24hrs. Slant culture was observed for the growth and coloration of the medium, positive with blue colour and negative with green colour [18].

2.10. Sugar Fermentation of the Bacteria Isolated fromMacrobrachium vollenhovenii

This test shows the ability of microorganisms to ferment certain sugars. Five sugars were used; mannitol, sucrose, maltose, galactose and fructose.

MANNITOL: 3g of peptone powder was dissolved in 180ml of distilled water in appropriately labelled conical flask and 0.5g of phenol red was added. 1g of Mannitol sugar was added into the conical flask and shaken thoroughly. The solution was dispensed in 5ml amounts into test tubes with inverted Durham's tubes and autoclaved for 15minutes. The test tubes were then inoculated with loop full of test organisms and incubated at 37℃ for maximum of 48 hours.. The test was observed for acid production leading to colour change (red to yellow) as well as gas production that causes the displacement of the liquid in the inverted Durham's tubes which indicates a positive test [19].

SUCROSE: : 3g of peptone powder was dissolved in 180ml of distilled water in appropriately labelled conical flask and 0.5g of phenol red was added. 1g of Sucrose sugar was added into the conical flask and shaken thoroughly. The solution was dispensed in 5ml amounts into test tubes with inverted Durham's tubes and autoclaved for 15minutes. The test tubes were then inoculated with loop full of test organisms and incubated at 37℃ for maximum of 48 hours. The test was observed for acid production leading to colour change (red to yellow) as well as gas production that causes the displacement of the liquid in the inverted Durham's tubes which indicates a positive test [19].

MALTOSE: : 3g of peptone powder was dissolved in 180ml of distilled water in appropriately labelled conical flask and 0.5g of phenol red was added. 1g of Maltose sugar was added into the conical flask and shaken thoroughly. The solution was dispensed in 5ml amounts into test tubes with inverted Durham's tubes and autoclaved for 15minutes. The test tubes were then inoculated with loop full of test organisms and incubated at 37℃ for maximum of 48 hours.. The test was observed for acid production leading to colour change (red to yellow) as well as gas production that causes the displacement of the liquid in the inverted Durham's tubes which indicates a positive test [19].

GALACTOSE: 3g of peptone powder was dissolved in 180ml of distilled water in appropriately labelled conical flask and 0.5g of phenol red was added. 1g of Galactose sugar was added into the conical flask and shaken thoroughly. The solution was dispensed in 5ml amounts into test tubes with inverted Durham's tubes and autoclaved for 15minutes. The test tubes were then inoculated with loop full of test organisms and incubated at 37℃ for maximum of 48 hours.. The test was observed for acid production leading to colour change (red to yellow) as well as gas production that causes the displacement of the liquid in the inverted Durham's tubes which indicates a positive test [19].

FRUCTOSE: 3g of peptone powder was dissolved in 180ml of distilled water in appropriately labelled conical flask and 0.5g of phenol red was added. 1g of Fructose sugar was added into the conical flask and shaken thoroughly. The solution was dispensed in 5ml amounts into test tubes with inverted Durham's tubes and autoclaved for 15minutes. The test tubes were then inoculated with loop full of test organisms and incubated at 37℃ for maximum of 48 hours. The test was observed for acid production leading to colour change (red to yellow) as well as gas production that causes the displacement of the liquid in the inverted Durham's tubes which indicates a positive test [19].

2.11. Starch Hydrolysis Test of Bacteria Isolated fromMacrobrachium vollenhovenii

Nutrient agar was prepared and the isolates were inoculated onto the plates with sterile inoculating loop using streak method. The plates were incubated at 37^0C for 24hrs, after incubation the plates were flooded with Gram's iodine. Plates were observe for clear zone around the test organisms [15].

2.12. Antibiotic Susceptibility Testing of Bacteria Isolated from Macrobrachium vollenhovenii

Muella-Hinton agar was prepared according to manufacturer's specification and sterilized at 121°C for 15minutes. The medium was then poured into appropriate Petri dishes aseptically. Antibiotics susceptibilities for Gram positive bacteria and Gram negative bacteria were determined according to Clinical and Laboratory Standard Institute (CLSI) using the disc diffusion method [20].

With the aid of inoculating loop, suspected colonies were picked and inoculated in each plate and spread evenly to form a lawn culture, then the antibiotic disc were placed on the inoculated plates, incubated at 37°C for 24 hrs. the growth and zone of inhibition after 24 hours were recorded. Susceptibility test of the isolates was determined by using the following antibiotic discs; Pefloxacin (PEF, 10µg), Gentamycin (CN, 10µg), Ampiclox (APX, 30µg), Zinnacef (Z, 20µg), Amoxicillin (AM, 30µg), Rocephin (R, 25µg), Ciprofloxacin (CPX, 10µg), Streptomycin (S, 30µg), Septrin (SXT, 30µg), Augmentin (AU, 30µg), Tarivid (OFX, 10µg). The antibiotic disc used was produced by Maxicare Medical Laboratory, Nigeria. Zones of inhibition were determined in accordance with procedure of the [20]. The isolates were categorized as susceptible or resistant [20].

