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Bacteria Flora in the Gut and Respiratory Organs of *Clarias gariepinus* in Fresh and Brackish Water Habitats of Ondo State, South/West Nigeria

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***Abstract*—**Bacteria flora of *Clarias gariepinus* collected from two natural habitats namely Owena River (freshwater) and Igbokoda lagoon (brackish water) were examined using standard microbiological procedures. Thirteen bacterial species were identified. The result indicated that from the identified bacteria isolated, *Vibrio* sp, *Proteus* sp. *Shigella* sp. and *E. coli* were present in both habitats (fresh and brackish waters). Others were habitat-selective such as *Salmonella* sp., *Pseudomonas* sp, *Enterococcus* sp, *Staphylococcus* sp. that were found only in freshwater habitat. While *Branhamella* sp, *Streptococcus* sp. and *Micrococcus* sp. were found in brackish water habitat. Bacteria load from Owena river (freshwater) was found to be the highest load recorded at 6.21 x 10^4cfu. T-test analysis also revealed that there was a marked significant difference between bacterial load in guts of sampled *Clarias* from fresh water and brackish water habitats.

***Keywords*—**Bacteria flora, gut, *Clarias gariepinus*, Owena river.

I. Introduction

CLARIAS GARIEPINUS (BURCHELL, 1822) belong to the family *Claridae*, the air breathing catfish. The species are characterized by the ability to grow on a wide range of artificial and natural foods, attainment of a large size within short time, high yield potentials, hardness and tolerance to low dissolved oxygen and other aquatic conditions [1].

It is widely distributed throughout Africa. In Nigeria, catfish is widely cultured [2] because of its high growth rate, ability to withstand stress, disease and ability to spawn easily. *C. gariepinus* is one of the most suitable aquaculture species in Nigeria.

Disease control is an inherent part of any animal production system, however, in the aquatic environment, the intimate relationship between bacteria and their hosts and the frequent use of open production systems add to this challenge.

Bacteria, including the non-pathogenic as well as pathogenic forms, are usually present in small numbers in many fish species. Bacteria in normal situation seldom cause any problem as the fish's own immune system is more than capable of fending off any infection which may become chronic [3].

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Fish gills functions as both respiratory and osmo-regulatory organs. Basically, they consist of a network of capillaries where blood is separated from the surrounding water by only one or two layers of cells.

One of the limiting factors in fish production is mortality due to bacterial infections [4]. Bacteria, which are ubiquitous, can be normal flora of fish skin and its environment.

This study focused on the isolation, characterization and the quantification of the bacterial population present in the gut and respiratory organs of *Clarias gariepinus* in fresh water and brackish water environment in Ondo State, South Western Part of Nigeria. The results will be of tremendous interest in identifying the types of bacteria flora associated with freshly collected samples of *C. gariepinus* from the two water bodies.

II. Materials and Methods

A. Collection of Specimens

Adult samples of *Clarias gariepinus* were collected from a freshwater habitat, Owena River and also obtained from fishermen at Igbokoda lagoon a brackish water habitat both in Ondo State, Nigeria.

Collections were kept fresh in ice flakes in a vacuum flask (25 litres) during transportation to the laboratory prior to analysis. The microbial analyses were carried out at the limnology laboratory in Department of Fisheries and Aquaculture, Federal University of Technology, Akure, Ondo State, Nigeria.

The visual examination of the whole body was carried out to determine the fitness. The total length, standard length cm and the total weigh (g) Samples were taken using tape rule to the nearest 1.0cm and weighing balance to the nearest 1.0g.

The glass wares (pipettes, test tubes), were sterilized in an oven at 160^{o}C for 1½ hours. Absolute alcohol was used to surface disinfect the working table. Each of the specimens was dissected aseptically to remove the gills and gut.

B. Serial Dilution of Samples

Each organ was placed in a sterile bottle containing 5ml sterile distilled water and vigorously shaken to allow the contained bacteria dislodge into the water. Then from each suspension, 0.1ml was pour-plated using freshly prepared molten nutrient agar (N.A.) (MERCK) 20ml. The plates, after being covered, were gently swirled to evenly mix-up and allowed to gel. These were inverted and incubated at 37^{o}C for 24 hours. Microbial count (Bacteria load) colonies which

developed after incubation were subjected to counting using Gallenkamp colony counter (CX-300) and were also expressed in colony forming unit (CfU).

