Bacterial Loads and Bacterial Distribution Pattern Associated with *Clarias gariepinus* Juveniles Cultured in Concrete Fish Ponds in Port Harcourt

Ifeoma Laeticia Okoliegbe, Carol Nchedo Ariole and Gideon Chijioke Okpokwasili

Department of Microbiology, Faculty of Sciences, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria
E-mail: laetymaria@yahoo.com

Abstract: The aim of this study was to investigate the bacteriological quality and taxonomic composition of bacterial populations associated with pond rearing water, gills, feed and intestine of healthy African catfish, *Clarias gariepinus,* cultured in concrete ponds in Port Harcourt metropolis of Nigeria. Bacterial numbers were deciphered using standard microbiology methods. Bacteria identification was carried out using molecular methods. Total culturable heterotrophic countsin rearing water ranged from $2.0 \pm 0.1 \times 10^4$ to$2.6 \pm 0.2 \times 10^4$ cfu/ml; in sediment; in gill filaments, $1.8 \pm 0.2 \times 10^5$ to $2.7 \pm 0.3 \times 10^5$ cfu/g; in intestine, $2.9 \pm 0.4 \times 10^4$ to $2.6 \pm 0.3 \times 10^6$ cfu/g and in feed $2.3 \pm 0.1 \times 10^6$ to $2.8 \pm 0.4 \times 10^6$cfu/g. The bacterial species isolated during the study were identified as *Shewanella algae, Vibrio* sp., *Providencia vermicola, Bordetella pertussis, Escherichia coli, Alcaligenes fecalis*, *Proteus mirabilis*and *Alcaligenes* sp. *Shewanella algae* had the highest prevalence.
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1. Introduction

The world population is currently more than seven billion and is projected to grow up to nine billion in 2050. A commensurate sustainable development of aquaculture is neede to measure up with the demands of the growing population. According to FAO (2016b), Nigeria is the largest producer of African catfish in the whole world and is followed consecutively by Netherlands, Brazil, Hungary, Kenya, SyrianArab Republic, South African, Cameroon, and Mali. African catfish is a commercially popular fish in Nigeria, thus the need for improvement of its aquaculture.

The growth and development of aquaculture in Nigeria mainly involves the improvement of African catfish aquaculture (Dauda *et al.* (2018). The success of aquaculture largely depends on water quality which is a function of bacterial load, taxonomic composition and physico-chemical properties of the fish rearing water. Aquatic microorganisms influence the water quality and also closely affect the physiological status of the fishand postharvest quality (Amande and Nwaka, 2013). Aquatic animals gulp in a large number of bacteria into their gastrointestinal tracts and gills from water, sediment and feed. However, beneficial bacteria can be helpful to the host organism when administered as probiotics both in fish, animals and humans (Okoliegbe *et al.,* 2017).

There are three possible outcomes of microorganisms coming in contact with the fish. The microorganisms may enter the mouth with water or food and only pass through the gastrointestinal tract and go out through the anus (allochthonous microorganisms) and/or colonise the walls of the gastrointestinal tract (autochthonous). The microorganisms coming into contact with fish surfaces may be inhibited by the fish normal flora.

The microorganisms may colonize the fish skin with higher concentration on damaged skin (Austin, 2006) or by natural inhibitory compounds present on or in the fish (Austin and Austin (1987). Microbiota refers to the different microbial communities which inhabit the body sites in which surfaces and cavities are open to the environment. Fish microbiota aids in digestion, nutrition, disease resistance and maintenance of mucosal tolerance. Fish microbiota also plays a vital role in the synthesis of vitamins B and K and in the metabolism of bile acids, other sterols and xenobiotics (Cummings and Macfarlane, 1997).

The microbiota and bacterial distribution patterns of a healthy fish differ from that of an unhealthy fish. However, some pathogens have been found in healthy fish without causing disease in the fish. Pathogens such as *Salmonella*, *Flavobacterium*, *Aeromonas, Klebsiella* and *Vibrio* have been isolated from healthy fish. Some microorganisms in water, sewage and sediments that come in contact with the fish influence the microflora of the external surfaces as well as the internal cavities of the fish intestine.