3. Results

Two (2) distinct samples of fresh crayfish was collected from two different fishermen at Asejire river in Ikire, Osun state. Also fresh handlers were also collected from each fisherman with the use of swab stick containing peptone water and river water sample was also collected in a sterile sample bottle at different sampling sites. These five different samples were collected in order to compare or know the differences in the bacteria load and also to determine if the microorganisms present in the freshwater crayfish is also similar to that of their handlers and the riverwater i.e, to determine the comparative study of microbial load associated with Fresh Crayfish, their Handlers and the River.Twenty three (23) species of microorganisms were isolated altogether. *Staphylococcus aureus* was the most isolated bacteria present with a larger percentage of 13.0%. other isolated bacteria include *Micrococcus luteus* 4.3%, *Alcaligenes latus* 4.3%, *Citrobacter diversus* 4.3%, *Listeria grayi* 4.3%, *Bacillus cereus* 4.3%, *Citrobacter freundii*4.3%, *Proteus vulgaris* 4.3%, *Salinicoccus roseus* 4.3%, *Marinococcus hispanicus 4.3%*, *Morganella morganii*4.3, *Micrococcus halobius* 4.3, *Alteromonas espejiana* 4.3%, *Corynebacterium cystitidis* 4.3%, *Listeria mesenteroides* 4.3%, *Micrococcus varians* 4.3%, *Salmonella choleraesuis* 4.3%, *Vibrio cholera* 8.7%, *Marinococcus halophilus*4.3%, *and Klebsiella planticola 4.3%.*

Table 1 Shows the colony forming unit (CFU) of isolates gotten from *Macrobrachium vollenhovenii*. Bacteria colonies which developed after incubation (24hrs) were subjected to counting and were expressed in Colony Forming Unit (cfu/ml). Twenty-three(23) bacterial isolates were isolated from *Macrobrachium vollenhovenii* using Nutrient agar as the media used for culturing the isolated microorganisms. In some petri dishes, the number of colonies formed due to morphological shape may be two or three colonies of microorganisms while most of the isolated are basically one colony of microorganism.Some of the microbial colony was too outrageous to be easily counted, so the plates were divided into four for easy counting. While in some plates, there was no colony formed. The colony Forming Unit of a particular organism can be calculated by multiplying the number of colonies and the dilution factor and divide them by the 0.5ml of the sample suspension. The river water was moderately alkaline (pH 8.8) and within the permissible limit (pH 6.5-8.5) of drinking water standards of WHO. In this study, the comparative analysis of freshwater crayfish, handlers and the river with respect to their microbial load is still within the similar range of growth. The microbial load of Fresh crayfish increases when exposed to environmental condition and refrigeration for the second day analysis.

Table-1. Average Total of Bacteria Count in *Macrobrachium Vollenhovenii* Samples in Colony Forming Unit/Ml(Cfu/Ml)

ISOLATE	NUMBER OF COLONIES	CFU/ml
FCA3	38	3.8×10^{4}
FCA5	3	3×10^{3}
FCB3	64	6.4×10^{4}
FCB5	16	1.6×10^{6}
FCB7	3	3×10^{7}
FHA3	15	1.5×10^{4}
FHA5	9	9×10^{5}
FHB1	21	2.1×10^{2}
FHB3	3	3×10^{3}
FHB5	1	1×10^{5}
RIVER A	45	4.5×10^{4}
RIVER B	6	6×10^{5}
RIVER C	2	4×10^{3}
RCA3	340	34.0×10^{2}
RCB3	15	1.5×10^{4}
ECA1	224	22.4×10^{2}
ECB3	12	1.2×10^{4}
ECA(2)3	3	3×10^{3}

KEYS: FCA3=Fresh crayfish sample A RCB= Refrigerated crayfish sample B
FCB= Fresh crayfish sample B ECA= Exposed crayfish sample A
FHA= Fresh handler sample A ECB= Exposed crayfish sample B
FHB= Fresh handler sample B ECB2= Exposed sample B second day
RIVER A=River sample A RCA= Refrigerated crayfish sample A
RIVER B= River sample B ECA2= Exposed crayfish sample A second day
RIVER C= River sample C

Table 2: INTERPRETATION OF STATISTICAL ANALYSIS

There was significant difference (P<0.05) in the means of the treatment groups at all dilution levels. At dilution factor 10^{-3}, the treatment means separated into three classes with FC, FH and RV in a group, and RC and EC in separate groups. At this level, bacterial load decreased thus: FC (51.00± 13.00)> RV (45.00± 00)>FH (18.00±3.00)> RC (15.00± 00)> EC (12.00± 00).

At dilution factor 10^{-5}, only two separate mean groups were observed: RC-FH-RV-FC and EC. Bacterial loads decreased from FC to EC thus: FC (9.50±3.00)>FH (6.00±3.00)> RV (6.0±0.00)> RC (3.0±0.00)> EC (0.00±0.00).

Two mean groups were also observed at 10^{-7} which is RC-EC in one group, and FC and RV in the other group. Bacterial load decreased thus: FC (3.0±0.00)> RV (2.0±0.00) >FH (1.00±0.00)>RC (0.00±0.00) and EC (0.00 ±0.00).

Dilution factors with (0.00 ±0.00) have no growth after cultured, so there was no colony to count.

Table 3: Shows the morphological and macroscopy characteristics of isolates gotten from *Macrobrachium vollenhovenii* in Ikire, Osun state. This was done by physically examining Bacteria colonies which grows after 24hrs of incubation for surface appearance, colony colour, colony edge, colony shape and appearance of colony in light (either translucent or opaque). Most of the colony colours formed were milky in colour and only few were yellow in colour.

