Original Research Article

Characterization and Isolation of Bacteria from Adult African Catfish (*Clarias gariepinus*) in selected fish farms of Rivers state, Nigeria.

ABSTRACT

This research examined the bacterial loads in the intestine, skin and gills of cultured *Clarias gariepinus* in some selected private farms at Eleme, Emohua, Ikwerre, Obio/Akpor and Port Harcourt Local Government Areas of Rivers State, Nigeria. Determination of bacterial loads species characterization in fish parts was done using standard microbiological procedures. Results of the assay showed high bacterial count in the gill, gut and skin of fish from the different locations, especially at Emuoha Local Government Area. The total heterotrophic bacteria counts of fish: gills ranged between 1.7×10^8 and 3.1×10^7; gut ranged between 1.4×10^7 and 7.2×10^6 and skin ranged between 1.5×10^6 and 6.8×10^6 in all the stations under consideration (Eleme, Emohua, Ikwerre, Obio/Akpor and Port Harcourt city Local Government Areas of Rivers State, Nigeria.). Bacterial genera isolated includs: *Escherichia coli, Salmonella* sp. *Enterica* sp., *Staphylococcus* sp., *Bacillus* sp., *Shigella* sp., *Enterobacter* sp., *Klebsiella* sp.and *Citrobacter* sp.

Key words: *Escherichia coli, Salmonella* sp. *Enterica* sp., *Staphylococcus* sp., *Bacillus* sp., *Shigella* sp., *Enterobacter* sp., *Klebsiella* sp.and *Citrobacter* sp.. Eleme, Emohua, Ikwerre, Obio/Akpor and Port Harcourt Local Government Areas

1. INTRODUCTION

Like humans and other animals, fish suffer from diseases [7]. Fish defences against disease are specific and non-specific. Non-specific defences include skin and scales, as well as the mucus layer secreted by the epidermis that traps microorganisms and inhibits their growth [7]. Specific defences are specialised responses to particular pathogens recognised by the fish's body, that is adaptative immune responses [1]. Bacterim is a type of biological cell and constitute a large domain of prokaryotic microorganisms [2]. A prokaryote is a unicellular organism that lacks a membrane-bound nucleus, mitochondria, or any other membrane-bound organelle [2]. Typically a few micrometres in length, bacteria have a number of shapes, ranging from spheres to rods, spirals; live in symbiotic and parasitic relationships with plants and animals [6]. Virtually all animal life on earth is dependent on bacteria for their survival as only bacteria and some archea possess the genes and enzymes necessary to synthesize vitamin B12, also known as cobalamin, and provide it through the

food chain [3]. Vitamin B12 is a water-soluble vitamin that is involved in the metabolism of every cell of the human body [4]. In humans and most animals, the largest numbers of bacteria exist in the gut, and a large number on the skin. The vast majority of the bacteria in the body are rendered harmless by the protective effects of the immune system, though many are beneficial, particularly in gut flora [5]. However, several species of bacteria are pathogenic and cause infectious diseases. Examples are the *renibacterium salmoninarum* which causes bacterial kidney disease. Sometimes referred to as Fin and Tail Rot, bacterial infections are the second-most common diseases aquarium fish experience after parasites [6]. They often follow parasite infestations, abrasions or physical injury, but can also be brought on by chronic exposure to poor water quality and/or poor diet [9]. Removal of a fish's protective mucous membrane or scales during netting is another common cause. Bacteria are responsible for many fatal diseases in fishes like furunculosis, columnaris, fin and tail rot, vibriosis, dropsy, cotton mouth disease and tuberculosis. They could be gram positive or gram negative [10].

Gram-positive bacteria are bacteria that give a positive result in the Gram stain test, which is traditionally used to quickly classify bacteria into two broad categories according to their cell wall while Gram-negative bacteria are bacteria that do not retain the crystal violet stain used in the Gram staining method of bacterial differentiation [8].

Bacterial infections manifest in many ways, but common signs include a white film on the fish's body or fins, cloudy eyes, tattered fins, and hemorrhaging (bloody patches) or open sores (ulcers) on the body and mouth [11]. *Furuculosis* disease is caused by *Aeromona salmonicida* in salmon fishes [13]. It is a non-motile, gram-negative bacterium. This disease frequently appears to infect fishes living in the dirty waters containing a large amount of decaying matter [12]. The first symptoms of this disease are appearance of boil like lesions. Others symptoms are blood-shot fins, blood discharge from the vent, haemorrhages in muscles and other tissues and necrosis of the kidney [20]. Bursting of boils allow the spread of this disease among other fishes and also offer suitable areas for fungus growth. Fishes severely infected with the bacteria die in good number [13].

2. MATERIALS AND METHODS

Study area: Rivers State is a predominantly low-lying pluvial state in southern Nigeria, located in the eastern part of the Niger Delta on the ocean ward extension of the Benue Trough. The inland part of the state consists of tropical rainforest, and towards the coast, the typical Niger Delta environment features many mangrove swamps. Rivers State has a total area of 11,077 km^2 (4,277 squar mitre), making it the 26th largest state in Nigeria. Surrounding states are

Imo, Abia and Anambra to the north, Akwa Ibom to the east and Bayelsa, Delta to the west. On the south, it is bounded by the Atlantic Ocean. Its topography ranges from flat plains, with a network of rivers to tributaries.

2.1 Sampling Method: Fifteen live *Clarias gariepinus* adults (1.25kg average weight) were randomly collected from ponds of 5 privately owned farms in Emohua, Eleme, Ikwerre, Obio/Akpor and Port Harcourt Local Government Areas of Rivers State, Nigeria. Specimens were caught using a hand net and transferred alive in plastic containers to the laboratory for examination and collection of samples. In the laboratory, bacteria were isolated from the gills, fish guts and skin using standard methods.

2.2 Preparation of Media: Nutrient Agar (obtained commercially in powdered form) was used for the isolation of bacteria from the fish samples. The media was prepared according to the manufacturers guide; 28.0g was dissolved in 1L of distilled water and sterilized by autoclaving at 121 0^c for 15 minutes. The media was allowed to cool and then poured into sterile disposable petri dishes and allowed to solidify.

2.3 Preparation of Samples/ Microbiological Analysis: The fish were killed and placed on a clean foil paper where 1g of skin, 1g of fish guts and 10g of gills were collected. Fish tissues where then put into 9ml of distilled water to give 1:10 dilution and shaken thoroughly. 1ml of the pond water samples was also pippeted into 9ml of distilled water to also give 1:10 dilution. The stock solution was serially diluted up to 10-5 as described by Willey *et al*. (2008). Plating (spread plate method) was done by inoculating 0.1ml of the dilution on nutrient agar in duplicate plates using 10-4 and 10-5 and spreading with a sterile glass spreader, the plates were then incubated for 18-24 hours at ambient temperature. The plates were examined after incubation and the number of colony forming units (CFU) that developed were counted and recorded.

2.4 **Isolation**: Isolation of the colonies was done by sub-culturing representative colonies on a freshly prepared nutrient agar. This was then incubated at 31 0^C for 24 hours to obtain pure cultures.

2.5 Characterization of Isolates: The characterization of the organisms was based on colonial, morphological and biochemical characteristics of colonies. Microscopic examination of surface colonies on nutrient agar medium was used to determine the shape and arrangement of microorganisms. Morphological characteristics were studied on the oil immersed slide under the microscope after gram staining.

2.6 Biochemical Tests

2.6.1 Catalase Tests: This test is used to determine the ability of an organism to breakdown hydrogen peroxide (H_20_2) into oxygen and water. Only organisms that have the enzyme catalase are able to catalyse the reaction. The presence of the enzyme in a bacterial cell is evident when a small inoculum is introduced into a 3 % hydrogen peroxide solution and rapid production of effervescence occurs. The absence of catalase is evident by a weak production of effervescence.