Moreso, the microorganisms present in the environment of the fish egg or larval stage also shape the bacterial numbers and taxonomic composition of the gut microbiota (Austin, 2006). Thus, one important

aim of studying the gastrointestinal microbiota of fish is to be able to manipulate the microbial communities of fish gastrointestinal tract in order to promote fish health and correct some health disorders thus improving fish yield. This study was aimed at investigating the bacterial species associated with the pond water, gills, intestine and feed of *Clarias gariepinus* cultured in some concrete fish ponds in Port Harcourt metropolis.

2. Materials and Methods

2.1 Study Area

The study area is three concrete ponds within Port Harcourt metropolis which is situated approximately on latitudes $4^{o}40^{1}N$ $–50^{1}$ N and between longitude $70.00^{1}E$ – $7^{o}10^{1}E$. The source of water to these ponds is borehole. The ponds are densely stocked with healthy *Clarias gariepinus* juveniles. *Clarias gariepinus* is widely consumed by the populace in this area.

Thus, the improvement of *Clarias gariepinus* aquaculture necessitated this study. The rearing water was changed every morning and evening to ensure good sanitary condition. There is no vegetation in the ponds. The fish were fed *ad libidum* three times daily on commercial artificial feed.

2.2 Sample Collection

2.2.1 Fish

Ten samples of healthy fish species *Clarias gariepinus* (average weight:160g; average length: 24cm) were collected from each of the three ponds. The samples were carefully carried inside a sterile bucket containing the fish rearing water and transported to the laboratory for analysis. Samples were analyzed immediately, within two hours of sampling.

2.2.2 Feed

A spoonful of fish feed was collected from five different points in the feed sack and then taken to the laboratory for analyses.

2.3 Sample preparation

2.3.1 Fish (Gills and Intestine)

The juveniles were starved for 24 hours in order to achieve complete gut clearance which helped to prevent contamination from undigested food. The fish was killed by physical destruction of the brain. The intestine and the gills were aseptically excised with the aid of sterilized scissors and then analyzed.

2.4 Bacteriological analysis

2.4.1 Water

Bacteriological analysis was performed on the rearing water samples of the three ponds separately. Serial dilutions of the water samples were made (10^{-1} to 10^{-6}) with sterile physiological saline (0.85% w/v NaCl) in deionized water. 0.1 ml aliquots of the appropriate dilutions were inoculated onto tryptone soya agar, MacConkey agar, thiosulphate citrate bile salt (TCBS) agar and Nutrient agar plates in triplicate by the spread plate method.

2.4.2 Fish (gills and intestine)

Appropriate amount of the whole gills and intestine were taken aseptically, and macerated in a sterilized mortar. Then 1 g of each homogenate was shaken in 25 ml of sterile physiological saline. Aliquots of 1 ml of the resultant homogenate solution were serially diluted (10^{-1} to 10^{-7}) and plated out. 0.1 ml aliquots were spread on media plates and incubated at $37^{o}C$ for 24 h after which bacterial counts were taken.

2.4.3 Fish feed

A spoonful of fish feed was grinded and 1 g was dissolved in 25ml of sterile physiological saline solution. One milliliter aliquots of the homogenate solutions were serially diluted (10^{-1} to 10^{-7}) using normal saline and plated out as previously described in the water sample.

2.5 Incubation and colony count

All the inoculated plates (water, feed, gills and intestine) were incubated at 37 °C for 24 h and bacterial counts were obtained. Plates having 30 - 300 colonies were used to calculate bacterial population numbers, recorded as cfu per unit of sample. The colonies were sorted out on the basis of microscopic and morphological differences – (colour, texture, elevation, edges, consistency, shape, opacity and size) and the number of colonies of each recognizable type was counted.