C. Isolation of Pure Culture and Preparation of Stock Cultures

From the original plates, distinct colonies were picked with sterilized wire loop and streaking into freshly prepared nutrient agar plates to obtain pure isolates of the organism after an incubation period of 24 hours at 37°C. The stock cultures were then stored in the refrigerator at 4°C pending identification test.

D. Cultural/Biochemical Tests on Bacteria Isolates

The biochemical tests that were performed on the bacteria isolates includes, gram reaction, catalase, coagulate, nitrate reduction, starch hydrolysis, indole (lactose, sucrose, glucose, maltose, galactose and dextrose) using standard microbiological techniques [5].

E. Data Analysis

Biological data obtained were subjected to analysis of variance (ANOVA) and also T-test was used to determine if there was a significant difference between bacterial loads of

(i) Gills and guts
(ii) Habitats (fresh and brackish water)

III. Results and Discussion

The results of the cultural/biochemical test carried out on the bacterial isolates were presented in Table I.

TABLE I
BIOCHEMICAL TESTS FOR THE IDENTIFICATION OF THE BACTERIAL ISOLATES

Colonial Colour	Grey	Yellow	Grey	Grey	White	Green	Green	Green	Green	Green
Gram Reaction	-	+	-	-	-	-	+	-	+	+
Cell Morphology	Cocci	Cocci	S. rod	M. rod	M. rod	Cocci	S. rod	S. rod	L. rod	Cocci
Spore	-	-	-	-	-	-	-	-	-	-
Motility	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	-	-	-	-	+	-	+	-
Indole	-	-	-	-	+	-	-	-	+	+
VogesProskaeur	-	-	-	-	+	-	-	+	-	-
Methyl Red	-	A		AG	AG	-	-	-	-	-
Glucose	-	A	-	AG	AG	-	-	AG	-	-
Sucrose	AG	A	AG	-	AG	-	-	AG	-	A
Lactose	-	A	AG	AG	-	-	-	A	-	A
Mannitol	-	A	-	-	-	-	-	A	-	A
Fructose	-	A	-	-	-	-	-	A	-	-

The following bacteria were identified; *Salmonella* sp., *Pseudomonas* sp., *Proteus* sp., *Klebsiella* sp., *Escherichia coli, Vibrio* sp., *Enterococcus* sp., *Staphylococcus* sp., *Citrobacter* sp., *Shigella* sp, *Branhamella*, *Streptococcus* and *Micrococcus.*

The incidence of occurrence of the identified bacteria isolates in the gut and gill of *Clarias* from each of the two habitats investigated were presented in Table II. The result indicated that from the identified bacteria isolated, *Vibrio* sp., *Salmonella* sp., *Proteus* sp, *Pseudomonas* sp. And *E. coli* occurred in the gut and gill of *Clarias gariepinus* from Owena River (fresh water habitat). However, *E. coli* and *Branhamella* sp. were common in the gut and gill of *Clarias gariepinus* from Igbokoda lagoon (Brackish water) (Table II). *Vibrio* sp, *Proteus* sp, *Shigella* sp and *E. coli* were present in both habitats (fresh water and brackish water) as shown in Table II. The results also indicated that *E. coli* was present in both the gut and gills of the *Clarias* from the two locations, this confirmed recent pollution and of fecal origin. *E. coli* is known to cause *gastroenteritis* in humans [6]. The occurrence of *Salmonella* sp. and *Shigella* sp. indicates contamination during handling by vendors, is also an indication that the water where they lived was contaminated. Similar observation was made by Hatha [7] who reported that *Salmonella* and *Shigella* sp. existed on the skin, gills and intestine of catfish but the most potential reservoir of *Salmonella* was the intestine.

The identified micro-flora of *Clarias gariepinus* from the two habitats similar to the reports of Musefiu et al. [8], in their study of bacteria flora of *Clarias* and Tivkaa Joseph and Samson [9] in Ibadan South/West, and Uyo metropolis of South-South, Nigeria respectively.

The commensal bacteria flora included facultative pathogens which under stress could give rise to fish disease. These isolates are potential pathogens to humans as well. *E. coli*, *Salmonella* sp. and *Streptococcus* sp. are implicated to be fish borne.

A. Bacteria Load

The bacteria load in the organs of *C. gariepinus* from the two habitats is shown in Fig 1. The *Clarias* collected from Owena River (Fresh water) had mean bacterial population of 4.81×10^4 and 1.56×10^4 on the gut and gills respectively.

The mean bacterial population of specimen from Igbokoda lagoon (brackish water) is 1.31×10^4 and 1.25×10^4 for the gut and gills respectively.

