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Genetic Diversity Studies and Sensitivity to Antibiotics of Bacteria in Different Parts of Advanced Juveniles *Clarias gariepinus* from Idogo on Yewa River

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Abstract: The study was conducted to determine the genetic relationship of the bacteria isolated from different parts of *Clarias gariepinus* using Random Amplified Polymorphic DNA (RAPD). PCR analysis as well as the antibiotics resistance profile was done. Bacteria isolates were taken from the skin, gills and gut of *C. gariepinus*. DNA was extraction from the bacteria isolates using CTAB method. The DNA extracted was subjected to RAPD-PCR analysis using five (5) RAPD primers. The RAPD gels were scored, analyzed using NTSYS program and the phylogenetic trees were constructed. The antibiotics resistance profile of these bacterial isolated were determined using disc diffusion method. Twenty bacteria species were isolated from the gill, gut and the skin of the *Clarias gariepinus* include *Citrobacter murlinae* strain, *Escherichia coli* strain, *Morganella morganii* strain, *Alkaligenes feacalis* strain, *Citrobacter freundii*, *Citrobacter freundii* strain, *Citrobacter freundii* strain, *Morganella morganii* stain, *Erwinia tasmaniensis* stain, *Morganella morganii* strain, *Akaligenes feacalis* strain, *Proteus penneri* strain, *Citrobacter braaki* strain, *Morganella morganii* strain, *Citrobacter amalanoticus* strain and *Citrobacter freundii* stain. The study revealed a genetic diversity which is required for populations to be more adaptive with the environmental changes. Eight (8) antibiotic substances were examined for the susceptibility of the species. These include Ceftazidime, Cefuroxime, Gentamicin, Ofloxacin, Augumentin, Nitrofurantion, Ciprofloxacin, Cefixime. However, Ciprofloxacin and Ofloxacin were found to be more effective as bacteria species were more resistance to other antibiotics.
Keywords: *Clarias gariepinus*, Random Amplified Polymorphic DNA (RAPD), *Clarias gariepinus*

INTRODUCTION

The estimation of Nigeria fish resources as well as potential yield estimate per annum from rivers, flood plains, pools and reservoirs is 226,550 metric tonnes FDF [1]. Among the commercially important fresh water fish generally in Nigeria are the *Tilapia, Lates, Chysichthys, Mormyrus, Lutjanus, Hemichromis, Hydrocynus and Clarias.* These species of fish depending on the size is of high economic value in Nigeria.

Micro organisms include viruses and bacteria. But certain algae and fungi can also be considered as microorganisms because they are microscopic in size [2]. It is well recognized that microorganism occurred in captured fisheries of tropical waters and infections compared with fishes from the temperate waters [3]. Essentially, bacteria may occurs in fresh water bodies as pathogenic, natural and spoilage organisms [4]. Majority of these organisms are non-pathogenic, causing only spoilage of fish but there are some which are pathogenic causing food poisoning. Quality standards have been prescribed for the fish and fishery products meant for export and they are being monitored strictly [5].

Several works have revealed the presence and occurrence of bacteria flora in pond waters. This shows that gram-negative rod shaped bacteria are found in a cultured population (pond system) Naim *et al.*, [6]. The study further stated that pond water bacteria had a reflection on the bacterial composition of the gills and intestine (gut) of catfish (*Clarias gariepinus).*

Under natural water environment, microbes such as bacteria occurs in both manmade and natural fresh water bodies as pathogenic, natural and spoilage micro organisms. It is important to study their occurrence on these commercially important fish species with the intention to investigate the tendency to cause food poisoning or death from the consumption of fish.

Therefore, the objective of this study is to determine the genetic relatedness and diversity of bacteria associated with fish species using Random Amplified Polymorphic DNA, as well as sensitivity and resistance of isolated bacteria to specific antibiotics.

MATERIALS AND METHODS

Study area/Collection of Samples

The study was carried out at Yewa river, Idogo, Yewa South Local Government Area of Ogun state. It lies between longitude $2^{0}51^{11}$ and Latitude $6^{0}25^{11}$ S of the equator. Twenty fish samples were taken from Idogo station. Bacteria from each fish samples were obtained from the gill surfaces, gut and skin using swab sticks. All swab sticks were streaked on both Nutrient agar and Mac Conkey agar and the samples were later incubated overnight at 37^{0}C.