Three representatives of each colony type were then streaked on nutrient agar plates repeatedly until pure isolates were obtained. Pure cultures obtained were transferred to nutrient agar slants. Cultures on nutrient agar slants were kept at $4^{o}C$ for stock purposes and these were transferred to new slants every six weeks.

2.6 Phenotypic identification of bacteria

All the purified isolates were observed for cell shape, motility, spores, and Gram staining. The isolates were then subjected to biochemical tests (oxidase, catalase, sugar fermentation, indole, methyl red, Vogues-Proskauer and H_2S production) following the criteria described in the Bergey's Manual of Systematic Bacteriology (Holt *et al*., 1994) for identification to genus or species level.

2.7 Molecular identification of isolates

2.7.1 DNA extraction

DNA was extracted using the boiling method where 2 mL of overnight pure Luria Bertani broth cultures were transferred to sterile eppendorf tubes and centrifuged at 13,000 rpm for 3 min. The supernatant was discarded and cells re-suspended in 200 µL sterile distilled water. The cell solution was then heated at 100 °C in an Accu dri-block (Lasec, SA) for 10 min,

followed by centrifugation at 13,000 rpm for 2 min to pellet the cells]. The supernatants were transferred to clean, sterile tubes and used directly as templates for PCR assay.

2.7.2 16S rRNA amplification

Each 25 µL PCR reaction mix constituted 12.5 µL of 2X PCR master mix, 0.5 µL each of both reverse and forward primers, 6.5 µL nuclease-free water and 5 µL of template DNA. PCR was conducted in a T1000 Touch Thermal Cycler (Bio-Rad, Hercules, CA, USA). Cycling conditions are 5 min 94 °C initial denaturation, 60 s 94 °C denaturation, 30 s 54 °C annealing, 60 s 72 °C extension, 5 min 72 °C final extension; 35 cycles. The PCR products were separated by agarose gel electrophoresis in 1% agarose, stained with ethidium bromide.

A 100 basepair DNA ladder was included in each run. The amplified DNA was sequenced in the laboratory. The sequence of the primer that was used is 27F (5^1AGA TTTGATCCTGGCTCAG3^1) and 1492 R (5^1GGTTACCTTGTTACGACTT 3^1) (Weisburg, 1991). The sequences obtained were submitted to NCBI Gene Bank databases.

2.7.3 Sequencing and Phylogenetic analysis

The amplified PCR product was purified using a Genei PCR purification kit (Genei, Bangalore). The amplified 16S rRNA gene sequence was obtained on a 3500 genetic analyser using the Bigdye-Termination technique. The resultant sequence was edited using Bioedit and a BLAST search was done in the National Centre for Biotechnology Information (NCBI) database to identify the nearest neighbor of the amplified sequence. The result of the sequencing was used for homology searches. The evolutionary history of the species having the sequences was inferred using the neighbor-joining method. The evolutionary distances were computed using the Jukes-Cantor method. The obtained nucleotide sequences of the identified species were deposited in the Genbank database.

3. Results

3.1 Bacterial loads observed in this study

The trend of bacterial numbers observed in this study in decreasing order of magnitude was: Feed > Intestine > Gills > Water. The feed had the highest Total Viable Count followed by the intestine. The lowest bacterial numbers observed in the study was in the rearing water. Pond 2 had the highest bacterial count in gills and feed. The trend of Total Coliform count was: Pond water > Gills > Intestine > Feed with the highest number observed in the pond water. There were no Coliforms in the feed of Ponds 1 and 2.

The *Vibrio* count was highest on the gills in Ponds 2 and 3 while in Pond 1, the *Vibrio* count was highest in the intestine of the fish. There was no *Vibrio* in the feed of the three ponds. The quantitative estimation of aerobic heterotrophic bacteria in pond water, gill filaments, intestine and feed of *Clarias gariepinus* is given in Table 1. During the period of study, bacterial load in pond water ranged from $2.0 \pm 0.1 \times 10^4$ to$2.6 \pm 0.2 \times 10^4$ cfu/ml; in gill filaments, $1.8 \pm 0.2 \times 10^5$ to $2.7 \pm 0.3 \times 10^5$ cfu/g; in intestine, $2.9 \pm 0.4 \times 10^4$ to $2.6 \pm 0.3 \times 10^6$ cfu/g and in feed $2.3 \pm 0.1 \times 10^6$ to $2.8 \pm 0.4 \times 10^6$cfu/g.

