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

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**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 ± 0.1 × 104 to2.6 ± 0.2 × 104 cfu/ml; in sediment; in gill filaments, 1.8 ± 0.2 × 105 to 2.7 ± 0.3 × 105 cfu/g; in intestine, 2.9 ± 0.4 × 104 to 2.6 ± 0.3 × 106 cfu/g and in feed 2.3 ± 0.1 × 106 to 2.8 ± 0.4 × 106cfu/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.

[Okoliegbe, I. L., Ariole, C. N. and Okpokwasili, G. C. **Bacterial Loads and Bacterial Distribution Pattern Associated with *Clarias gariepinus* Juveniles Cultured in Concrete Fish Ponds in Port Harcourt.** *N Y Sci J* 2019;12(1):77-83]. ISSN 1554-0200 (print); ISSN 2375-723X (online). <http://www.sciencepub.net/newyork>. 9. doi:[10.7537/marsnys120119.09](http://www.dx.doi.org/10.7537/marsnys120119.09).

**Keywords:** [Bacterial flora](https://www.tandfonline.com/keyword/Bacterial+Flora), [intestine](https://www.tandfonline.com/keyword/Intestine), [gills](https://www.tandfonline.com/keyword/Gills), fish feed, [*Clarias*](https://www.tandfonline.com/keyword/Saudi+Arabia) *gariepinus*

**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 4o401N –501 N and between longitude 70.001E – 7o101E. 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 37oC 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 4oC 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 H2S 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 (51AGA TTTGATCCTGGCTCAG31) and 1492 R (51GGTTACCTTGTTACGACTT 31) (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 ± 0.1 × 104 to2.6 ± 0.2 × 104 cfu/ml; in gill filaments, 1.8 ± 0.2 × 105 to 2.7 ± 0.3 × 105 cfu/g; in intestine, 2.9 ± 0.4 × 104 to 2.6 ± 0.3 × 106 cfu/g and in feed 2.3 ± 0.1 × 106 to 2.8 ± 0.4 × 106cfu/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 ± 0.2 × 104to 2.3 ± 0.4×105cfu/ml in pond water; 1.9±0.2×104to 2.5±0.2×104cfu/g in gill filaments; 1.5±0.6×103to 2.1±0.2×104cfu/g in intestine and in the feed 0to 2.2±0.1×103cfu/g. The quantitative estimation of total *Vibrio* in pond water, gill filaments, intestine and feeds ranged from 1.4±0.1×104to 2.2 ± 0.2×104in pond water; 1.8 ± 0.1 × 104 to 3.1±0.2×105cfu/g in gill filaments; 1.9 ± 0.1 × 104to 2.5 ±0.1×104 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 ± 0.1 × 104 | 1.8±0.2 × 105 | 2.6 ± 0.3 × 106 | 2.3±0.1× 106 |
| 2 | 2.3 ± 0.2 × 104 | 2.7 ± 0.3 × 105 | 2.9 ± 0.4 × 104 | 2.8 ± 0.4 × 106 |
| 3 | 2. 6± 0.2 × 104 | 2.3 ± 0.3 × 105 | 2.6 ± 0.1× 105 | 2.4 ±0.3× 106 |

(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±0.2×104 | 1.9±0.2×104 | 1.5±0.6×103 | 0 |
| 2 | 2.8±0.1×104 | 2.04±0.6×104 | 1.5±0.1×104 | 0 |
| 3 | 2.3±0.4×105 | 2.5±0.2×104 | 2.1±0.2×104 | 2.2±0.1×103 |

(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±0.2×104 | 1.8±0.1×104 | 2.5 ± 0.1 × 104 | 0 |
| 2 | 2.2±0.1×104 | 3.1±0.2×105 | 2.1 ± 0.2 × 104 | 0 |
| 3 | 1.4±0.1×104 | 2.7±0.6×105 | 1.9 ± 0.1 × 104 | 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 |  |  | Methyl red | Vogues-Proskauer | Citrate | Oxidase | Urease | Sugar Fermentation | Triple Sugar Iron | | | Phenotypic Identity |
| Glucose | Sucrose | Lactose | Motility | Indole | Butt | Gas | H2S |
| 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*  *Bordetella*  *Escherichia*  *Providencia*  *Vibrio* | 14  (17.1)  11  (13.4)  9  (11.0)  10  (12.2)  10  (12.2) | 7  (7.5)  12  (12.9)  12  (12.9)  0  18  (19.4) | 10  (10.5)  9  (9.5)  9  (9.5)  13  (13.7)  15  (10.5) | 10  (11.9)  11  (13.1)  16  (19.0)  7  (8.3)  10  (11.9) | 15  (15.3)  12  (12.3)  8  (8.2)  8  (8.2)  17  (17.3) | 11  (17.5)  0  10  (15.9)  0  12  (19.0) | 12  (14.3)  10  (11.9)  7  (8.3)  5  (6.0)  10  (14.3) | 9  (11.5)  16  (20.5)  12  (15.4)  0  10  (12.8) | 14  (16.9)  13  (15.7)  8  (9.6)  10  (12.0)  8  (9.6) | 0  0  0  0  0 | 0  0  0  0  0 | 0  0  8  (22.2)  15  (41.7)  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 106. Ubiebi (2017) analyzed fish feeds sold in Abraka, Delta State and obtained bacterial counts of 104to 105 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 104 – 106 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 102 – 107(FAO, 1979; Adams and Moss, 1999). However, Akinwale *et al.,* (2007) obtained higher bacterial counts of 1012 and 1010 in *Clarias gariepinus* intestine and gills respectively while Al-Harbi and Uddin (2010) obtained counts within the range of 106 to 107 in gills and 108 to 1010 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.