Further analysis indicated that gut form Owena River (fresh water) had the highest bacterial population (6.21×10^4) and the lowest bacteria population was recorded in the gill of the

C. gariepinus from Igbokoda lagoon (brackish) location (1.51 x 10^4).

The mean (fresh water) bacterial population of Owena Gut 4.81 x $10^4$is higher than the mean bacteria population of Igbokoda lagoon gut (1.31 x10^4). Also, the mean bacterial population of Owena gill (1.56 x 10^4) is higher than gill from brackish water with 1.25 x 10^4 of mean bacteria population. The result of bacterial population of 10^4 conforms to the finding of Wantanabe [10] who reported lower counts in the range of 10^4 – 10^5 for *Clarias* sp. and Tivkaa Joseph [9] who reported 1.2 x 10^4 – 2.16 x 10^4. However, this results was low when compared with the findings of Adedeji *et al.* [11] who reported counts in the range of 10^{12}- 10^{13}cfu and also with the result of Egbere et al. [12] who reported counts in the range of 10^6 – 10^8 in their study of catfish ponds in Jos, metropolis, Northern Nigeria [9].

T-test was used to analyze the level of significance of bacteria population in organs of *C. gariepinus* and their locations. There is no significant difference ($P<0.05$) in the bacteria load between gut and gill of *C. gariepinus* from the brackish habitat (Igbokoda lagoon) whereas there is marked significant difference in the gut and gill from Owena river fresh water habitat.

The results indicated a marked significant difference between bacterial load on the gut of samples from Owena River and Igbokoda lagoon, which are fresh and brackish habitat respectively whereas, there was no significant difference between gills of sampled fish from Owena River and Igbokoda lagoon.

TABLE II
INCIDENCE OF OCCURRENCE OF BACTERIA ISOLATES ON THE GUT AND GILL OF *CLARIAS GAERIEPINUS* FROM THE TWO HABITATS

	Fresh Water (Owena River)		Brackish Water (Igbokoda River)	
Bacteria Isolates	Gut	Gill	Gut	Gill
Vibrio sp	+	+	+	-
Salmonella	+	+	-	-
Klebsiella	-	+	-	-
Proteus	+	+	-	+
Pseudomonas sp	+	+	-	-
Enterococococcus	-	+	-	-
Citrobacter	-	-	-	+
Shigella	+	-	+	-
Staphylococcus	+	-	-	-
Escherichia coli	+	+	+	+
Branhamella	-		+	+
Streptococcus	-	-	-	+
Micrococicus	-	-	-	+

Key
Present – (+)
Absent – (–)

The analysis revealed that bacteria load is higher in the fresh water habitat than the lagoon; it also proved that there is significant difference in the bacterial loads of the two habitats under investigation. This could be as a result of effluent discharge into the municipal rivers. This is in agreement with Egbere et al., [12] that most contaminated water source for catfish could result from indiscriminate disposition of human and animal excreta. During raining seasons, environmental wastes including fecal matter are washed from polluted lands into water bodies.

IV. Conclusion

The identified bacteria isolated from *Clarias gariepinus* in fresh and brackish water in this research were from the Southwestern part of Nigeria.

Same bacteria were isolated by various researchers in different region of Nigeria, namely, Jos, Ibadan, Uyo, that is, North, West, South/East parts of the country respectively. It can therefore be concluded that, these are the micro flora bacteria of *Clarias gariepinus* in Nigeria. It can also be concluded that fresh water habitat has the higher bacteria load than the brackish water of Ondo State coastal region.

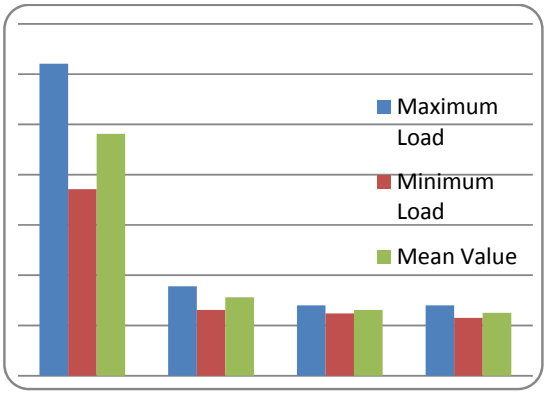

Fig.1 Bacterial load of the gut and gills organ of *Clarias gariepinus* from Owena River (Freshwater habitat) and Igbokoda Lagoon (Brackish water habitat)

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