2.6.2 Citrate Test: The citrate test is used to determine the ability of an organism to utilize sodium citrate as its sole source of carbon and inorganic ammonium salt as its only source of nitrogen. Bacteria that grow on this medium turn the bromothymol blue indicator from green to blue. Simmon citrate agar is prepared in a capped test tube according to manufacturer's instruction. A sterile wire loop is used to pick a loopful of the test organism and streaked on slant surface. The tube is incubated at 37°C for 48h. Change in colour from green to blue is indicative of positive result while no change in colour is indicative of a negative result.

2.6.3 Indole Test: This test is used to determine the ability of an organism to split the amino acid tryptophan to form pyruvic acid, ammonia and indole using the enzyme tryptophanase. A loopful of the test organism is inoculated into sterile peptone water medium and incubated at 37°C for 48h. Thereafter, 0.3-0.5 ml of Kovac's reagent is added using a Pasteur's pipette. Appearance of red ring layer on medium is indicative of positive test while development of a yellow ring is indicative of negative result.

2.6.4 Methyl Red/Voges Proskauer (MR/VP) Test: This test is used to determine the ability of an organism to produce and maintain stable acid end products from glucose fermentation and to determine the ability of some organisms to produce neutral end products such as acetyl-methyl carbinol or acetoine from glucose fermentation. The MR/VP broth medium is used for this test. A loopful of test organism is inoculated into 10 ml sterile MR/VP broth medium prepared according to manufacturer's instructions. The tube is incubated at 35-37 °C for 48 h. After incubation, the broth culture is shared into two equal parts (5 ml), one part is used for methyl red test, and the other part for Voges Proskauer test. To the part for MR, 5-6 drops of methyl red reagent is added and to the part for VP, 0.6 ml (6 drops) of 5 % a-naphthol and 0.2ml (2 drops) of 40% KOH reagent are added and observed for 5 minutes. Development of bright red colouration is indicative of positive MRVP tests.

2.6.5 Motility Test: This test is to determine if an organism is motile. An organism must possess flagella (a locomotory organelle) to be motile. Semi-solid nutrient agar is used for this test. Half strength of the medium is prepared

following manufacturer's direction. A young (fresh) colony is picked with a sterile straight wire (inoculating needle) and inoculated by stabbing into the centre of medium. Thereafter, the tube incubated at 37 °C for 24-48h. Growth (in diffused form) from line of stab into the medium is indicative of positive result, whereas growth only along the line of stab is indicative of a negative result.

2.6.6 Oxidase Test: This test is used to determine the presence of bacterial cytochrome oxidase using the oxidation of the substrate tetramethyl-p-phenylenediamine dihydrochloride to indophenols. A filter paper soaked with 1% tetramethyl-p-phenylenediamine dihydrochioride is used to perform the test. A platinum wire loop is used to pick a small portion of the test organism which is then rubbed on the soaked filter paper. Observation for 10 seconds for development of purple colouration on the smeared portion is indicative of positive result for oxidase test while no change in colour of portion was indicative of negative oxidase test.

2.6.7 Coagulase Test: This test is used to differentiate Staphylococcus aureus (positive) from coagulase negative staphylococci (negative). S. aureus produces two forms of coagulase: bound and free. Bound coagulase or "clumping factor," is bound to the bacterial cell wall and reacts directly with fibrinogen. This results in an alteration of fibrinogen so that it precipitates on the staphylococcal cell, causing the cells clump when a bacterial suspension is mixed with plasma. The presence of bound coagulase correlates well with free coagulase, an extracellular protein enzyme that causes the formation of a clot when S. aureus colonies are inoculated with plasma. The clotting mechanism involves activation of a plasma coagulase-reacting factor (CRF), which is a modified or derived thrombin molecule, to form a coagulase CRF complex. This complex in turn reacts with fibrinogen to produce the fibrin clot. To perform this test, a loopful of test organism is inoculated into rabbit plasma, this is observed in 5 to 10 seconds for clumping. Positive result is denoted by clumping of the staphylococcal cells, while no clumping is indicative of negative coagulase test.

2.6.8 Starch Hydrolysis Test: This test is used to determine the ability of an organism possessing the enzyme amylase to hydrolyze starch. The presence of this enzyme in any bacterial isolate (or culture) gives it the ability to use up the starch in the medium that is used to grow the organism. A 1 % starch agar is used to carry out this test. This starch is incorporated in nutrient agar medium, autoclaved at 121 °C, poured into sterile Petri-dish, allowed to solidify and dried. Each test organism is then streaked on the agar surface and incubated at 37 °C for 24-48 h. Thereafter, the plate is flooded with iodine solution. A positive result is shown by a clear zone around the lines of streak of the organism.

2.6.9 Triple Sugar Iron Acar (TSIA) Test: This test is used to determine whether the bacterial isolates could ferment glucose and lactose or sucrose and form hydrogen sulfide (H_2S), gas, and acids. The Triple Sugar Iron Agar (TSIA) is used for this test. The slant was prepared according to manufacturer's recommendation. To perform this test, a straight sterile inoculation needle is used to pick the organism which is then inoculated by first stabbing the centre of the medium (not to the bottom of the tube) and streaking the surface of the agar slant using wire loop. Thereafter, the tubes were incubated at 35-37 °C for 24-48 h. Change in colour of medium from pink to yellow indicate acid production resulting from glucose fermentation. When in addition to glucose, lactose and/or sucrose are fermented; the large amount of fermented products formed on the slant will neutralize the alkaline amines and render the slant acidic (yellow). Black colouration indicates production of hydrogen sulfide (H_2S) and cracks in the medium or complete upward shift of the agar slant from bottom of tube indicates positive gas production.

2.6.10 Urease Test: This test is used to determine the ability of an organism to produce the enzyme urease,, which hydrolyzes urea. Hydrolysis of urea produces ammonia and CO_2. The formation of ammonia alkalinizes the medium, and the pH shift is detected by the colour change of phenol red from light orange at pH 6.8 to magenta at pH 8.1. Urea agar prepared according to manufacturer's standard is used for this test. A loopful of an overnight test organism is streaked on the agar slant and incubated 35^0 C for 48hour to 7 days. change in colour of medium from orange to magenta is indicative of positive urease test while no change in colour of medium is a negative result.

2.6.11 Sugar Fermentation: This test is used to determine the ability of an organism to ferment a specific carbohydrate that is incorporated in a basal medium, thereby producing acid and gas. Peptone broth (1%) is the basal medium incorporated in 1% sugar used for the test. Bromocresol purple (0.3%) indicator is added to the sugar medium with Durham's tube added in the tube in inverted (upside down) position. After sterilization, a loopful of test organism is introduced into the medium using a sterile wire loop. The test tube is then incubated at 35-37 °C for 24-48 h. Change in colour from purple to yellow is an evident of acid production resulting from sugar fermentation while gas production is detected in the Durham's tube.

2.6.12 Gram Staining: The Grain's staining technique is carried out to differentiate the bacterial isolates into Gram positive and Gram negative bacteria based on their cell wall composition. A loopful of a 24h culture is picked and used to make a smear on a clean, greased slide. The smear was heat fixed by passing over a flame and subsequently, the smear is flooded with crystal violet for one minute 'and rinsed under slow running tap. The smear is

flooded with iodine for one minute and rinsed under slow running water. Thereafter, the smear is decolorized using 95% alcohol for 30 seconds or until smear no longer washes off if held at an angle and rinsed with water. Furthermore, the smear is counter stain with safranin for 30 seconds and rinsed with water. Then it is allowed to air dry. Thereafter, a drop of immersion oil is placed on the smear and viewed under the light microscope using oil immersion objective lens. Cells that appeared purple were recorded as Gram- positive cells while cells that appeared pink under the microscope are taken as Gram-negative cells.