Water Test and Morphometric Features of Fish Samples

The standard length, head length and total length in centimeters (cm) were measured and recorded after weighing the fish samples in grams (g). The water quality was evaluated on Temperature, Dissolved Oxygen and pH.

Isolation of Bacteria and Extraction of DNA Using CTAB Method

The media for microbiological analysis were weighed out according to the manufacturers' specifications. The media were properly weighed and dissolved in conical flask containing distilled water covered with aluminum foil and sterilized in the autoclave at 121^{0}C for 15 minutes. Agar media were allowed to cool to 45^{0}C before pouring into sterile plastic petri dishes for solidification and finally dry in an incubator. The colonies from original culture on Nutrient agar plates were picked by sterile inoculating loop and streaked the isolate on sterile Nutrient plates. The plates were incubated at 37^{0}C for 24 hours for pure microbial growth. Bacteria isolates grown overnight were transferred to eppendorf tube and it was spun down at 14,000rpm for 2mins, the supernatant was discarded and the extraction of DNA was done using CTAB method Thottappily *et al.,* [7]. The DNA was later resuspended in 100µl of sterile distilled water. DNA concentration of the samples were measured and the genomic purity were determined. The DNA was further check on 1.0 % agarose gel and was visualised on UV light source.

RAPD-PCR amplification and electrophoresis

PCR analysis was done using MJ Research Thermal Cycler (PTC-200 model). The RAPD primers used for PCR amplification were OPB-12, OPB-20, OPH-08, OPH-12 and OPH-19. The reaction mix was carried out in 20µl final volume containing 60ng - 80ng genomic DNA, 0.1 µM of the primers, 2mM $MgCl_2$, 125µM of each dNTP and 1 unit of Taq DNA polymerase. The thermocycler profiles has an initial denaturation temperature for 3mins at 94^{0}C, followed by 45 cycles of denaturation temperature at 94^{0}C for 20seconds, annealing at 37^{0}C for 40 seconds and primer extension at 72^{0}C for 40seconds, followed by final extension temperature at 72^{0}C for 5 minute was added. PCR amplicon electrophoresis was carried out by size fractionation on 1.2% agarose gels. The gel electrophoresis was done at 100V for 2 hours. The DNA was visualized and photographed on UV light source.

Antibiotics Sensitivity Test

Fresh Nutrient Agar was prepared according to the manufacturer`s specification and it was sterilized at 121^{0}C for 15 minutes. The bacteria isolates kept on nutrient agar slant were retrieved and streaked on a fresh Nutrient Agar plates, an antibiotic disc was introduced into each Nutrient Agar plates and these were incubated at 37^{0}C for 24 hours. After sometime a clear zone (incubation zone) was observed and the diameter was measured.

Statistical Analysis

The bands obtained from the gel was transferred to numerical figures, 1 represents the presence of a band while 0 is absence of a band, it was later subjected to analysis using NTSYS software to draw the dendogram for the bacteria isolates. Data from the morphometrics and water parameter were analyzed using descriptive statistics.

RESULTS AND DISCUSIONS

Water Test and Morphometric Features

Table 1 showed that the mean value for the Total Length was 29.2 $\pm$0.715cm, the Standard Length was 25.51 $\pm$ 0.49cm and Head Length was 5.20 $\pm$ 0.46cm. Table 2 showed that Temperature, Dissolved Oxygen and pH were 31.2^{0}C, 3.6ppm and 7.2 respectively.