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**References**

1. Adams, M.R. and Moss,M.O. (1999). Food Microbiology (Royal society of Chemistry, Cambridge, 1999).
2. Akinwale, M.M.A., Keke, R.I. and Chidiebere, E. (2007). Bacterial microflora of the african catfish (*Clarias gariepinus*) juveniles raised under fish cum pig integrated farming system in freshwater earthen ponds during the dry season. *Nigerian Journal of Fisheries,* 4 (2) 2007: 91-104.
3. Ahmed H. Al-Harbi & M. Naim Uddin (2010) Bacterial Populations of African Catfish, *Clarias gariepinus* (Burchell 1822) Cultured in Earthen Ponds. *Journal of Applied Aquaculture*, 22:3, 187-193.
4. Amande, T.J. and Nwaka, S.U. (2013). Bacterial flora of African catfish (*Clarias gariepinus* ) harvested from ponds in Uyo South-South Nigeria*. IOSR Journal Of Environmental Science, Toxicology And Food Technology (IOSR-JESTFT),*5(3) (Jul. - Aug. 2013):72-76.
5. Apun, K., Yusof, A.M., and Jugang, K. (1999) Distribution of bacteria in tropical freshwater fish and ponds. *International Journal of Environmental Health Research,*9, 285–292.
6. Ariole C. N., Nwogu H. A., Chuku P. W. (2014). Enzymatic Activities of Intestinal Bacteria Isolated from Farmed *Clarias gariepinus. International Journal of Aquaculture*, 4(18); 108-112.
7. Austin, B. (2006). The Bacterial Microflora of Fish, Revised. *The Scientific World Journal,* 6: 931–945.
8. Austin, B. and Austin, D.A. (1987) Bacterial Fish Pathogens, Disease of Farmed and Wild Fish*. Ellis Horwood*, Chichester.
9. Cummings, J.H. and Macfarlane, G.T. (1997). Role of intestinal bacteria in nutrient metabolism. *Journal of Parenteral and Enteral Nutrition*, 21(6);357-65.
10. Dauda AB, Natrah I, Karim M, Kamarudin MS, Bichi AH (2018). African Catfish Aquaculture in Malaysia and Nigeria: Status, Trends and Prospects. *Fish Aqua* J 9: 237.
11. Diler, O., Altun, S., Calikusu, F., and Diler, A. (2000) A study on qualitative and quantitative bacterial flora of therainbow trout (*Oncorhynchus mykiss*) living in different fish farms. *Turkish. Journal of Veterinary and Animal Science,*24, 251–259.
12. Diyaolu D.O. (2015). Aerobic bacterial flora and survival of African catfish *Clarias gariepinus* during early life stages in the hatchery. *International Journal of Fisheries and Aquatic Studies,*3(1);380-384.
13. FAO. (1979). Manuals of food quality control. FAO. Food nutrition paper1 4/4, 1979.
14. FAO (2016b) Cultured Aquatic Species Information Programme: *Clarias gariepinus*. Fisheries and Aquaculture Department, Food and Agricultural Organization of the United Nations, Rome.
15. Holt, J.G., Krieg, N.R. Sneath, P.H.A., Staley, J.T. Williams, S.T. (1994). Bergey's manual of determinative bacteriology.
16. Lee, S. and Lee, Y. (1995) Identification of intestinal microflora in rainbow trout. *J. Microbiol*. 33, 273–277.
17. Novotny, L., Dvorska, L., Lorencova, A., Beran, V., and Pavlik, I. (2004) Fish: a potential source of bacterial pathogens for human beings. *Vet. Med.* 49, 343–358.
18. Okoliegbe, I. L., Solomon, L. and Dick, A. A. (2017). Exploiting Microbial Communities Associated with Marine Fish: An Indispensable Approach to Sustainable Aquaculture. *Nature and Science,*15(4):84-91.
19. Pelzar, M.J., Chan, E.C.S. and Noel,R. K. (1986). General Microbiology, ( Singapore: *McGraw-Hill Book Company*, 1986).
20. Ubiebi, C.O. (2017). Isolation and identification of bacterial isolates from poultry and fish feeds sold in Abraka, Delta State, Nigeria. *Journal of Industrial Technology.* 2017, 2(1): 14-20.
21. Uddin, N. and Al-Harbi, A.H. (2012). Bacterial flora of polycultured common carp, *Cyprinus carpio* and African catfish, *Clarias gariepinus*. International *Aquatic Research*, 4, 2012, 10.
22. Umana, S.I., Okon, U.C., Uko, M.P. and Bassey, M.P. (2017). Occurrence of bacterial contaminants in fin-fishes (*Clarias gariepinus* and *Coptodon guineensis*) from humic freshwater ecosystem of Eniong River, Itu, Akwa Ibom State, Nigeria. *Journal of Advances in Microbiology,*6(1): 1-12.

1/20/2019