Table-2. The Bacteria Colony Count for each Samples

ISOLATES	FC	RC	EC	RIVER	FH
10^{3}	51.00 ± 13.00^{c}	15.00 ± 0.00^{b}	12.00 ± 0.00^{a}	45.00 ± 0.00^{c}	18.00 ± 3.00^{b}
10^{5}	9.50 ± 3.50^{b}	3.00 ± 0.00^{b}	0.00 ± 0.00^{a}	6.00 ± 0.00^{b}	6.00 ± 3.00^{ab}
10^{7}	3.00 ± 0.00^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2.00 ± 0.00^{c}	1.00 ± 0.00^{b}
**Values are mean ± S.D., n=3;*					
***Means with different superscripts down a column are significantly different at P<0.05*					

KEYS:
FCA= Fresh Crayfish Sample A RIVER= River water sample,
RC= Refrigerated Crayfish, EC= Exposed (Opened- Air) Crayfish, FH= Fresh handler

Table-3. Morphological and Cultural Characteristics of Bacterial Isolates from River and Crayfish

ISOLATES	SURFACES	PIGMENTATION	EDGE	SHAPE	APPEARANCE
FCA 10^{-3}A	Smooth	Yellow	Undulate	Circular	Opaque
FCA 10^{-3}B	Smooth	Milk	Undulate	Rhizoid	Translucent
FCA 10^{-5}A	Smooth	Milk	Lobate	Irregular	Opaque
FHA 10^{-3}A	Smooth	Milk	Entire	Rhizoid	Translucent
FHA 10^{-7}A	Rough	Milk	Crenated	Irregular	Translucent
FHA 10^{-7}B	Dull	Milk	Lobate	Irregular	Opaque
FCB 10^{-3}A	Smooth	Milk	Fimbrate	Rhizoid	Translucent
FCB 10^{-5}A	Rough	Milk	Lobate	Circular	Translucent
FCB 10^{-5}B	Dull	Milk	Fimbrate	Circular	Translucent
FCB 10^{-5}C	Rough	Milk	Entire	Circular	Opaque
FCB 10^{-5}D	Glistering	Yellow	Fimbrate	Circular	Translucent
FHB 10^{-3}A	Smooth	Milk	Undulate	Filamentous	Translucent
RC 10^{-1}A	Smooth	Yellow	Entire	Filamentous	Opaque
RC 10^{-1}B	Rough	Yellow	Fimbrate	Circular	Translucent
EC 10^{-1}A	Smooth	Milk	Undulate	Rhizoid	Translucent
EC 10^{-3}A	Smooth	Yellow	Entire	Rhizoid	Translucent
EC 10^{-5}A	Smooth	Yellow	Undulate	Irregular	Translucent
RC2 10^{-3}B	Smooth	Milk	Undulate	Rhizoid	Translucent
RIVER 10^{-1}A	Smooth	Milk	Lobate	Irregular	Translucent
RIVER 10^{-3}A	Smooth	Milk	Undulate	Rhizoid	Translucent
RIVER 10^{-5}A	Dull	Milk	Lobate	Filamentous	Opaque
FHA 10^{-3}B	Smooth	Milk	Fimbrate	Filamentous	Opaque
FCB 10^{-1}A	Smooth	Milk	Undulate	Filamentous	Translucent
FCA 10^{-3}A	Smooth	Milk	Undulate	Rhizoid	Translucent

Table 4: Showsthe microscopy examination of isolates from*Macrobrachium vollenhovenii* in Ikire, Osun state. This was achieved using the Gram staining techniqueto determine if the isolates are either gram positive or gram negative organism and to indicate if they are chain cocci, short or long rod bacteria. Ten(10) of the isolates are Gram negative bacteria and others are Gram positive bacteria.

Table 5: Showing results of the biochemical test carried out in the isolation of microorganisms from *Macrobrachium vollenhovenii* in Ikire, Osun state. The biochemical analysis of the isolates includes Catalase, Starch Hydrolysis, Oxidase, Indole, Urease and Citrate. This test really helps in the identification of the isolates.

Table 6: Showing the sugar fermentation tests of the isolates from *Macrobrachium vollenhovenii* which aids in the identification of probable microorganisms. The sugar fermentation includes glucose, fructose, manitol, galactose and sucrose.

Table **7:** Showing distribution and identification of probable microorganisms isolated from *Macrobrachium vollenhovenii* after carrying out the biochemical and sugar fermentation tests, macroscopy (physical) observation of the colonies, identification of isolates under the microscope and Bergey's manual. Some of the Organisms identified may be as a result of faecal contaminants which are *Vibrio cholerae, Citrobacter freundii, and Klebsiella planticola*.

Table 8**:** Showing the frequency table i.e the number of times each identified microorganisms occur in the isolates. The percentage frequency for *Micrococcus luteus,Alcaligenes latus,Citrobacter diversus, Listeria grayi, Bacillus cereus, Morganella morganii,Proteus vulgaris,Salinicoccus roseus, Micrococcus halobius, Alteromonas espejiana, Corynebacterium cystitidis, Micrococcus varians, Salmonella choleraesuis, Marinococcus halophilus and Klebsiella planticola*were 4.3%because they occur only ones and the percentage frequency for *Vibrio cholerae* was 8.6% and *Staphylococcus aureus* was 13.0%.

Table-4. Microscopy Examination of Bacteria Isolated from *Macrobrachium Vollenhovenii* in Ikire

ISOLATES	GRAM STAINING
FCA 10^{-3}A	+ve cocci
FCA 10^{-3}B	+ve cocci
FCA 10^{-5}A	-ve rod
FHA 10^{-3}A	+ve rod central spore
FHA 10^{-7}A	+ve rod
FHA 10^{-7}B	+ve cocci in cluster
FCB 10^{-3}A	-ve rod
FCB 10^{-5}A	-ve rod
FCB 10^{-5}B	+ve rod
FCB 10^{-5}C	+ve cocci
FCB 10^{-5}D	+ve rod in spore
FHB 10^{-3}A	-ve rod
RC 10^{-1}A	+ve cocci in chain
RC 10^{-1}B	+ve cocci in chain
EC 10^{-1}A	-ve cocci
EC 10^{-3}A	+ve rod in chain
EC 10^{-5}A	+ve cocci in chain
RC2 10^{-3}B	+ve cocci
RIVER 10^{-1}A	-ve rod
RIVER 10^{-3}A	-ve rod
RIVER 10^{-5}A	-ve rod
FHA 10^{-3}B	-ve rod
FCB 10^{-1}A	-ve rod