The quantitative estimation of total coliform bacteria in pond water, gill filaments, intestine and feed is given in Table 2. The total Coliform bacterial count ranged from $2.0 \pm 0.2 \times 10^4$to $2.3 \pm 0.4\times10^5$cfu/ml in pond water; $1.9\pm0.2\times10^4$to $2.5\pm0.2\times10^4$cfu/g in gill filaments; $1.5\pm0.6\times10^3$to $2.1\pm0.2\times10^4$cfu/g in intestine and in the feed 0to $2.2\pm0.1\times10^3$cfu/g. The quantitative estimation of total *Vibrio* in pond water, gill filaments, intestine and feeds ranged from $1.4\pm0.1\times10^4$to $2.2 \pm 0.2\times10^4$in pond water; $1.8 \pm 0.1 \times 10^4$ to $3.1\pm0.2\times10^5$cfu/g in gill filaments; $1.9 \pm 0.1 \times 10^4$to $2.5 \pm0.1\times10^4$ cfu/g in intestine and none in feed (Table 3). The morphological, biochemical and tentative identification of the isolates are presented in Table 4.

Table 1: Bacterial Loads in Pond Water, Sediments, Gills, Intestine and Feed of the studied fish, *Clarias gariepinus*.

Pond No.	Water (cfu/mL)	Gills (cfu/g)	Intestine (cfu/g)	Feed (cfu/g)
1	$2.0 \pm 0.1 \times 10^4$	$1.8\pm0.2 \times 10^5$	$2.6 \pm 0.3 \times 10^6$	$2.3\pm0.1\times 10^6$
2	$2.3 \pm 0.2 \times 10^4$	$2.7 \pm 0.3 \times 10^5$	$2.9 \pm 0.4 \times 10^4$	$2.8 \pm 0.4 \times 10^6$
3	$2.6\pm 0.2 \times 10^4$	$2.3 \pm 0.3 \times 10^5$	$2.6 \pm 0.1\times 10^5$	$2.4 \pm0.3\times 10^6$

(Values are given as Mean ± SD)

Table 2: Total Coliforms in Pond water, Sediments, Gills, Intestine and Feed of the studied fish *Clarias gariepinus*

Pond No.	Water (cfu/mL)	Gills (cfu/g)	Intestine (cfu/g)	Feed (cfu/g)
1	$2.0\pm0.2\times10^4$	$1.9\pm0.2\times10^4$	$1.5\pm0.6\times10^3$	0
2	$2.8\pm0.1\times10^4$	$2.04\pm0.6\times10^4$	$1.5\pm0.1\times10^4$	0
3	$2.3\pm0.4\times10^5$	$2.5\pm0.2\times10^4$	$2.1\pm0.2\times10^4$	$2.2\pm0.1\times10^3$

(Values are given as Mean ± SD)

Table 3: Total *Vibrio* Counts in Pond water, Sediments, Gills, Intestine and Feed of the studied fishes; *Clarias gariepinus*.

Pond No.	Water (cfu/mL)	Gills (cfu/g)	Intestine (cfu/g)	Feed (cfu/g)
1	$2.0\pm0.2\times10^4$	$1.8\pm0.1\times10^4$	$2.5 \pm 0.1 \times 10^4$	0
2	$2.2\pm0.1\times10^4$	$3.1\pm0.2\times10^5$	$2.1 \pm 0.2 \times 10^4$	0
3	$1.4\pm0.1\times10^4$	$2.7\pm0.6\times10^5$	$1.9 \pm 0.1 \times 10^4$	0

(Values are Given as Mean ± SD)