2.6.13 Procedures for Microbiological Analysis

2.6.13.1 Total Heterotrophic Bacteria (THB): 1g of sample was weighed into 9ml sterile diluent (0.85% NaC1) under aseptic condition. It was then agitated to Ahomogenize and serially diluted. Then 0.1 ml aliquot of the inoculum was collected using a sterile pipette, inoculated on Nutrient Agar (NA) medium. The inoculum was spread evenly with a sterile bent glass rod. Plates were incubated at 37°C for 24 hours. Thereafter, colonies were counted to obtain Colony Forming Unit (CFU) value per gram of sample. Distinct colonies with different morphological patterns were picked and streaked or subculture on freshly prepared nutrient agar medium to obtain pure culture after 24 hours incubation at 37°C. The pure cultures were gram stained for microscopic examination. It was also used to carry out biochemical tests for characterization/identification of the isolates.

3. RESULTS

3.1 Isolation and identification of bacterial species: In this study, bacterial isolates, namely, *Escherichia coli, Salmonella* sp. *Enterica* sp.*, Staphylococcus* sp.*, Bacillus* sp.*, Shigella* sp.*, Enterobacter* sp.*, Klebsiella* sp.and *Citrobacter* sp.*,* representing 8 bacterial species were accessed through fish gills, guts and skin from 15 fish samples (3 per farm) of the 5 farms studied at a farm per LGA (Eleme, Obio/Akpor, Emuoha, Ikwerre and Port Harcourt City) – Fig. 1. This study shows that *Escherichia coli* count was high in the gills, fish guts and skin of fish from all sampled locations, except in the fish guts of Eleme Local Government Area while in Obio/Akpor, *Bascilus* sp. was observed in both the fish guts and skin but was not found in the gills. At Emohua, *Bascillus* sp. was seen in the gills, fish guts and skin of the specimen. At Eleme, it was not observed on the gills and fish guts but on the skin. At Port Harcourt, the species was present in the gills only. In Ikwerre Local Government Area, the species had it's highest occurrence, as it was seen in the gill, fish gut and skin. *Klebsiella* sp. was absent in the fish guts of all the locations sampled except at Port Harcourt. It was found to be present in the gills of samples taken from

Obio/Akpor and Emohua; and present only in the skin of fish from Ikwerre LGA. *Enterobacter, Salmonella, Shigella and Citobacter* sp were all absent in the gills, guts and skin of samples from Obio/Akpor. *Staphylococcus* sp was observed on the gill and skin in samples from Obio/Akpor and Port Harcourt; Skin of fish from Emuoha; Guts and skin of fish from Eleme; Gills of fish from Port Harcourt City; Guts and skin of fish from Ikwerre. *Salmonella* sp was observed on fish gut and skin of fish from Eleme and Port Harcourt City and gill and guts of that from Ikwerre LGA. *Enterobacter* sp was seen on the gill of fish from Emuoha; Skin of fish from Port Harcourt and gill of fish from Ikwerre LGA while *Shigella* sp was recorded in fish guts from Emuoha; Gill and fish guts from Eleme; Gill in Port Harcourt and gill and guts from Ikwerre LGA. *Citrobacter* sp was only observed on the skin of fish from Eleme LGA (Tables 1,2,3). The highest Total Heterotrophic Bacteria density occurred in fish gut at Ikwerre Local Gvernment Area (7.2×10^6), followed by 6.8×10^6 on skin at Eleme Local Government Area. Generally, Total Heterotrophic Bacteria density was more on the skin of fish from Eleme, Emohua, Obio/Akpor and Port Harcourt Local Government Area when compared with other entry sites (Table 4). As *Citrobacter* sp. was completely absent in the gill and fish guts, *E. Coli*. formed 29.41% of bacteria followed by *Baccillus* sp (17.65%); Klebsella, *Enterobacter, Shigella* and *Staphilococcus* sp. were within the same range (11.76%) in the gills while in the guts 23.53% of *E. Coli*. formed the bulk (23.53%) followed by *Baccillus* sp , *Salmonella* and *Shigella* sp. (17.65%), *Klebsella* and *Staphylococcus* sp. (11.76%), were the least. *Enterobacter* sp. was completely absent in the gut. *Salmonella* sp. was least in occurrence in the gills. *Baccillus, Staphilococcus sp* and *E.colli*. occurred most on the skin (23.81%) followed by *Klebsella, Enterobacter, Shigella* and *Citrobacter* sp. (4.96%) -Table 5. *E. colli* was most abundant in the gills (35.71%), skin (35.71%) and guts (28.57%). On the skin, it was observed that *Baccillus* sp. was 45.45% and the same in the gills and guts (27.27%). *Klebsella* and *Enterobacter* sp. occurred most in the gills (40.00 and 66.67%) and guts (40.00%) when compared with the skin (20.00 and 33.33%). *Enterobacter* sp was not found on the skin. In the fish gut, *Salmonella* sp and *Shigella* sp (50.00% each) when compared with that of gills (16.67 and 33.33%) and skin (33.33% each). *Staphylococcus* sp occurred most on the skin (55.56%) when compared with that of gills and guts (22.22% each) while *Citrobacter* sp was only found on the skin (100.00%) – Tables 6 and 7.

Table 1: Total bacteria count in the gills of *C. gariepinus* from the different locations

Code	Gram Reaction	Cell morphology	Catalase	Oxidase	Indole	Citrate	Motility	H_2S	Gas	Slant	Butt	Cilu	Lac	Suc	Probable Genera
Emo.gill	-	Rod	-	-	+	-	-	-	+	A	A	A/G	A/G	A/G	*E. coli*
	+	Rod	+	-	-	+	+	-	-	B	A	A	-	A	*Bacillus* sp.
	-	Rod	-	-	-	+	-	-	+	A	A	A/G	A/G	A/G	*Klebsiella* sp.
	-	Rod	+	+	-	+	+	-	+	A	A	A/G	A/G	A/H	*Enterobacter* sp.
E1em.gill	-	Rod	+	-	-	-	-	-	-	B	A	A	-	A	*Shigella* sp.
	-	Rod	-	-	+	-	-	-	+	A	A	A/G	A/G	A/G	*E. coli*
Ikwe. gill	-	Rod	-	-	+	-	-	-	+	A	A	A/G	A/G	A/G	*E. Coli*
	+	Rod	+	-	-	+	+	-	-	B	A	A	-	A	*Baccillus* sp.
	-	Rod	+	-	-	+	+	+	+	B	A	A/G	A/G	A/G	*Salmonella* sp.
	-	Rod	+	+	-	+	+	-	+	A	A	A/G	A/G	A/H	*Enterobacter* sp.
	-	Rod	-	-	-	-	-	-	-	B	A	A	-	A	*Shigella* sp.
Obio. gill	+	Rod	+	-	-	+	-	-	-	A	A	A	A	A	*Staphylococcus* sp.

	-	Rod	-	-	+	-	-	-	+	A	A	A/G	A/G	A/G	*E. Coli*
	-	Rod	-	-	-	+	-	-	+	A	A	A/G	A/G	A/G	*Klebsiella* sp.
PHC. gill	-	Rod	-	-	+	-	-	-	+	A	A	A/G	A/G	A/G	*E. coli*
	+	Rod	+	-	-	-	+	-	-	A	A	A	-	A	*Bacillus*
	+	Cocci	+	-	-	+	-	-	-	A	A	A	A	A	*Staphylococcus* sp.