Keys: +ve = positive -ve = Negative

FCA 10^{-3} A= Fresh crayfish sample A 10^{-3} subculture 1, FCA 10^{-3} B = fresh crayfish sample A 10^{-3} subculture 2

FCA 10^{-5}A = Fresh crayfish sample A 10^{-5} subculture 1, FHA 10^{-3}A= Fresh handler sample A 10^{-3} subculture 1

FHA 10^{-7}A= Fresh handler sample A 10-7 subculture 1, FHA 10^{-7}B= Fresh handler sample A 10-7 subculture 2

FCB 10^{-3}A= Fresh crayfish sample B 10-3 subculture 1, FCB 10^{-5}A= Fresh crayfish sample B 10^{-5} subculture 1

FCB 10^{-5}B= Fresh crayfish sample B 10-5 subculture 2, FCB 10^{-5}C= Fresh crayfish sample B 10^{-5} subculture 3

FCB 10^{-5}D= Fresh crayfish sample B 10-5 subculture 4, FHB 10^{-3}A= Fresh handler sample B 10^{-3} subculture 1

RC 10^{-1}A= Refrigerated crayfish sample 10-1 subculture 1, RC 10^{-1}B= Refrigerated crayfish sample 10^{-1} subculture 2

EC 10^{-3}A= Exposed crayfish sample 10-3 subculture 1, EC 10^{-5}A= Exposed crayfish sample 10-5 subculture 1,

RC2 10^{-3}B = Refrigerated crayfish sample second day 10-1 subculture 2, FHA 10^{-3}B= fresh handler A 10-3 subculture 2

FCB 10^{-1}A= fresh crayfish sample B 10-1 subculture 1,RC2 10^{-3}B= refrigerated crayfish sample 10-3 subculture 2

RIVER 10^{-1}A= River sample 10-1 subculture 1, RIVER 10^{-3}A= River sample 10-3 subculture 1

RIVER 10^{-5}A= River sample 10-5 subculture 1

Table-5. Biochemical Charateristics of Bacteria Isolated From*macrobrachium Vollenhovenii* in Ikire

Isolates	Catalase	Starch hydrolysis	Oxidase	Indole	Urease	Citrate	Organism identify
FCA 10^{-3}A	+	+	+	+	-	+	*Micrococcus luteus*
FCA 10^{-3}B	+	+	-	-	-	+	*Alcaligenes latus*
FCA 10^{-5}A	+	+	-	+	-	+	*Citrobacter diversus*
FHA 10^{-3}A	+	+	-	+	-	+	*Listeria grayi*
FHA 10^{-7}A	+	+	-	-	-	+	*Bacillus cereus*
FHA 10^{-7}B	+	+	-	+	-	+	*Staphylococcus aureus*
FCB 10^{-3}A	+	+	-	+	-	-	*Citrobacter freundii*
FCB 10^{-5}A	+	+	-	-	-	+	*Proteus vulgaris*
FCB 10^{-5}B	+	+	+	-	-	+	*Salinicoccus roseus*
FCB 10^{-5}C	+	+	-	+	-	+	*Marinococcus hispanicus*
FCB 10^{-5}D	+	+	-	+	-	+	*Staphylococcus aureus*
FHB 10^{-3}A	+	+	-	+	-	+	*Morganella morganii*
RC 10^{-1}A	+	+	-	+	-	+	*Staphylococcus aureus*
RC 10^{-1}B	+	+	+	+	-	+	*Micrococcus halobius*
EC 10^{-1}A	+	+	-	+	-	+	*Alteromonas espejiana*
EC 10^{-3}A	+	+	-	-	-	+	*Corynebacterium cystitidis*
EC 10^{-5}A	+	+	-	+	-	+	*Listeria mesenteroides*
RC2 10^{-3}B	+	+	-	-	-	-	*Micrococcus varians*
RIVER 10^{-1}A	+	+	-	-	-	+	*Salmonella choleraesuis*
RIVER 10^{-3}A	+	+	+	-	-	+	*Vibrio cholera*
RIVER 10^{-5}A	+	+	+	-	-	+	*Vibrio cholera*
FHA 10^{-3}B	+	+	+	+	-	-	*Marinococcus halophilus*
FCB 10^{-1}A	+	+	-	+	-	+	*Klebsiella planticola*

Table-6. Sugar fermentation of isolated bacteria from *macrobrachium volllenhovenii* in ikire

Isolates	Glucose	Fructose	Manitol	Sucrose	Galactose	Organism identify
FCA 10^{-3}A	+g	+	+ g	+	+	*Micrococcus luteus*
FCA 10^{-3}B	+g	+g	+g	+ g	+ g	*Alcaligenes latus*
FCA 10^{-5}A	+ g	+	+g	+g	+ g	*Citrobacter diversus*
FHA 10^{-3}A	+	+	+ g	+ g	+	*Listeria grayi*
FHA 10^{-7}A	+g	+g	+g	+ g	+ g	*Bacillus cereus*
FHA 10^{-7}B	+	+	+ g	+g	+g	*Staphylococcus aureus*
FCB 10^{-3}A	+	+	+ g	+ g	+g	*Citrobacter freundii*
FCB 10^{-5}A	+	+	+g	+	+g	*Proteus vulgaris*
FCB 10^{-5}B	+g	+g	+g	+	+g	*Salinicoccus roseus*
FCB 10^{-5}C	+	+	+	+g	-g	*Marinococcus hispanicus*
FCB 10^{-5}D	+g	+	+	+	+	*Staphylococcus aureus*
FHB 10^{-3}A	+g	+	+	+	-g	*Morganella morganii*
RC 10^{-1}A	+	+g	+	+	+	*Staphylococcus aureus*
RC 10^{-1}B	+g	+	+	+	+	*Micrococcus halobius*
EC 10^{-1}A	-	-	+g	-	+g	*Alteromonas espejiana*
EC 10^{-3}A	+g	+	+	+	+	*Corynebacterium cystitidis*
EC 10^{-5}A	+g	+	+	+	+g	*Listeria mesenteroides*
RC2 10^{-3}B	+g	+g	+g	+g	+g	*Micrococcus varians*
RIVER 10^{-1}A	+	+g	+	+g	+	*Salmonella choleraesuis*
RIVER 10^{-3}A	+g	+g	+g	+g	+g	*Vibrio cholera*
RIVER 10^{-5}A	+g	+g	+g	+g	+g	*Vibrio cholera*
FHA 10^{-3}B	+	+	+	+	+	*Marinococcus halophilus*
FCB 10^{-1}A	+	+	-	+	-	*Klebsiella planticola*