Table 4: Molecular identification of the isolates

Isolate Code	Phenotypic Identity	Molecular Identity	Gene Bank Ascession Number of close relative	Maximum identity (%)	Gene Bank Ascession Number of isolate
B3	*Shewanella algae*	*Shewanella algae* strain G1	KY774312.1	100	MH734824
B4	*Vibrio* sp.	*Vibrio* sp. Strain Prvy 108	MF948980.1	100	MH734825
B6	*Providencia stuartii*	*Providencia vermicola* strain Bp Dy5	MF966261.1	99.4	MH734827
B14	*Bordetella pertussis*	*Bordetella pertussis* strain SFS A64	KX138518.1	96	MH734835
B16	*Escherichia coli*	*Escherichia coli* strain IBBI	MG557808.1	90	MH734837
B17	*Alcaligenes* sp.	*Alcaligenes faecalis* strain VITSIM 2	KF641858.1	98	MH734838
B18	*Proteus mirabilis*	*Proteus mirabilis* T18	ALC385636.1	98	MH734839
B20	*Alcaligenes* sp.	*Alcaligenes* sp. strain JLT1515	KX302626.1	100	MH734841

3.2 Molecular Identification of isolates

Bacterial isolates recovered from pond water, gills, intestine and feed of *Clarias gariepinus*were identified to species level using morphological, biochemical (Table 4) and molecular methods (Table 5). A total of sixty two samples were screened; fish gills (n = 30), fish intestine (n = 30), rearing water (pooled sample) and fish feed (pooled sample) of which 48 isolates were obtained by culture on different media.

The isolates were narrowed down to eight using morphological and biochemical tests, which were then identified using molecular methods (Table 5). Molecular identification of the isolates revealed that the isolates were closely related to *Shewanella algae* strain G1, *Vibrio* sp. Strain Prvy 108, *Providencia vermicola* strain Bp Dy5, *Bordetella pertussis* strain SFS A64, *Escherichia coli* strain IBBI, *Alcaligenes faecalis* strain VITSIM 2, *Proteus mirabilis* T18 and *Alcaligenes* sp. strain JLT1515 (Table 5).

3.3 Frequency of isolation of isolates and bacterial flora distribution

All the isolates occurred in the three ponds. *Shewanella* had the highest frequency of occurrence in the gills, intestine and water followed by *Proteus*. *Shewanella, Alcaligenes, Bordetella, Escherichia, Providencia* and *Vibrio* did not occur in the feed of Ponds 1 and 2.

Only *Proteus* occurred in the feed of Ponds 1 and 2 while only *Proteus, Escherichia* and *Providencia* occurred in the feed of Pond 3. *Bordetella* did not occur in the intestine of the fish in Pond 3 and *Providencia* did not occur in the intestine of the fish in Pond 3. *Vibrio* occurred in all samples except feed. The distribution of bacteria among the samples - rearing water, gills, intestine, sediments and fish feeds is shown in Table 6.

Table 5: Morphological and Biochemical characteristics of the isolates

Isolate No	Ponds/Source	Sample	Colonial Morphology	Sugar Fermentation			Gram reaction	Motility	Indole	Methyl red	Vogues-Proskauer	Citrate	Oxidase	Urease	Sugar Fermentation	Triple Sugar Iron			Phenotypic Identity
				Glucose	Sucrose	Lactose										Butt	Gas	H_2S	
B3	1,2,3	Intestine	3mm,opaque,flatsmooth,mucoid,white	+	-	-	-	+	-			-	+	+	O	A	+	-	*Shewanella algae*
B4	1,2,3	Intestine	3mm,shiny,yellow,raised	+	+	+	-	+	+	-	-	+	+		OF		-	A	*Vibrio* sp.
B6	1,2,3	Feed	3mm,opaque,flat, wet,rough,milky	+	-	-	-	+	+	-	+	-	-	+	OF	A	-	+	*Providencia vermicola*
B14	1,2,3	Feed	3mm,opaque,flat rough,shiny,silver	-	-	-	-	+	-	-	-	+	+	+	OF		+	-	*Bordetella pertussi*
B16	1,2,3	Intestine	2mm,raised,creamround,shiny	+	+	+	-	-	+	-	-	+	-	+	O	A	+	-	*Escherichia coli*
B17	1,2,3	Intestine	3mm,opaque,convex,smooth,white	+	+	-	-	+	-	-	+	+	+	+	O	A	-		*Alcaligenes faecalis*
B18	1,2,3	Feed	4mm,opaque, round,smooth, wet, white	+	-	-	-	+	-	+	-	+	-	+	OF	A	+	+	*Proteus mirabilis*
B20	1,2,3	Intestine	4mm,opaque,convex,smooth,shiny,milk	+	-	-	-	+	-	-	+	+	+	+	O	A	-	-	*Alcaligenes* sp.