Table 2: Spread of bacteria in the Fish guts of of *C. gariepinus* from the different locations

Emo.gut	-	Rod	-	-	-	+	-	-	+	A	A	A/G	A/G	A/G	*Klebsiella* sp.
	-	Rod	-	-	-	-	-	-	-	B	A	A	-	A	*Shigella* sp.
	-	Rod	-	-	+	-	-	-	+	A	A	A/G	A/G	A/G	*E. coli*
Elem.gut	-	Rod	+	-	-	+	+	+	+	B	A	A/G	A/G	A	*Salmonella* sp.
	+	Rod	+	-	-	-	-	-	-	B	A	A	-	A	*Shigella* sp.
	+	Cocci	+	-	-	+	-	-	-	A	A	A	A	A	*Staphylococcus* sp.
Ikwe.gut	-	Rod	+	-	-	-	-	-	-	B	A	A	-	A	*Shigella* sp.
	-	Rod	+	-	-	+	+	-	-	B	A	A	-	A	*Bacillus* sp.
	-	Rod	-	-	+	-	-	-	+	A	A	A/G	A/G	A/G	*E. coli*

	+	Cocci	+	-	-	+	-	-	-	A	A	A	A	A	*Staphylococcus* sp.
	-	Rod	+	-	-	+	+	+	+	B	A	A/G	A/G	A	*Salmonella* sp.
Obio.gut	-	Rod	-	-	+	-	-	-	+	A	A	A/G	A/G	A/G	*E. coli*
	+	Rod	+	-	-	+	+	-	-	B	A	A	-	A	*Bacillus* sp.
	+	Rod	+	-	-	-	+	-	-	A	A	A	-	A	*Bacillus* sp.
PHC.gut	-	Rod	+	-	-	+	+	+	+	B	A	A/G	A/G	A	*Salmonella* sp.
	-	Rod	-	-	+	-	-	-	+	A	A	A/G	A/G	A/L	*E. coli*
	-	Rod	-	-	-	+	-	-	+	A	A	A/G	A/G	A/G	*Klebsiella* sp.

Table 3: Total bacteria count on the skin of *C. gariepinus* from the different locations

Emo.skin	+	Rod	+	-	-	+	+	-	-	B	A	A	A	A	*Bacillus* sp.
	-	Rod	-	-	+	-	-	-	+	A	A	A/G	A/G	A/G	*E. coli*
	+	Cocci	+	-	+	+	-	-	-	A	A	A	A	A	*Staphylococcus* sp.
Elem.skin	-	Rod	+	-	-	+	+	+	+	B	A	A/G	A/G	A	*Salmonella* sp.
	+	Rod	+	-	-	+	+	-	-	B	A	A	-		*Baccilus* sp.
	-	Rod	-	-	-	-	-	-	+	A	A	A/G	A/G	A/G	*E. Coli*

	+	Cocci	+	-	-	+	-	-	-	A	A	A	A	A	*Staphylococus* sp.
	-	Rod	+	+	-	+	+	+	+	A	A	A/G	A/G	A	*Citrobacter* sp.
Ikwe.skin	-	Rod	-	-	+	-	-	-	+	A	A	A/G	A/G	A/G	*E. coli*
	+	Cocci	+	-	-	+	-	-	-	A	A	A	A	A	*Staphylococcus* sp.
	+	Rod	+	-	-	+	+	-	-	B	A	A	-	A	*Bacillus* sp.
	+	Rod	+	-	-	-	+	-	-	A	A	A	-	A	*Bacillus* sp.
	-	Rod	+	-	-	+	+	+	+	B	A	A/G	A/G	A	*Klebsiella* sp.
	-	Rod	+	-	-	+	-	-	-	A	A	A	A	A	*Staphylococcus* sp
Obio.skin	-	Rod	-	-	+	-	-	-	+	A	A	A/G	A/G	A/G	*E. coli*
	+	Rod	+	-	-	+	+	-	-	B	A	A	-	A	*Bacillus* sp
	+	Cocci	+	-	-	+	-	-	-	A	A	A	A	A	*Staphylococcus* sp.
PHC.skin	-	Rod	+	-	-	+	+	+	+	B	A	A/G	A/G	A	*Salmonella* sp.
	-	Rod	+	-	-	-	-	-	-	B	A	A	-	A	*Shigella* sp.
	-	Rod	+	+	-	+	+	-	+	A	A	A/G	A/G	A/G	*Enterobacter* sp.
	+	Rod	-	-	+	-	-	-	-	A	A	A/G	A/G	A/G	*E. coli*

Key: Emo. gill = Emohua gill, E1em. Gill = Eleme gill; Ikwe. Gill = Ikwerre gill; Obio. Gill = Obio Akpor gill; PHC.gill = Port Harcourt gill; Emo. gut = Emohua gut; Elem. gut = Eleme gut; Ikwe.gut = Ikwerre gut; Obio. gut = Obio/Akpor gut; PHC gut = Port Harcourt gut; Emo. skin = Emohua skin; Elem. skin = Eleme skin; Ikwe. skin = Ikwerre skin; Obio.skin = Obio/Akpor skin; PHC. skin = Port Harcourt skin.

Table 4: Total Heterotrophic Bacteria (THB) Densities in Gill, Fish guts and Skin of *C. gariepinus* from the different locations.

	THB (cfu/g)		
LGA	**Gill**	**Fish guts**	**Skin**
EleLGA	3.1×10^{7}	4.2×10^{6}	6.8×10^{6}
EmoLGA	1.7×10^{8}	2.2×10^{7}	5.2×10^{6}
IkweLGA	3.1×10^{7}	7.2×10^{6}	1.5×10^{6}
ObioLGA	1.7×10^{6}	1.4×10^{7}	4.5×10^{6}
PHCLGA	2.8×10^{7}	3.5×10^{6}	4.8×10^{6}

sp Goernment Area; IkweLGA= Ikwerre Local Goernment Area; ObioLGA= Obio/Akpor Local Goernment Area; PHCLGA= Port Harcourt City Local Goernment Area: CFU/g = Colony Forming Unit per gram;

Table 5: Percentage occurrence of each bacterial Isolate in the gill, gut and on skin of *Clarias gariepinus*

Bacteria	Gills(%)	Fish guts(%)	Skin(%)
E. Coli.	29.41	23.53	23.81
Baccillus sp	17.65	17.65	23.81
Klebsella sp	11.76	11.76	4.96
Enterobacter sp	11.76	0.00	4.96
Salmonella sp	5.88	17.65	9.52
Shigella sp	11.76	17.65	4.96
Staphylococcus sp	11.76	11.76	23.81
Citrobacter sp	0.00	0.00	4.96

Table 6: Percentage occurrence of bacterial Isolates in body tissues of *Clarias gariepinus* across locations

Bacteria	Gills(%)	Fish guts(%)	Skin(%)
E. Coli.	35.71	28.57	35.71
Baccillus sp	27.27	27.27	45.45
Klebsella sp	40.00	40.00	20.00
Enterobacter sp	66.67	0.00	33.33
Salmonella sp	16.67	50.00	33.33
Shigella sp	33.33	50.00	16.67
Staphylococcus sp	22.22	22.22	55.56
Citrobacter sp	0.00	0.00	100

Table 7: Identification of bacteria Isolates at different locations

Bacteria	Obio/Akpor			Emohua			Eleme			Port City Harcourt			Ikwerre		
	Gill	Fish gut	Skin	Gill	Fish gut	Skin	Gill	Fish gut	Skin	Gill	Fish gut	Skin	Gill	Fish gut	Skin
E. coli	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Bacillus sp	-	+	+	+	+	+	-	-	+	+	-	-	+	+	++
Klebsiella sp	+	-	-	+	-	-	-	-	-	-	+	-	-	-	+
Salmonella sp	-	-	-	-	-	-	-	+	+	-	+	+	+	+	-
Enterobacter sp	-	-	-	+	-	-	-	-	-	-	-	+	+	-	-
Shigella sp	-	-	-	-	+	-	+	++	-	-	-	+	+	+	-
Staphylococcus sp	+	-	+	-	-	+	-	+	+	+	-	-	-	+	+
Citrobacter sp	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-