Keys: -=Absent
+= Present g= Gas production through bubbles

Table-7. Probable Microorganism Isolated From *Macrobrachium Vollenhovenii*

Isolates	**Probable Organisms**
FCA 10^{-3}A	*Micrococcus luteus*
FCA 10^{-3}B	*Alcaligenes latus*
FCA 10^{-5}A	*Citrobacter diversus*
FHA 10^{-3}A	*Listeria grayi*
FHA 10^{-7}A	*Bacillus cereus*
FHA 10^{-7}B	*Staphylococcus aureus*
FCB 10^{-3}A	*Citrobacter freundii*
FCB 10^{-5}A	*Proteus vulgaris*
FCB 10^{-5}B	*Salinicoccus roseus*
FCB 10^{-5}C	*Marinococcus hispanicus*
FCB 10^{-5}D	*Staphylococcus aureus*
FHB 10^{-3}A	*Morganella morganii*
RC 10^{-1}A	*Staphylococcus aureus*
RC 10^{-1}B	*Micrococcus halobius*
EC 10^{-1}A	*Alteromonas espejiana*
EC 10^{-3}A	*Corynebacterium cystitidis*
EC 10^{-5}A	*Listeria mesenteroides*
RC2 10^{-3}B	*Micrococcus varians*
RIVER 10^{-1}A	*Salmonella choleraesuis*
RIVER 10^{-3}A	*Vibrio cholera*
RIVER 10^{-5}A	*Vibrio cholera*
FHA 10^{-3}B	*Marinococcus halophilus*
FCB 10^{-1}A	*Klebsiella planticola*

Table-8. Frequency Distribution of Bacterial Isolates From*macrobrachium Vollenhovenii*in Asejire Dam

Isolates	**Frequency**	**Percentage of Occurrence (%)**
Micrococcus luteus	1	4.3%
Alcaligenes latus	1	4.3%
Citrobacter diversus	1	4.3%
Listeria grayi	1	4.3%
Bacillus cereus	1	4.3%
Staphylococcus aureus	1	4.3%
Citrobacter freundii	1	4.3%
Proteus vulgaris	1	4.3%
Salinicoccus roseus	1	4.3%
Marinococcus hispanicus	1	4.3%
Staphylococcus aureus	3	13.0%
Morganella morganii	1	4.3%
Micrococcus halobius	1	4.3%
Alteromonas espejiana	1	4.3%
Corynebacterium cystitidis	1	4.3%
Listeria mesenteroides	1	4.3%
Micrococcus varians	1	4.3%
Salmonella choleraesuis	1	4.3%
Vibrio cholera	**2**	8.6%
Marinococcus halophilus	1	4.3%
Klebsiella planticola	1	4.3%

Table 9**:** Showing the Antimicrobial sensitivity test of the 20 Microorganisms isolated from *Macrobrachium vollenhovenii* in Ikire. Zone diameter measurements without an interpretive category should not be reported. Recommended interpretive categories for various MIC and zone diameter values are identified with the use of keys for each organism group are based on evaluation of data.The “susceptible” category implies that isolates are inhibited by the usually achievable concentration of antimicrobial agent when the dosage recommended to treat the site of infection is used, resulting in likely clinical efficacy. The “intermediate” category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels. And for which response rates may be lower than for susceptible isolates. This category includes buffer zone. The “resistant” category implies that isolates are not inhibited by the usually aachievable concentration of agent with normal dosage schedules or that demonstrate MICs or zone diameters that fall in the range where specific microbial resistance mechanisms are [21]. Two tables were drawn both for the Antibiotics susceptibility of Gram negative and Gram positive bacteria.

Table-9a. Antibiogram profiles of Gram positive bacteria against common antibiotics (measured in mm) ± SEM Antibiotic (concentration in µg)

Probable Organisms	E	PEF	CN	APX	Z	AM	R	CPX	S	SXT
Micrococcus luteus	0	0	16	0	10	12	20	0	20	20
Alcaligenes latus	0	15	0	0	0	0	14	16	0	0
Listeria grayi	20	20	20	0	0	0	20	20	20	20
Bacillus cereus	20	20	20	0	0	0	20	20	20	20
Staphylococcus aureus	20	20	20	0	0	0	20	20	20	20
Salinicoccus roseus	20	20	20	0	0	0	20	0	15	12
Marinococcus hispanicus	17	20	20	0	0	0	20	0	13	13
Staphylococcus aureus	17	15	12	0	0	0	20	20	20	20
Staphylococcus aureus	20	20	20	0	10	11	20	20	20	20
Micrococcus halobius	0	0	0	11	0	0	0	10	0	0
Corynebacterium cystitidis	20	20	20	0	0	0	20	20	20	20
Listeria mesenteroides	17	15	10	0	0	0	17	15	16	0
Micrococcus varians	20	16	20	0	0	0	20	20	16	18