Key: O – Oxidation; F – Fermentation; A – Acid; - Negative; + Positive

Table 6: Site specific frequencies of isolation of bacterial genera from the ponds

Genus	Gills			Intestine			Water			Feed		
PONDS	1	2	3	1	2	3	1	2	3	1	2	3
Proteus	10 (12.2)	16 (17.2)	13 (13.7)	12 (14.3)	17 (17.3)	14 (22.2)	21 (25.0)	7 (9.0)	12 (14.5)	19 (100)	7 (100)	13 (36.1)
Shewanella	18 (22.0)	28 (30.1)	26 (27.4)	18 (21.4)	21 (21.4)	16 (25.4)	19 (22.6)	24 (30.8)	18 (21.7)	0	0	0
Alcaligenes	14 (17.1)	7 (7.5)	10 (10.5)	10 (11.9)	15 (15.3)	11 (17.5)	12 (14.3)	9 (11.5)	14 (16.9)	0	0	0
Bordetella	11 (13.4)	12 (12.9)	9 (9.5)	11 (13.1)	12 (12.3)	0	10 (11.9)	16 (20.5)	13 (15.7)	0	0	0
Escherichia	9 (11.0)	12 (12.9)	9 (9.5)	16 (19.0)	8 (8.2)	10 (15.9)	7 (8.3)	12 (15.4)	8 (9.6)	0	0	8 (22.2)
Providencia	10 (12.2)	0	13 (13.7)	7 (8.3)	8 (8.2)	0	5 (6.0)	0	10 (12.0)	0	0	15 (41.7)
Vibrio	10 (12.2)	18 (19.4)	15 (10.5)	10 (11.9)	17 (17.3)	12 (19.0)	10 (14.3)	10 (12.8)	8 (9.6)	0	0	0
Total	82 (100)	93 (100)	95 (100)	84 (100)	98 (100)	63 (100)	84 (100)	78 (100)	83 (100)	19 (100)	7 (100)	36 (100)

Values are the mean of frequencies of occurrence in the three ponds; Numbers in parenthesis represent the percentage frequencies

4. Discussion

Investigation of the bacterial flora of fish has been confined largely to marine species with little attention paid to fresh water fish. In this study the bacterial numbers and taxonomic composition of bacterial populations associated with *Clarias gariepinus* was investigated. There were variations in the bacterial load of pond water, gills, intestine and feed of the three ponds. Fish feeds had the highest bacterial counts containing bacterial populations of 10^6. Ubiebi (2017) analyzed fish feeds sold in Abraka, Delta State and obtained bacterial counts of $10^4$to 10^5 and also isolated *Escherichia coli* and *Proteus* from the samples.