4. Discussion

The fish samples (skin, fish gut and gill) examined in this study looked physically healthy based on their appearances but they were all infected with bacteria. [14] reported that bacteria are abundant in the environment in which fish live and it is therefore impossible to avoid them being a component of their diet. Results of the assay showed a high bacterial count in the gill, gut and skin of fish taken from all the Local Government Areas under consideration (Emohua, Eleme, Ikwerre, Obio/Akpor and Port Harcourt City). The heterotrophic bacteria count in these LGA(s) ranged from 7.2×10^6 CFU/g in the in the gut to 6.8×10^6 CFU/g on the skin. Heterotrophic bacteria counts obtained in the gills ranged from 1.7×10^6 CFU/g to 3.1×10^7 CFU/g, the counts obtained in the intestine ranged from 1.4×10^7 to 4.2×10^6 and in the skin, the counts ranged from 1.5×10^6 to 6.8×10^6 respectively. Gram-positive rod-shaped bacteria dominated the populations, this contrasts with the report by [15,38,39]. The bacteria entering along with the diet of fish during ingestion may adapt themselves in the gastrointestinal tract and form a symbiotic association within the digestive tract of fish, in which large numbers of microbes are present. This indicates that the digestive tracts of fish provide favourable ecological niches for these organisms [16,36,37]. In this work, the gills from each location were found to have the least count for bacterial load. This is in agreement with [17,34,35] who reported that the gill has the least count of bacterial count. The occurrence of these species in different organs of fish may be associated with some predisposing factors such as poor handling, feed and feeding, improper pond management, and excessive amounts of decomposing organic matter in the pond [18,32,33]. In a study carried out on bacterial infection on *C. gariepinus,* isolated and identified *Staphylococcus aureus, E. coli , Staphylococcus epidermis and Pseudomonas fluorescens,* it was observed that *E.coli* dominated [18,30,31]. This is in agreement with this work. The high bacterial load in the gill, gut and intestine of fishes observed during the rainy season in this study could be attributed to cool atmospheric environment and pond water temperature, which is conducive for proliferation of mesophilic organisms [15,40,41]. Almost every year health disorders in freshwater fish are recorded on many farms in Nigeria [7,42,43]. The causes of the diseases may be various factors; however, the most important among bacterial infections are those caused by motile Aeromonas (*A. hydrophila, A. sobria, and A. caviae*), [19,44,45] whereas in this study *Escherichia coli, Salmonella* sp*, Staphylococcus* sp*, Bacillus* sp*, Shigella* sp*, Enterobacter* sp*, Klebsiella* sp and *Citrobacter* sp could be primarily noted to be responsible for the fish diseases in the four local government areas in Rivers state. Clinical symptoms observed during health disorders vary depending on the type of disease. Generally, infections caused by *Aeromonas* and *Staphylococcus* spp.

are the most common among bacterial fish diseases, causing motile aeromonas septicaemia (MAS), motile aeromonas infection (MAI), Staphylococcus and furunculosis; skin ulceration, gill and fin lesions are most frequently noticed [46,47,7]. If the disease becomes a systemic infection, then it is called MAS. The other psychrophilic Aeromonas, *Aeromonas salmonicida*, causes furunculosis in *Oreochromis niloticus*, which is manifested in ulcers on the skin. During infection, high mortalities reaching about 80% of stock are also observed [7,49,48]. Fish diseases, are commonly found in various environments including water, and therefore fish are constantly exposed to bacteria [20]. In this work *E. Coli., Baccillus* sp*, Klebsella* sp*, Enterobacter* sp*, Salmonella* sp *Shigella* sp, *Staphylococcus* sp and *Citrobacter* sp respectively formed: 29.41, 17.76, 11.76, 11.76, 5.88, 11.76, 11.76 and 0.00% of bacteria occcurence in the gills; 23.53, 17.65, 11.76, 0.00, 17.65,17.65,11.76 and 0.00% in the gut and 23.81, 23.81,4.96,4.96,9.52,4.96,23.81 and 4.96% on the skin across all the locations. Interaction with bacteria is especially dangerous under conditions of stress, which include unfavourable environmental conditions as well as human intervention in catching, sorting, and transporting of the fish [50, 51,17]. In recent years, the number of infections caused by Gram positive bacteria, especially in rainbow trout, has increased significantly in Nigria [7,52,53]. This coroborates with this work in that across the stations 35.71, 28.57, 35.71% of *E. Coli. ;*27.27, 27.27, 45.45% of *Baccillus* sp*;* 40.00,40.00, 20.00% of *Klebsella* sp*;* 66.67,0.00, 33.33% of *Enterobacter* sp*;* 16.67, 50.00, 33.33% of *Salmonella* sp*;* 33.33,50.00, 16.67% of *Shigella* sp*;* 22.22, 22.22, 55.56% of *Staphylococcus* sp and 0.00, 100% of *Citrobacter* sp were respectively found in the gill, gut and skin across the five LGA(s). Among many species of Gram-positive microorganisms, two of them are of particular importance in the pathology of bacterial fish diseases: *Lactococcus garviae* and *Streptococcus iniae* [21,54,55] and these bacterial species cause serious health disorders in different species of freshwater and marine fish: rainbow trout and other salmonids from the Oncorhynchus family, eels, and fish belonging to the Ictaluridae (catfish) and Cichlidae (tilapia) families. The source of bacterial infection for fish can be both water and sediments [56,57,7]. Although bacteria might be present in the environment and also in fish throughout the year, their related disorders occur during summer when the water temperature increases and reaches optimal values in the range of 18ºC–25ºC. Therefore, it is assumed that temperature as well as the sanitary state of the aquatic environment are the most important factors conducive to the occurrence of disease symptoms [7,59,58], agrees with this work in that, *Citrobacter* sp was absent in the gill and fish gut while *Enterobacter* sp was not found in the gut. Several factors have contributed to microbiological pollution of the ponds, including runoff and storm-water that contain deposits from wildlife, agriculture, urban, forestry and rural settlements. These factors have contributed to the escalating population of

human-fish microbes within Rivrs State [17,70]. Most of these fish farms within the State are found less than 50m away from densely populated settlement areas which are prone to flooding. Lack of proper sewage amenities together with a high water table has permitted untreated sewage to enter the farms either through runoff, storm-water or by fishing forks used by people fishing in the farms. Pathogenic human *Enterobacteriaceae* deposited in the fish ponds attach to the vegetation as well as lodge on the gills and body surfaces of fish. In this study, the fish farms in Obi/Akpor and Port City Harcout Local Government Areas (LGA) are seen to have the highest *Salmonella* and *Shigella* distribution. The two LGAs are densely populated and are at close proximity to people that generates a lot of waste; thus the water circulation in the region is poor. [18,68,69] reported that studies have shown that 5.2% prevalence of diarrhoeal diseases are caused by *Enterobacteriaecea* which correlates well with the high *E. coli* and *Enterobacteriaceae* population in this study. This situation is also compounded by the fact that over 81% of the total number of households in the LGA (s) under consideration dumps their wasts on the road sides that sometimes run dry without water [7,66,67]. The remaining 19% may bag their waste. The same scenario is observed in Eleme and Obio/Akpor LGA, where there are fewer waste bins shared among households. Untreated sewage effluent is disposed directly on roads sides because of the debilitated sewage treatment system in the Rivers State. Thus the distribution of *E. coli* in the population (gill - 35.71%, gut – 28.57%, skin - 35.71%), *Baccillus* sp (gill and gut – 27.27 each, skin – 45.45%), *Klebsella* sp (gill and gut - 40.00% each, skin – 20.00%), *Enterobacter* sp (gill – 66.67%, gut – 0.00 and skin – 33.33%), *Salmonella* sp (gill - 16.67%, gut – 50.00%, skin – 33.33%), *Shigella* sp (gill – 33.33%, gut – 50.00% and skin – 16.67%), *Staphylococcus* sp (gill and gut – 22.22% each and skin – 55.56%), and *Citrobacter* sp (gill and gut – 0.00, skin – 100%) can be seen as an indicator of faecal run off contamination amongst other coliforms. This observation correlates well with the diarrhoea prevalence of 8.7% of the total number of outpatients recorded in 2006. Transportation of fish from contaminated farms, the packaging of fish in dirty baskets and plastic basins, and the display of fish on dirty nylon sheets for buyers 20 meters from the fish farms could also enhance post-harvest contamination of the fish by the fish farmers [22,64,65]. In this study, it was observed that 14.29% of microbes were found on the skin of fish at Emohua LGA, 23.81% on fish skin at Eleme, 28.57% on fish skin at Ikwerre, 14.29% at Obio/Akpor and 19.05% at Port Harcourt City Local Government Area. Also, 23.53, 11.76, 29.41,17.65,17.65% of bacteria were respectively found in the fish gill at Emuoha, Eleme, Ikwerre, Obio/Akpor and Port Harcourt City LGA while at Emuoha, Eleme and Port Harcourt city, 18, 7 and 5% each formed the bacteria load in the gut were as Ikwerre and Obio/Akpor respectively formed 31.25% and 12.50% of microbes. This depicts that bacteria abundance peaked on fish skin, gill and gut of fish at