PCR amplification using RAPD primers

There were 57 polymorphic markers generated from the five RAPD markers in table 5. There were also 10 monomorphic markers from the primers. A total number of 67 markers were generated. Fourteen alleles were obtained from the first primer OPB-12, 16 alleles from the second primer OPB-20 and 17 alleles from the third primer OPH-08. Eight alleles were from the fourth primer OPH-12 and 12 alleles from the fifth primer Table 4. The dendogram in Figure 4 shows that all the bacteria isolates obtained from the fish at Idogo station were separated into two major groups at 70.2% similarities. Group 1 consist of 19 bacteria isolates while Group 2 consist of only one isolate (*E. coli*). Group 1 was further divided into two sub groups; Group 1A and Group 1B. Group 1A consist of 18 isolates while Group 1B has just one isolate (*Erwinia tasmaniensis*)

Antibiotics Sensitivity Patterns

The sensitivity test showed that the resistance of 100% was recorded after sensitivity with Cefuroxime and Augumentin while, only 85% and 82% resistance was observed to Ceftazidime and Gentamicin respectively in Figure 1. Highest susceptibility of 75%

was recorded by Ciprofloxacin while the least susceptibility rate of 10% and 15% was recorded for Ceftazidime and Gentamicin respectively.

Table 1: Summary of Morphometrics Characteristics

Morphometrics (cm)	Readings (Mean ± SE)
Total length	29.2 ±0.715
Standard length	25.51±0.49
Head length	5.20±0.46

Table 2: Water parameter

Water parameters	Reading
Temperature (^{0}C)	31.2
Dissolved Oxygen (ppm)	3.6
pH	7.2

Table 3: Percentage Antibiotic Sensitivity Patterns of Bacterial Isolate from *C. gariepinus*

Antibiotics	S %	I %	R %
Ofloxacin	50.0	20.0	30.0
Augumentin	0.0	0.0	100
Nitrofurantion	25.0	15.0	60.0
Ciproflocxacin	75.0	10.0	15.0
Ceftazidime	10.0	5.0	85.0
Cefuroxime	0.0	0.0	100.0
Gentamicin	15.0	5.0	80.0
Cefixime	5.0	20.0	75.0

I-Intermediate, R- resistance and S- sensitive

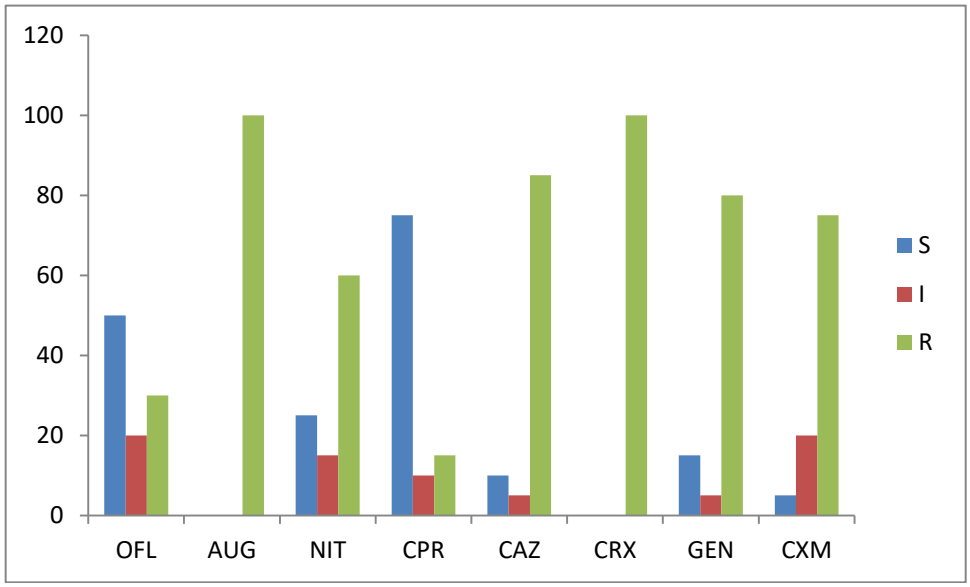

Fig-1: Antibiotics Resistance and Susceptibility Pattern of Bacteria Isolates (%)

Antibiotics: OFL-Oflaxacin, AUG- Augumentin, NIT- Nitrofurantion, CPR- Ciprofloxacin, CAZ- Ceftazidime, CRX- Cefuroxime, GEN- Gentamicin, CXM- Cefixime.