KEYS: Susceptibility (S) ≥16 Intermediate (I) 15-12 Resistance (R) ≤11
S – Streptomycin 30µg APX – Ampiclox 30µg 0.00 – No Inhibition
SXT – Septrin 30µg Z – Zinnacef 20µg E – Erythromycin 10µg
AM – Amoxacillin 30µg PEF – Pefloxacin 30µg R – Rocepin 25µg
CN – Gentamycin 10µg CPX – Ciprofloxacin 10µg

Table-9b. Antibiogram Profiles of Gram negative Bacteria against Common Antibiotics (measured in mm) ± SEM

Probable Organisms	CH	SP	CPX	AM	AU	CN	PEF	OFX	S	SXT
Citrobacter diversus	0	0	0	0	14	16	20	20	20	13
Citrobacter freundii	0	11	13	0	0	0	16	0	0	0
Proteus vulgaris	20	20	20	0	0	0	20	20	20	20
Morganella morganii	0	15	0	0	0	0	14	16	0	0
Alteromonas espejiana	0	11	13	0	0	0	0	16	0	0
Klebsiella planticola	0	20	16	0	0	0	20	15	16	0
Salmonella choleraesuis	16	16	11	0	0	0	12	16	0	0
Vibrio cholera	20	16	20	0	0	0	20	20	16	18
Marinococcus halophilus	20	20	20	0	14	15	17	20	20	14

KEYS: Susceptibility (S) ≥16 Intermediate (I) 15-12 Resistance (R) ≤11
S – Streptomycin 30µg AU – Augmentin 30µg SXT – Septrin 30µg
SP – Sparfloxacin 10µg AM – Amoxacillin 30µg 0.00 – No Inhibition
PEF – Pefloxacin 30µg CPX – Ciprofloxacin 10µg CN – Gentamycin 10µg
OFX – Tarivid 10µg CH – Chloramphenicol 30µg

4. Discussion

This study presents the isolation and characterization of thirteen (13) Gram positive and ten (10) Gram negative bacteria from *Macrobrachium vollenhovenii* in Ikire as the base line. A total of five (5) samples were collected such as fresh crayfish samples from two different fishermen and two different handler samples and the river sample inclusive which were tested quantitatively and qualitatively. From Table 1, the total average of bacterial count fromsample B of Fresh crayfish, Fresh handler, River sample and Fresh crayfish sample A simultaneouslyshows highnumber of microbial load. In the study research, Fresh crayfish sample B(FCB7) has the highest number of microbial load which is 3×10^7 followed by FCB5 which has 1.6×10^6 followed by Fresh Handler which has a CFU of 9×10^5. The Exposed samples have the lowest Colony Forming Unit followed by the Refrigerated samples.The high microbial load in the freshwater crayfish samples depicts a deplorable state of poor hygienic and sanitary practices employed in the process of experiment. The decrease in the microbial loads of Exposed and Refrigerated samples may be as a result of some dead microorganisms which cannot survive outside the aquatic region. Most microflora of freshwater crayfish were absent in the ice treatment and open environment maybe because they cannot survive in an unfavourable condition. Nevertheless, ice treatment cannot control Psychrophilic Microbes such as *Staphylococcus aureus* and *Micrococcus sp*, and also in an open air environment, *Listeria sp* cannot be controlled.The isolated bacteria namely: *Micrococcus luteus*, *Alcaligenes latus*, *Citrobacter diverticus*, *Listeria grayi*, *Bacillus cereus*, *Citrobacter freundii*, *Proteus vulgaris*, *Salinicoccus roseus*, *Marinococcus hispanicus*, *Morganella morganii*, *Micrococcus halobius*, *Alteromonas espejiana*, *Corynebacterium cystitidis*, *Listeria mesenteroides*, *Micrococcus*

varians, Salmonella choleraesuis, Vibrio cholerae,Staphylococcus aureus, Marinococcus halophilus and Klebsiella planticola are of great importance.

From this study it really shows that some of the organisms isolated were also common in the study carried out by Akintola and Bakare [22]. The higher load of bacteria associated with *Macrobrachium vollenhovenii* from Asejire dam may be due faecal pollution of the aquatic environment to a lesser extent, the natural aquatic environment and industrial discharge into the water dam. The organisms isolated such as *Micrococcus luteus* causes an infection to human health which is endocarditis involving prosthetic valves [23]. It can also act as an opportunistic pathogens in immunocompromised patients to cause Peritoneal Dialysis(PD) failure [24].

The natural flora of crayfish that play a predominant role in spoilage include the genera *Pseudomonas,Vibrio* and *Micrococcus*, while *Pseudomonas sp.* are among the major spoilage bacteria at near freezing temperatures [25]. In order to prolong the shelf life of crayfish, it is essential to control these spoilage bacteria [26].

Most normal flora bacteria of fresh crayfish are Gram positive and this could explain their prevalence on their handlers.This shows that 45 to 75% samples collected were contaminated. This could be as a result of the fact that during handling of crayfish, the natural flora of crayfish environment had been contaminated with organisms associated with man, such as *Staphylococcus aureus* which was isolated in this study. For instance, *Staphylococcus aureus* was isolated from Fresh Handler Sample A (FHA7), Fresh Crayfish Sample B (FCB7) and Refrigerated Crayfish Sample (RCA). And *Staphylococcus aureus* happens to be a normal flora of human. It must have contaminated the crayfish through direct contact with human. This study revealed the prevalence rate of bacteria as follows: *Staphylococcus aureus* 13%,*Micrococcus species* 13%,*Marinococcus species* 8.6%, *Vibrio cholerae* 8.6%, *Citrobacter species* 8.6%, *Listeria species* 8.6%, *Bacillus* species 4.3% and *Klebsiella* species 4.3% respectively for the samples (Table 3). This study is in agreement with the findings of Israelwho found the presence of bacteria like *Staphylococcus aureus* as the predominant organism isolated (Israel *et al.*, 2016). The results showed that *Staphylococcus aureus*were more isolated compared to other bacterial species due to their ability to invade fresh crayfish through faecal contaminant. The study demonstrates that Gram positive bacteria are the most common contaminating microbes while ten (10) of the isolates were Gram negative.