Ikwerre LGA followed by Emuoha, Eleme and Obio/Akpor LGA which agrees with [23,62,63] who reported that the effect of runoff and storm-water that collect a lot of animal, plant and human wastes from point and non-point sources and channel these to the rivers and the lake create an environment that supports microbiological pathogens These factors increase the eutrophication process thus creating a vast conducive environment for the survival of microbes which eventually infect the fishes, which in recent years have been isolated from various fresh and marine waters contaminated by urban sewerage [60,61,24]. It was therefore imperative to isolate enteric pathogenic bacteria from fish that might be transmitted to humans after handling, or consumption of such fish.. Gutting and washing of the fish using dirty water collected directly from the fish farms due to lack of proper municipal piped water could exacerbate the contamination process within the environment [25,70,71]. The report that the prevalence of diarrhoea was 6.8% of the total outpatient diseases in 2006 and the fact that the highest distribution of *Shigella* spp (32%) followed by *Salmonella* spp. and *E. coli* was also noticed [72,73,26] agrees with this work.The population else where in Nigeria shares the same problem affecting River state in particular. However, in Emohua LGA , contamination prevalence was 23.33% and *Enterobacteriacea* distribution was equally low in fish collected from the farm. This observation is attributed to the sewage area being open, where there is adequate mixing of pond-water with that of river-water from the run-off, which passes a few meters way from the areas of human settlement. In this study, it was realized that the waters of the highly populated areas in the city received large quantities of untreated wastewater discharged from human and industrial sources. In addition, since rainfall could introduce enteric pathogens from distant sources into the environmnt, it was believed that they contributed to the fish farms pollution [27,76, 77]. *Salmonella enterica* has been considered the causal agent of the largest number of enteric infections in the world [28,74,75]. Raw foods and cross-contamination of ready-to-eat products are the main routes of fish diseases transmission [29, 78.79]. It is therefore concluded that isolation of *Escherichia coli, Salmonella* sp*, Staphylococcus* sp*, Bacillus* sp*, Shigella* sp*, Enterobacter* sp *, Klebsiella* sp and *Citrobacter* sp in fish harvested from the fish ponds in all the five LGA(s) was an indication of contamination of the waters by pathogens. The sources of all these pathogens are poorly understood, and this study provides vital data that is critical in assessing and controlling the risk associated with the presence of bacteria in Rivers state environment. Assuming that the main source of *Escherichia coli, Salmonella enterica, Staphylococcus aureus, Bacillus, Shigella, Enterobacter, Klebsiella* and *Citrobacter* sp contamination in the fish farms environment is of human or animal origin, the different population structures may be attributed to the different rates of growth and survival of these microbes in the farms environment and this necessitates a pilot study to

establish the source of the isolates. It is also concluded that, *E.coli* was the clinically important serovar mostly identified in this study which attests to its capacity of adaptation and survival in this environment, as has been suggested by other authors. The presence of different enteric bacteria in the aquaculture environments suggests that strict hygiene procedures should be followed during the handling and processing of fish from the culture systems to prevent the transfer of potentially pathogenic bacteria to humans. Thus there is need for a code of practice for fish growers in tropical aquaculture systems to ensure safe food sources.

5. Conclusion

Escherichia coli, Salmonella sp*, Staphylococcus* sp*, Bacillus* sp*, Shigella* sp*, Enterobacter* sp*, Klebsiella* sp and *Citrobacter* sp observed in this work are established fish/human pathogens. *E. coli* has an assertive effect in *Clarias gariepinus* ponds which is evident from their wide spread prevalence across all the 5 Local Government Areas under consideration and among all samples analysed (gill, gut and skin). Bacteria abundance peaked on fish skin, gill and gut of fish at Ikwerre LGA followed by Emohua, Eleme and Obio/Akpor LGA Study on the antibiotic resistance of these enteric pathogens could establish the real threat posed by these organisms. However, detection of diverse group of enteric bacteria including potential pahogens in the *Clarias gariepinus* production ponds suggests that strict hygienic procedures should be followed during handling and processing of fish from the similar culture systems and proper cooking prior to consumption to prevent the transfer of potentially pathogenic bacteria to humans.

REFERENCES

1. Metz H Water as a vector of infection: waterborne bacteria (in German). *Zentralbl Bakteriol Mikrobiol Hyg* (B). 1980; 172: 255–274.

2. Bhaftopadhyay P. Fish – catching and handling. In: Robinson R.K. (ed.): Encyclopedia of Food Microbiology. Vol. 2, *Academic Press, London.* 1547. 2000

3. Acha PN, Szyfres B. Zoonoses and communicable diseases common to man and animals. Vol. I. Bacterioses and mycoses. 3rd ed. Scientific and Technical Publication No. 580, Pan American Health Organization, Regional Office of the WHO, Washington, USA. 384.2003.

4. Lipp EK, Rose JB.. The role of seafood in food-borne diseases in the United States of America. Rev Sci Tech OIE. 1997; 6: 620–640.

5. Zlotkin A, Eldar A, Ghifino C, Bercovier H. Identification of Lactococcus garvieae by PCR. J Clin Microbiol. 1998; 36: 983–985.

6. Von Reyn CF, Arbeit RD, TostesonA NA, Ristola MA, Barber TW, Waddell R, Sox CH, Brindle RJ, Gilks CF, Ranki A, Bartholomew C, Edwards J, Falkingham JO, O'Connor GT, Jacobs NJ, Maslow J, Lahdevirta J, Buhler S, Ruohonen R, Lumio J, Vuento R, Prabhakar P, Magnusson M. The international epidemiology of disseminated *Mycobacterium avium* complex infection in AIDS. AIDS. 1996; 10: 1025–1032.

7. Uedeme-Naa B. Quik check on fish: diseases, parasites and Feed. Jonny printing press, 79 shopping complex, Rivers State, Nigeria. 2020. (ISBN: 978 – 978 – 57312 – 3 - 0)

8. Minete HP. Salmonellosis in the marine environment. A review and commentary. *Int J Zoonoses*. 1986; 13: 71–75.

9. Fell G, Hamouda O, Lindner R, Rehmet S, Liesegang A, Prager R, Gericke B, Petersen L.An out-break of *Salmonella blockley* infections following smoked eel consumption in Germany. *Epidemiol Infect*. 2000 ; 125: 9–12.

10. Kauffman F. The bacteriology of *Enterobacteriacea*. Munksgaards, Copenhagen, Denmark. 1996.

11. Amavisit P, Boonyawiwat W, and Bangtrakulnont A. Characterization of *S*. enterica Serovar *Typhimurium* and Monophasic *Salmonella* Serovar 1, 4, [5], and 12: i: - Isolates in Thailand. J Clin Microbiol. 2005; 2736–2740. .