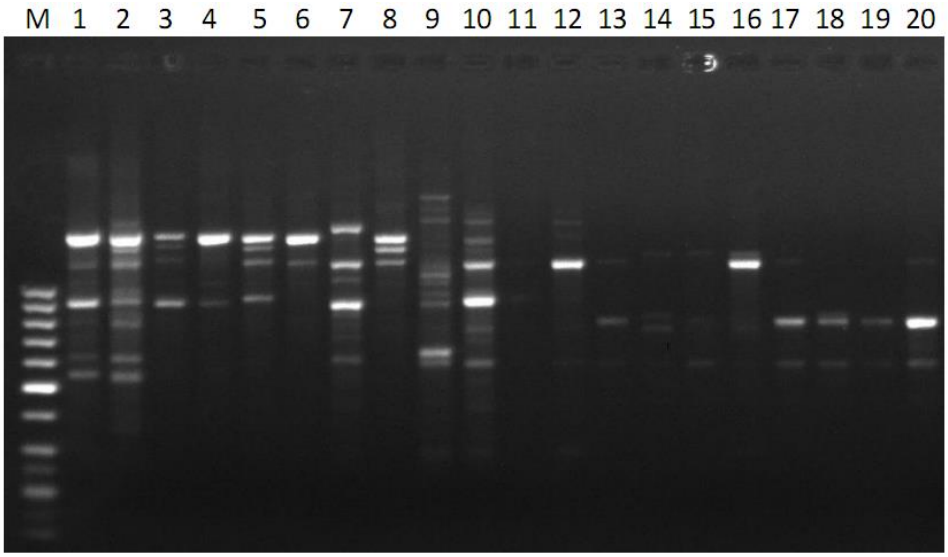

Fig-3: Electrophoresis gel for RAPD primer OPB 12

Table 4: Primer Sequences and Percentage Polymorphism

S/N	Primer name	Primer Sequences	No of Monomorphic markers	No of Polymorphic markers	Total no of markers	Percentage Polymorphism
1	OPB 12	CCTTGACGCA	Nil	14	14	100
2	OPB 20	GGACCCTTAC	01	15	16	94
3	OPH 08	GAAACACCCC	01	16	17	94
4	OPH 12	ACGCGCATGT	03	05	08	63
5	OPH 19	CTGACCAGCC	05	07	12	58
	Total		10	57	67	