The number of colony formed in this study is more prevalent in the refrigerated crayfish sample(RCA3=340) and exposed crayfish sample (ECA1=224) which was suggestively believed that the refrigerator used had been exposed to other microbes from old samples that are kept inside thereby leading to the inhibition of those microbes waiting to invade new samples and also because the organisms were isolated from serial dilution 10-1 for both samples. And the Exposed crayfish sample may be contaminated due to the unsterilized environment. The total isolates were observed morphologically on each plate in order to know their surfaces, pigmentation, edge, shape and appearance. Although, this morphological characteristics is never enough to identify the specific microorganisms present. To know or identified the specific organisms present, it is necessary to proceed to their microscopic characteristics with the use of microscope. In this study, thirteen (13) isolated samples were Gram positive bacteria and ten isolates were Gram negative bacteria.Nine isolated colonies are Gram negative rods. The genera *Listeria*, *Citrobacter*,*Proteus*, *Morganella*, *Alteromonas*, *Salmonella*, *Vibrio*, *Marinococcus* and *Klebsiella*are Gram negative bacteria while eight isolated colonies are Gram positive and five organisms are cocci.The microscopy characteristics is not enough to determine the specific microorganisms as there are many Gram positive and Gram negative bacteria that ever exist. To identified each of the isolates, biochemical test method is needed which includes the Catalase, Starch hydrolysis, Oxidase, Indole, Urease and Citrate test. These tests help to determine the probable organisms present. In this study, the results of the Urease test for all the isolates after examining it for 7days were all negative.

The antimicrobial sensitivity test for the 23 isolates from *Macrobrachium vollenhovenii* in Ikire reveals the zone of inhibition which determines whether a particular organism is susceptible or resistant to different antibiotics. TheZone diameter measurements without an interpretive category should not be reported. Recommended interpretive categories for various MIC and zone diameter values are included in tables for each organism group are based on evaluation of data. The susceptible" category implies that isolates are inhibited by the usually achievable concentration of antimicrobial agent when the dosage recommended to treat the site of infection is used, resulting in likely clinical efficacy The "intermediate" category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels. And for which response rates may be lower than for susceptible isolates and this category include buffer zone.The "resistant" category implies that isolates are not inhibited by the usually achievable concentration of agent with normal dosage schedules or that demonstrate MICs or zone diameters that fall in the range where specific microbial resistance mechanisms are [21]. Disk diffusion test was used to carry out the Antimicrobial sensitivity test. In this study, Gram negative bacteria show less sensitivity to the antibiotics used compare to Gram positive bacteria. Most of the Gram negative bacteria are highly resistant to Amoxaxillin, Gentamicin and Augmentin while most of them are highly sensitive to Ciprofloxacin, Streptomycin, Chloramphenicol and Tarivid. Most of the Gram positive bacteria are highly resistance to Amplicox, Zinnacef and Amoxacillin and almost all are highly susceptible to Erythromycin, Pefloxacin, Gentymycin, Rocepin, Ciprofloxacin, Streptomycin and Septrin.

5. Conclusion

From the result obtained from this study, contamination of fresh crayfish may be due to faecal pollution of the aquatic environment to a lesser extent, the natural aquatic environment and discharged from Industry, retail, restaurant, or home processing and preparation into the water bodies. Improper handling by the fishermen or consumers, environmental habitat and improper storage can also be the main reason for contamination. The river water could be used for irrigation but this is unsuitable for drinking purposes due to the presence of faecal

contaminant such as *Vibrio cholerae* which can cause cholera infection and *Salmonella sspecies*. With the result obtained, it has been shown that some of the isolated microorganisms were pathogenic which can cause an infection. Some can also cause opportunistic infection such as *Staphylococcus aureus, Bacillus sp* etc. It is concluded that the river is polluted as it is used as a sewer disposal site, but is also undergoing self-purification and has potential for significant improvement in water quality if discharges are ameliorated. Conclusively, it is imperative to maintain proper hygiene while handling freshwater crayfish and ensure proper cooking. With this result, freshwater crayfish (*Macrobrachium vollenhovenii*) is very safe for consumption since the microorganisms identified were not harmful if proper precautions can be followed and proper preservation of freshwater crayfish should be maintained if not consumed immediately because the normal microflora can easily causes the decay of the crayfish thereby leading to higher microbial load.

Recommendation

It is hereby recommended that fresh crayfish should be properly and effectively preserved and handled properly to prevent it from contamination. And proper hygienic measure should be carried out during and after aqua farming of fresh crayfish to avoid faecal contaminant such as Cholera and septicaemia.Crayfish should be properly cooked before consumption as this can result in allergic response. Likewise, proper cooking of crayfish can help to kill psychrophilic microbes such as *Staphylococcus* sp., *Bacillus cereus and Pseudomonas aeruginosa.* Isolation of fresh crayfish should be carried out without delay in order not to encourage the growth of other pathogenic organisms.This research work will also form a benchmark for environmental monitoring of the aquaculture zone hence improve the productivity of fresh crayfish as a proteinous source of food and also a source of calcium to the body

References

[1] Batt, W. H., 2016. "Saving the commons in an age of plunder." *American Journal of Economics and Sociology*, vol. 75.