12. Baudart J, Lemarchand K, Brisabois A, Lebaron P. Diversity of *Salmonella* strains isolated from the aquatic environment as determined by serotyping and amplification of the ribosomal DNA spacer regions. *Appl Environ Microbiol*. 2000; 66: 1544-1552.

13. O'Shea ML and Field R. Detection and disinfection of pathogens in storm-generated flows. *Can J Microbiol*. 1991; 38: 267-276.

14. Bell C and Kyriakides A. *Salmonella*: a practical approach to the organism and its control in foods. Publisher: Oxford; Malden, Mass. Blackwell Science Ltd. 2002*;* 59: 269

15. Dupray E, Derrien A. Influence of the previous stay of *Escherichia coli* and *Salmonella* spp. in waste water on their survival in seawater. *Water Res* . 1995; 29: 1005-1011.

16. Kozińska A., Pękala A. First isolation of *Shewanella putrefaciens* from freshwater fish – a potential new pathogen of the fish. Bull Eur Ass Fish Pathol 2004, 24, 199–203.

17. Godwin, A. O. M., Uchechi, U. Comparative study on bacterial load in intestine, gills and skin of cultured African catfish (Clarias gariepinus) from different locations in Rivers State, Nigeria. International Journal of Innovative Studies in Aquatic Biology and Fisheries,2016; 2(3), 21-29.

18. Austin B., Austin D.A.: Bacterial fish pathogens. Disease of farmed and wild fish, 6thed., Springer International Publishing Switzerland, 2016.

19. Park K.H., Kato H., Nakai T., Muroga K.:Phage typing of Lactococcus garvieae (formerly Enterococcus seriolicida) a pathogen of cultured yellowtail. Fisheries Sci (Tokyo) 1998, 64, 62–64.

20. .Eldar A., Bejerano Y., Bercovier H.: Streptococcus shiloi and Streptococcus difficile: two new streptococcal species causing a meningoencephalitis in fish. Curr Microbiol 1994, 28, 139–143.

21. Eldar A., Ghittino C.: Lactococcus garvieae and Streptococcus iniae infections in rainbow trout Oncorhynchus mykiss: similar, but different diseases. Dis Aquat Organ 1999, 36, 227–231.

22. Grawiński E.: Less notorious diseases of salmonids in Northern Poland territory. Życie Wet 2010, 85, 522–528

23. Olesen N.J., Vendramin N.: Overview of the disease situation and surveillance in Europe in 2015. In: 20th Annual workshop of the National Reference Laboratories for fish diseases, National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark,2016, pp. 13 15.

24. Aguado-Urda, M., López-Campos, G.H., Gibello, A., Cutuli, M.T., López-Alonso, V., Fernández-Garayzábal, J.F., Blanco, M.M. Genome sequence of *Lactococcus garvieae* 8831, isolated from rainbow trout lactococcosis outbreaks in Spain. Journal of Bacteriology*;* 2011; 193:4263–4264p.

25. Ali, E., Hashem, M. Al-Salahy, M. Pathogenicity and oxidative stress in Nile tilapia caused by *Aphanomyces laevis* and *Phoma herbarum* isolated from farmed fish. Diseases of Aquatic Organisms. 2011; 94(1), 17–28p.

26. Allen, D.A, Austin, B, Colwell, R.R. Aeromonas media, a new species isolated from river water. International Journal of Systematic and Evolutionary Microbiology; 1983; 33:599–604p.

27. Austin, B. and Austin, D.A. Bacterial Fish Pathogens; Disease of Farmed and Wild Fish (6th ed.). Springer International Publishing, Switzerland. 2016.

28. Barnes, A.C., Young, F.M., Horne, M.T. Ellis, A.E. *Streptococcus iniae*: serological differences, presence of capsule and resistance to immune serum killing. Diseases of Aquatic Organisms*;* 2003; 53:241–247p.

29. Bichi, A. Yelwa, S. Incidence of piscine parasites on the gills and gastrointestinal tract of clarias gariepinus (teugels) at bagauda fish farm, Kano. Bayero Journal of Pure and Applied Sciences, 2010; 3(1).

30. Bootsma, R., Clerx, J.P.M. Columnaris disease of cultured carp *Cyprinus carpioL.* Characteristics of the causative agent. Aquaculture. 1976; 7:371–384p.

31. Bott, T.L. Johnson, J.,Foster, E.M. Sugiyama, H. Possible origin of the high incidence of *Clostridium botulinum* type E in an inland bay (Green Bay of Lake Michigan). Journal of Bacteriology*;* 1968; 95:1542–1547p.

32.Bricknell, I. Types of pathogens in fish, waterborne diseases. In Fish diseases (pp. 53-80). Academic Press. 2017

33.Buller, N.B. Bacteria and Fungi from Fish and Other Aquatic Animals (2nd ed.). CABI International, Boston, USA.2014.

34.Candan, A., Küçüker, M.A., Karatas, S. Motile aeromonad septicaemia in salmo salar cultured in the Black Sea in Turkey. Bulletin of European Association of Fish Pathology; 1995; 15:195–196p.

35.Cann, D.C., and Taylor, L.Y. An outbreak of botulism in rainbow trout, *Salmo gairdneri* Richardson, farmed in Britain. Journal of Fish Diseases; 1982; 5:393–399p.

37. Cipriano, R. C. Bullock, G. L. Furunculosis and other diseases caused by Aeromonas salmonicida. National Fish Health Research Laboratory.2001.

38. Dulin, M.P. A review of tuberculosis (mycobacteriosis) in fish. Vet med/Small Animal Clinician. 1979; 74:735–737p.

39. .Durborow, R. M., Wise, D. J. Terhune, J. S. Saprolegniasis (winter fungus) and branchiomycosis of commercially cultured channel catfish. Southern Regional Aquaculture Center.2003.

40. Effiong, M. U. Isaac, I. N. Comparative study of the bacterial load and species diversity in the African catfish (Clarias gariepinus) cultured in contrasting aquaculture tanks in Uyo, Nigeria. Animal Research International, 2019; 16(3), 3443-3449.

41. Eldar, A., Bejerano, Y., and Bercovier, H. *Streptococcus shiloi* and *Streptococcus difficile*: two new streptococcal species causing a meningoencephalitis in fish. Current Microbiology. 1994; 28:139–143p.

42. Evelyn, T.P.T. First records of vibriosis in Pacific salmon cultured in Canada, and taxonomic studies of the responsible bacterium, *Vibrio anguillarum*. J Fish Res Board Can. 1971; 28:517–525p.

43. Feist, S. W. Longshaw, M.. Histopathology of fish parasite infections–importance for populations. Journal of Fish Biology, 2008 73(9), 2143-2160.

44. Gil, P., Vivas, J., Gallardo, C.S. Rodríguez, L.A. (2000). First isolation of *Staphylococcus warneri*, from diseased rainbow trout, *Oncorhynchus mykiss* (Walbaum), in Northwest Spain. Journal of Fish Diseases 2000; 23:295–298p.

45. Godwin, A. O. M. Uchechi, U. Comparative study on bacterial load in intestine, gills and skin of cultured African catfish (Clarias gariepinus) from different locations in Rivers State, Nigeria. International Journal of Innovative Studies in Aquatic Biology and Fisheries, 2016; 2(3), 21-29.

46. Gomez, S., Bernabe, A., Gomez, M.A., Navarro, J.A. Sanchez, J. Fish mycobacteriosis: morphological and immunocytochemical aspects. Journal of Fish Diseases. 1993; 16:137–141p.

47. Goodwin, A.E., Roy, S.J. jr, Grizzle, J.M., Terrell, Goldsby, Jr M. *Bacillus mycoides*: A bacterial pathogen of channel catfish. Diseases Aquatic Organisms; 1994; 18:173–179p..