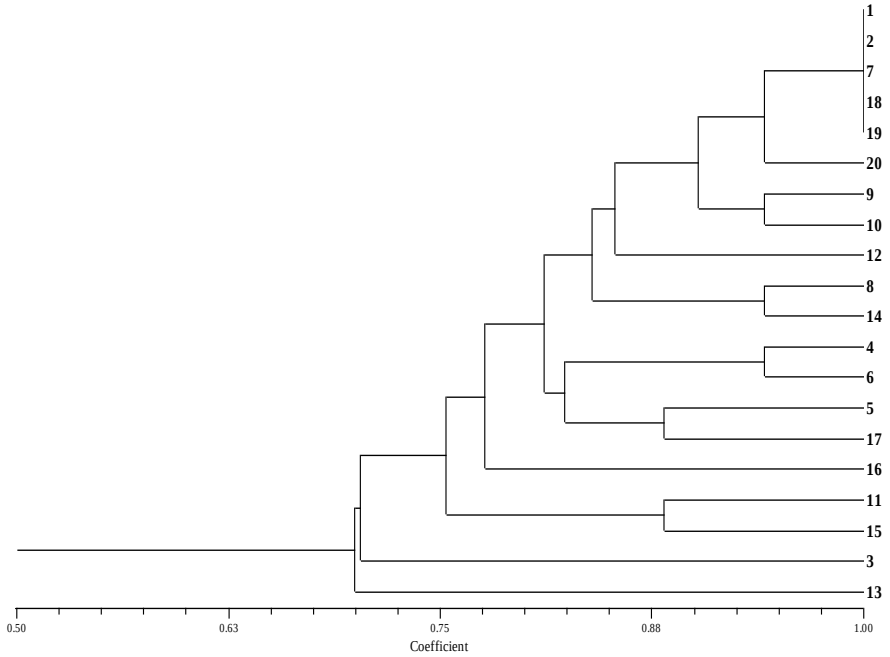

Fig-4: Dendogram for the 20 Bacteria Isolates

Key: 1: *Citrobacter amalanoticus* strain; 2: *Citrobacter murlianae* stain; 3: *Escherichia coli* strain; 4: *Morganella morganii* strain; 5: *Alkaligenes faecalis* strain; 6: *Citrobacter freundii* strain; 7: *Citrobacter freundii*; 8: *Citrobacter freundii* strain; 9: *Citrobacter freundii* strain; 10: *Citrobacter freundii* strain; 11: *Morganella morganii* strain; 12: *Citrobacter murlianae* stain; 13: *Erwinia tasmaniensis* strain; 14: *Morganella morganii* strain; 15: *Alkaligenes faecalis* strain; 16: *Proteus penneri* strain; 17: *Citrobacter braakii* strain; 18: *Morganella morganii* strain; 19: *Citrobacter amalanoticus* strain; 20: *Citrobacter freundii* strain.

DISCUSSION

It was revealed that the fish samples harboured bacteria growths of which the skin harboured the large percentage out of the 20 samples harvested from Idogo station. Antibiotic resistance is a significant public health issue. There have been many papers reporting a link between use in food animals, emergence of antibiotics resistance in *E. coli*, *Salmonella* and *Campybacter* in treated animals and transfer to humans via food chain. The result on morphometrics, contradict the work of [8] who reported a different morphometric characteristics in juvenile *C. gariepinus*

The antibiotics used in the sensitivity test were mostly third generation antibiotics. The result revealed a high resistance levels to the antibiotics by the isolates. The highest resistance was to Augumentin and Cefuroxime where all the isolates were totally resist. Ciprofloxacin was the antibiotic to which the leaser resistance (15%) was shown (fig 1). This information is similar to the finding of [9] in which the overall phenotypic antibiotic resistance profiles of gram negative bacteria species from the gut contents of the farmed *C. gariepinus* show multi-drug resistance pattern. Resistance to antimicrocrobial drugs may be as a result of indiscriminate use of these drugs in aquaculture at less than optimum dosage and such action leads to resistance in exposed pathogens. This poses a great problem to heath of the consumer who may be affected by the bacteria and infection from such bacteria and could be difficult to treat.

All the bacterial isolates found in fish from Idogo station were examined for their genetic relatedness. It was observed that the bacteria were divided into 2 major groups of which group 1 consist of nineteen (19) isolates while group 2 has just only one isolate (*E. coli*) furthermore, the group 1 bacteria was futher divided into 2 sub groups, group 1A and group 1B. Group 1A consist of 18 isolates . While only 1 isolate was found to belong to group 1B (*Erwinia tasmaniensis*). This diversity in their genetic sequences was similar to the report of [10], who reported similar genetic relatedness in bacteria. Contrarily, [11] reported different genetic diversity in bacteria isolate found in farm-raised C. *gariepinus* . This genetic relatedness found in fish bacteria from Idogo station could be caused by physio-chemical nature of the water (it serves as a way for population to adapt to changing environment) or their ecological niche (gut, skin and gills). The diversity suggests that these bacteria isolates could have been related based on their genetic composition.

CONCLUSION

This work provided information on bacterial flora from the gill, gut and skin of commercially important fresh water fish *C. gariepinus* which support huge artisanal and culture fisheries in Nigeria. Hence, this study confirm the existence of pathogenic bacteria organisms. *C. gariepinus* harboured microorganisms which are of pathogenic, food poisoning and food spoilage importance. The isolates have the potentials to cause serious infections to fish, to the animals that feed on them and finally to man. Thus, the need to ensure improved local method of processing fish so as to prolong the shelf life of fish through alteration of several factors that lower microbial proliferation in fresh fish, hence, reducing public health problems. Oflaxacin and Ciprofloxacin were the best antibiotic substances with the highest inhibition zones of 24mm and 30mm respectively to the bacterial isolates. Also this study revealed a genetic diversity which is required for populations to be more adaptive with the environmental changes. A RAPD technique is a valuable technique in investigating the genetic diversity in *C. gariepinus* populations. The genetic markers which were detected in the present study will be useful in utilization and management of genetic resources in *C. gariepinus*.

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