The counts obtained from the fish feeds may be as a result of the high nutritional content of the feed which proves to be conducive for the proliferation of the observed bacteria. The total culturable heterotrophic count observed in this study was higher in the intestine than in the gills and water. The bacterial counts in the intestine are within the range of $10^4 – 10^6$ which is exactly the range that Amande and Nwaka (2013) obtained from the intestine of *Clarias gariepinus.*

This is in line with the discovery of Syvokien J and Mick Jnien J (1998) who discovered that the total viable bacterial counts in water were many times lower than those in the intestine of fish indicating that the digestive tract provides favourable nutrient-rich environment for these microorganisms. These counts are within the food quality criteria stipulated range of $10^2 – 10^7$(FAO, 1979; Adams and Moss, 1999). However, Akinwale *et al.,* (2007) obtained higher bacterial counts of 10^{12} and 10^{10} in *Clarias gariepinus* intestine and gills respectively while Al-Harbi and Uddin (2010) obtained counts within the range of 10^6 to 10^7 in gills and 10^8 to 10^{10} in the fish intestine Fish with abundant and diverse microflora have considerable advantages such as production of vitamins B and K by the microbes and the ability to metabolize and assimilate a wider range of nutrients, thus enhancing their adaptive capacities and acquisition of vitamins. *Escherichia coli, Proteus* and *Vibrio* sp. have previously been isolated from *Clarias gariepinus* juveniles (Amande and Nwaka, 2013; Umana *et al.,* 2017).

The digestive tract of *Clarias gariepinus* has been reported to contain *Shewanella algae, Vibrio* sp. (Ariole *et al.* 2014; Al-Harbi and Uddin, 2010), *Providencia* (Nahiduzzaman, 2000), *Bordetella pertussis, Escherichia coli, Proteus mirabilis* (Diyaolu, 2015), *Alcaligenes* sp. (Lee and Lee, 1995). These bacteria were also isolated in this study. The nutrient-rich environment of the gastrointestinal tract may have enhanced the diversity and multiplication of resident bacteria. These bacteria may have been part of the fish normal flora developed right from birth or environmental bacteria having strong colonizing ability to have carved a niche for them in the gastrointestinal tract of the fish.

Moreso, in this study, the bacterial composition of the rearing water and the fish is very similar. This has also been noted by several authors (Uddin and Al-Harbi, 2012; Pelzar, 1986; Diler *et al.,* 2000; Apun *et al.,* 1999; Austin, 2006). The bacteria observed in the rearing water may have originated from the feed, leaks from nearby sewage tanks into the water source

underground, run-offs from farmlands, fish handling staff.

This could be the reason enteric microorganisms wereassociated with the fish samples. Some potential human pathogens have been detected in and around fish, namely *Aeromonas* spp., *Campylobacter jejuni, Clostridium botulinum, C. perfringens, Erysipelothrixrhusiopathiae, Edwardsiella tarda, Legionella pneumophila, Mycobacterium* spp., *Photobacteriumdamselae, Plesiomonas shigelloides, Staphylococcus aureus, Streptococcus iniae, V. cholerae, V.parahaemolyticus,* and *V. vulnificus* (Novotny *et al*., 2004). Some of the isolates obtained in this study such as *Proteus mirabilis, Vibrio* sp., *Escherichia coli* are potential fish and human pathogens. Some of these bacteria have been found to survive when undercooked and precooked fish foods were stored at freezing temperatures (Amande and Nwaka, 2013).

Some of the isolates obtained in this study are implicated in fish spoilage. Most researchers are of the opinion that fish muscle is sterile but after death, incorrect or inadequate handling can introduce bacteria to the flesh resulting in spoilage. The natural flora of fish that play a predominant role in spoilage include the genera *Pseudomonas, Vibrio, Micrococcus, Achromonas, Corynebacterium* and *Flavobacterium. Proteus* and *Pseudomonas* sp. are among the major spoilage bacteria at near freezing temperatures (Amande and Nwaka, 2013).

5. Conclusion

The bacterial loads observed in this study are within stipulated limits. Some fish and human pathogens were isolated from the *Clarias gariepinus* fish samples. To prevent associated public health risks, it is strongly recommended that catfish is properly cooked before consumption and cross- contamination avoided during preparation. The fish pond staff that handle the fish should ensure adequate hygiene when working in the fish pond.

Corresponding Author:
Ifeoma Laeticia Okoliegbe
Department of Microbiology, Faculty of Sciences, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria
Telephone: +2348140076113
E-mail: laetymaria@yahoo.com

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