48. Heo, G-J., Kawai, K., and Wakabayashi, H. Occurrence of *Flavobacterium branchiophila* associated with bacterial gill disease at a trout hatchery. Fish Pathology; 1990; 25:99–105p.

49. Hirvelä-Koski, V., Pohjanvirta, T., Koski, P. Sukura, A. Atypical growth of *Renibacterium salmoninarum* in subclinical infections. Journal of Fish Diseases. 2006; 29:21–29p.

50. Hossain, M. M., Uddin, M. N., Islam, M. N., Chakraborty, S. C. Kamal, M. Study on the intestinal bacteria of Labeo rohita (Ham.). Bangladesh Journal of Fisheries Research, 1999; 3(1), 63-66.

51. Ibrahim, B. U., Baba, J. Sheshi, M. S. Isolation and identification of bacteria associated with fresh and smoked fish (Clarias gariepinus) in Minna Metropolis, Niger State. Nigeria. Journal of applied and environmental microbiology, 2014; 2(3), 81-85.

52. Ke, X., Lu, M., Ye, X., Gao, F., Zhu, H. Huang, Z. Recovery, pathogenicity analysis of *Aerococcus viridans* isolated from tilapia (*Oreochromis niloticus*) cultured in Southwest of China. Aquaculture, 2012*;* 342–343:18–23p.

53. Khoa, L.V., Hatai, K., Yuasa, A. Sawada, K. Morphology and molecular phylogeny of *Fusarium solani* isolated from kuruma prawn *Penaeus japonicas* with black gills. *Fish Pathology;* 2005; 40(3), 103–109p.

54. Klinger, R.E. Floyd, R.F. (2002). Introduction to Freshwater Fish Parasites. [CIR716]. University of Florida IFAS Extension. 2002. Retrieved July, 2021 from http://fisheries.tamu.edu/files

55. Kocan, R., Hershberger, P., Sanders, G. Winton, J. Effects of temperature on disease progression and swimming stamina in Ichthyophonus-infected rainbow trout, Oncorhynchus mykiss (Walbaum). Journal of Fish Diseases, 2009; 32(10), 835-843.

56. Lansdell, W., Dixon, B., Smithin, N. Benjamin, L. Isolation of several Mycobacterium species from fish. Journal of Aquatic and Animal Health. 1993; 5:73–76p.

57. Larsen, J.L. Jensen, N.J.An Ae romonas species implicated in ulcer-disease of the cod (*Gadus morhua*). Nordisk Veterinaermedicin .1977; 29:199–211p.

58. Lõhmus, M. Björklund, M. Climate change: what will it do to fish—parasite interactions?. Biological Journal of the Linnean Society, 2015; 116(2), 397-411.

59. MacInnes, J.I., Trust, T.J. Crosa, J.H. Deoxyribonucleic acid relationships among members of the genus Aeromonas . Canadian Journal of Microbiology. 1979; 25:579–586p.

60. Morrison, C., Cornick, J., Shum, G. Zwicker, . Microbiology and histopathology of ‘saddleback’ disease of underyearling Atlantic salmon, Salmo salar L. Journal of Fish Diseases. 1981; 4:243–258p.

61. Muroga, K., Sugiyama, T. Ueki, N. (1977). Pasteurellosis in cultured black sea bream (*Myliomacro cephalus*). Journal of the Faculty of Fisheries and Animal Husbandry, Hiroshima University. 1977; 16:17–21p.

62. Nakai, T. Muroga, K. *Pseudomonas anguilliseptica* isolated from European eels (*Anguilla anguilla*) in Scotland. Fish Pathology; 1982. 17:147–150p.

63. Nigrelli, R.F. Vogel, H. Spontaneous tuberculosis in fishes and in other cold-blooded vertebrates with special reference to *Mycobacterium fortuitum* Cruz from fish and human lesions. Zoologica (New York); 1963; 48:130–143p.

64. Ogbukagu, C. M., Anaukwu, C. G., Ekwealor, C. C., Mba, A. N., & Ekwealor, I. A. Bacteriological Assessment and Antibiotic Susceptibility Profile of Bacteria Recovered from Clarias gariepinus Selected from Various Fish Farms in Anambra North Senatorial Zones in Anambra State, Nigeria. Advances in Microbiology, 22021; 11(5), 243-256.

65. Oladosu, G.A., Ayinla, O.A., Ajiboye, M.O. Isolation and pathogenicity of a *Bacillus sp*. Associated with a septicaemic condition in some tropical freshwater fish species. Journal Applied Ichthyology. 1884; 10:69–72p.

66. Olufemi, B.E. Roberts, R.J. Method for the isolation of Aspergillus species pathogens of fish from clinical materials. Veterinary Record. 1983; 112 (1), 15p.

67. Olufemi, B.E. Roberts, R.J.. Induction of clinical aspergillomycosis by feeding contaminated diet to tilapia, *Oreochromis niloticus* (L.). Journal of Fish Diseases. 1986; 9(2), 123–128p.

68. Pacha, R.E. Ordal, E.J. Myxobacterial diseases of salmonids. In: Snieszko SF (ed) A symposium on diseases of fishes and shellfishes, Special Publication No. 5. Washington D.C, *American Fisheries Society*, 1970; 243–257p.

69. Parisot, T.J. Wood, E.M. A comparative study of the causative agent of a mycobacterial disease of salmonid fishes. II. A description of the histopathology of the disease in chinook salmon (*Oncorhynchus tshawytscha*) and a comparison of the strain characteristics of the fish disease with leprosy and human tuberculosis. Annu Rev Respir Dis; 1960; 82:212–222p.

70. Pathak, S. Detection and diagnosis of fish pathogens using foldscope on freshwater fishes in the water bodies of the chirang district of assam, india. Foldscope and its Applications, 2019; 219.

71. Reay, G. A. Shewan, J. M. The spoilage of fish and its preservation by chilling. Advances in Food Research, 1949; 2, 343-398.

72. Rodger, H. D. Fish disease causing economic impact in global aquaculture. In Fish vaccines (pp. 1-34). Springer, Basel. 2016

73. Sugiyama, A. Kusuda, R. Studies on the characters of Staphylococcus epidermidis isolated from diseased fishes - II. Serological properties of the isolates. Fish Pathology. 1981; 16:25–33p.

74. Tison, D.L., Nishibuchi, M., Greenwood, J.D. Seidler, R.J. *Vibrio vulnificus* biogroup 2: a new biogroup pathogenic for eels. Applied and Environmental Microbiology; 1982; 44:640–646p.

75. Uddin, N. Al-Harbi, A. H. Bacterial flora of polycultured common carp (Cyprinus carpio) and African catfish (Clarias gariepinus). International Aquatic Research. 2012; 4(1), 1-9.

76. Udey, L.R., Young, E., and Sallman, B. Isolation and characterization of an anaerobic bacterium, *Eubacterium tarantellus* sp. nov., associated with striped mullet (*Mugil cephalus*) mortality in Biscayne Bay, Florida. J Fish Res Board Can. 1977; 34:402–409p.

77. Whipps, C.M., Butler, W.R., Pourahmad, F., Watral, V.G. Kent, M.L. 141. Yoshimizu, M., & Kimura, T. Study on the intestinal microflora of salmonids. Fish pathology, 1976; 10(2), 243-259.

78. Zdenek Lucky; Methods for the Diagnosis of Fish Diseases

79. Zhou, S.M., Xie, M.Q., Zhu, X.Q., Ma, Y., Tan, Z.L. Li, A.X. Identification and genetic characterization of *Streptococcus iniae* strains isolated from diseased fish in China. Journal of Fish Diseases; 2008; 31:869–875p.

Fig.1: Map of Rivers State showing all the Local Government Areas