[2] Yusuf, K. A., Oluwole, S. O., Abdusalam, I. O., and Majolagbe, A. O., 2013. "Assessment of spatial variation of surface water quality in Lagos, using multivariate statistical techniques." *Journal of Environment*, vol. 02, pp. 94-102.

[3] Ubwa, O. J., Abah, J., and Asemave, K., 2013. "An assessment of surface water pollution status around Gboko abattoir." *African Journal of Pure and Applied Chemistry*, vol. 7, pp. 131-138.

[4] Gholikandi and Hazrati, H., 2012. "Artificial neural network modeling for predict performance of pressure filters in a water treatment plant." *Desalination and Water Treatment*, vol. 39, pp. 192-198.

[5] Mustapha, A. and Nabegu, A., 2011. "Surface water pollution source identification using principal component and factor analysis in Getsi River, Kano, Nigeria." *Australian Journal of Basic and Applied Sciences*, vol. 5, pp. 1507-1512.

[6] Orogu, J. O., Aphair, A. E., and Oghonyon, E. I., 2018. "Bacteriological analysis of dried crayfish and stockfish in Ozoro market." *IndoAmerican Journal of Pharmaceutical Sciences*, vol. 5, pp. 5234-5239.

[7] Ibironke, S. I., Fashakin, J. B., and IGE, M. M., 2014. "Nutritional quality of animal polypeptide (Crayfish) formulated into complementary foods." *American Journal of Food and Nutrition*, vol. 2, pp. 9-42.

[8] Enang, B., 2014. "Analysis of crayfish price relationship in calabar. A bachelor of agriculture degree dissertation of the faculty of agriculture. Spp. In surface waters of southern alberta and its relation to manure source." *Canadian Journal of Microbiology*, vol. 49, pp. 326-335.

[9] Omomo, D., Sunday, R. M., and Kareem, I., 2014. "Effect of smoke drying techniques on the proximate and mineral composition of Macrobrachiumvollenhovenii (African River Prawn)." *World Academy of Science, Engineering and Technology International Journal of Biological, Food, Vertinary and Agricultural Engineering*, vol. 8, pp. 185-188.

[10] Gadotra, R., Gupta, V., Koul, M., and Gupta, S., 2013. "Impact of ascorbic acid and citric acid treatment on the shelf life of silver carp Hypophthalmichthys molitrix (Silver Carp) fillets in frozen Storage." *Int. J. Recent Sci. Res.*, vol. 4, pp. 1103-1108.

[11] Lauzon, H. L., Margeirsson, B., Sveinsdottir, K., Guojonsdottir, M., Karlsdottir, M. G., and Martinsdottir, E., 2010. "Overview on fish quality research. Impact of fish handling, processing, storage and logistics on fish quality deterioration. Iceland Matis Report." pp. 39-10.

[12] Can, O. P. and Begum, 2010. "Evaluation of the microbiological, chemical and sensory quality of carp processed by the sous vide method." *Int. J. Nutr. Food Eng*, vol. 4, pp. 477-482.

[13] Oyebamiji, O. F., Raheem, A. A., and Rasheed, Y. O., 2018. "Length-weight relationship, abundance and sex ratio of the giant river prawn macrobrachium vollenhovenii (herklots, 1857) from river Osun, Southwestern Nigeria." *J. Aquac. Res. Development*, vol. 9, p. 554.

[14] Collins, C. H., Lyne, P., Joseph, F., Taylor, I., and Francis, 2004. *The department of environmental science and technology*. London: Imperial College. p. 480.

[15] Bello, C. S. S., 2002. *Laboratory manual for student of medical microbiology*. 2nd ed. Jos: Satograhics Press. pp. 80-85.

[16] Cheesbrough, M., 2002. *District laboratory \practice in tropical countries, E. C.B.S* 2nd ed. Cambridge University Press, pp. 256-267.

[17] Fawole, M. O. and Oso, B. A., 2004. *Characterization of bacteria. Laboratory manual of microbiology*. 4th ed. Ibadan, Nigeria: Spectrum Book Limited. pp. 24-33.

[18] Willey, J. M., Sherwood, L. M., and Woolverton, C., 2008. *Prescott, harley and klein's microbiology*. New York: McGraw Hill Higher Education.

[19] Hoffman-Soommer, M., Migdalski, A., Rytka, J., and Kucharczyk, R., 2005. "Multiple functions of the vascular sorting protein Ccz1p in Saccharomyces cerrevisiae." *Biochem Biophys Res Commun*, vol. 329, pp. 197-204.

[20] CLSL, 2011. *Global health partnerships and international organisation for standardization*. PA: Clinical and Laboratory Standards Institute.

[21] CLSL, 2016. "Performance standards for antimicrobial susceptibility testing." In *26th supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute*.

[22] Akintola, S. and Bakare, S., 2011. *American Journal of Food Technology*, vol. 6, pp. 500-506.

[23] George, M. and Moses, E., 2011. "Native Valve Endocarditis due to Micrococcus luteus: a case report and review of the Literature." *Journal of Medical case Reports*, vol. 5, p. 251.

[24] Chih-Chin, K., Chih-Kang, C., and Jeng-Wen, H., 2014. "Micrococcus species-related Peritonitis in patients receiving peritoneal dialysis." *International Urology and Nephrology*, vol. 46, pp. 261-264.

[25] Adedeji, O. B., Adebisi, T., and Emikpe, B. O., 2011. "Bacteria load of the skin and stomach of clariasgariepinus and oreochromisniloticus from ibadan, south west nigeria: Public health implications." *Journal of Microbiology and Biotechnology Research*, vol. 1, pp. 52–59.

[26] Uddin, N. and Al-Harbi, A. H., 2012. "Bacterial flora of polycultured common carp, Cyprinus carpio and African catfish, Clarias gariepinus." *International Aquatic Research*, vol. 4, p. 10.