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Original Research Article

Molecular Characterization and Antibiotic Resistance Profile of Bacteria Associated with *Clarias gariepinus* (Burchell, 1822) From Ebute Iboro Location on Yewa River

ABSTRACT

Bacteria isolates associated with *Clarias gariepinus* were collected from fish sample gotten on Yewa River in Ebute Igbo-Aro. The isolates were assessed using 16S rRNA gene sequencing method to identify the starins and to construct the phylogenetic relationship. The morphometric characteristic such as weight, standard length, total length and head length of fish samples were measured. Bacteria were isolated from the gills, skin and gut of *Clarias gariepinius* using spread plate method. The DNA of the bacteria isolates were extracted using CTAB method, PCR amplification of 16S ribosomal RNA gene of isolates was carried out using universal primer for bacteria, purification of the PCR product using ethanol precipitation, thereafter sequenced using an automated DNA sequencer. The homologous of nucleotides was then searched at the gene bank of NCBI and the isolates were identified. The antibiotics sensitivity profile of the bacterial isolates was determined using disc diffusion method. There are three main type of bacteria found, these include *Citrobacter spp* had the highest number of bacteria associated with the water body, others are *Pseudomonas spp, Serratia spp* and *Aeromonas spp,* these bacteria were found dominantly on the skin. The sensitivity test showed that the identified bacteria isolates were sensitive to Ofloxacin (5mg), Nitrofurantoin (300mg) and Ciprofloxacin (5mg) which can be used to treat the bacteria isolate to reduce its pathogenic impact on human and to reduce disease outbreak when cultured. The bacteria isolate were 100% resistant to Cefixime (5mg) and Cefuroxime (30mg).

Keywords: Bacterial isolates, *C. gariepinus*, DNA extraction, Sequencing, Antibiotics and Sensitivity.

INTRODUCTION

Fish has been one of the main foods for human for many centuries and still constitute an important part of the diet in many countries (Leisner *et al.,* 1995). The advantages of fish as food are its easy digestibility and high nutritional value (Leisner *et al.,* 1995). These important attribute makes the community readily susceptible to microbial attack particularly bacterial (Adams *et al.,* 1999). This has two important consequences for spoilage. First, it limits degree of post mortem acidification of the tissue so that the ultimate pH of the muscle is 6.2 – 6.5 (Adams *et al.*, 1999). Fish are an important resource for human worldwide, especially in the rural area (Adeleye, 1992). Commercial and subsistence fishers hunt fish in wild fisheries or farm them in pond or cages in the ocean (aquaculture).

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Fish are susceptible to a wide variety of bacterial pathogen. Many of these bacterial capable of causing diseases are considered by some to be saprophytic in nature. These bacteria only become pathogens when fishes are physiologically unbalanced, nutritionally deficient, or there are other stressors, i.e., poor water quality, overstocking, which allow opportunistic bacterial infections to proceed. Disease breaks out in fish tank very quickly and you have to first identify the type of disease before you can take action. The bacterial are transmitted by fish that have made contact with other diseased fish. Bacterial fish disease and infections are very common and are one of the most difficult health problems to deal with (Douglas, 2007). Bacterial can enter the fish body through the gills, skin or it can stay on the surface of the body (Douglas, 2007). There are four types of bacterial infections. Bacterial gill disease: the gills are the primary target, systemic bacterial disease: bacteria invades the fish's body and damages internal organs, bacterial body ulcears: lesions on the fish body that can be shallow or deep and fin rot: most likely resulting from environmental stress. (Douglas, 2007).

Bacteria disease is one of the most important diseases in captive fishes and a significant cause of high fish morbidity and mortality rate. Different pathogenic, especially, gram negative bacterial has been reported to be isolated from naturally-infected fish. Many stress factor could contribute to bacterial infection in captive condition namely, poor water quality, crowding, transportation and inadequate nutrition. *Aeromonas hydrophila* is also considered a normal flora as well as a primary and secondary fish pathogen, including ornamental fish. Fish disease has caused substantial economic loss to fish farmers and the fisheries sector. Antibiotics have been used to prevent and treat the bacteria causing disease, but the success of treatment depends on antibiotic susceptibility of etiologic bacteria. Bacteria resistance is an important issue that need to be considered when choosing an appropriate antibiotic.

Therefore, the objective of this research work is to identify the bacteria isolates using 16SrRNA gene sequencing method and to determine the antibiotics susceptibility profile of the bacteria isolated from gills, guts and skin of *Clarias gariepinus* at Ebute Iboro station on Yewa River.

MATERIALS AND METHODS

Collection of the samples

The study was carried out at Ebute Iboro Yewa River which is a trans-boundary river between Republic of Benin and Nigeria. It lies approximately within latitude 6° 22'N and longitude 2^{O} 50'E of the Greenwich meridian. Twenty samples of C. *gariepinus* were collected at Ebute-Iboro landing site at Yewa River. Samples were collected for microbiological analysis by swabbing the gills, skin and guts of *Clarias gariepinus* with the swab sticks. All swab sticks were streaked on both Nutrient agar and Mac Conkey agar by BioMark Laboratory, the samples were later incubated for 24 hours at 37^{0}C.

Water Test and Morphometric Characteristic of fish samples

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The physcio-chemical parameters such as temperature (°C), conductivity (µs/cm) and pH of the water body were taken in-situ using Hanna multipurpose meter and recorded. Morphometric feature of fish sample such as weight, standard length and total length was determined.

Isolation of bacteria and Morphological Characterization

The media used in the experiment were weighed out according to the manufacturer's specification; these include Nutrient Agar and MacConkey Agar. The media were properly weighed and dissolved in conical flasks containing distilled water and boiled gently to dissolve the agar completely. The conical flask was plugged with cotton wool covered with Aluminum foil and sterilized in an autoclave at 121^{0}C for 15 minutes. The media were allowed to cool to 45^{0}C before pouring aseptically into sterile plastic petri dishes for solidification and finally dry in an incubator. Characteristics colonies from original culture on Nutrient agar and Mac Conkey agar plates was picked using sterile Aluminum wire loop and streaked the isolate onto sterile Nutrient and Mac Conkey Agar plates. These plates were incubated at 37^{0}C overnight for pure microbial growth. These isolates were placed to their appropriate agar slant labeled and incubated at 37^{0}C for 24 hours for growth after which they were kept in the refrigerator at 4^{0}C for identification.

Identification of the Bacteria Isolates by 16S rRNA Gene Amplification

Bacteria isolate grown overnight was transferred to eppendorf tube and it was spun down at 14,000rpm for 2mins, the supernatant was discarded and the DNA was extracted using CTAB method [8]. 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. PCR analysis was done using MJ Research Thermal Cycler (PTC-200 model).The primer used for PCR amplification was 16S universal primer for bacteria, the sequence for the forward primer was 5'AGAGTTTGATCCTGGCTCAG3' and reverse primer was 5'ACGGCTACCTTGTTACGACTT3' The PCR mix comprises of 1µl of 10X buffer, 0.4µl of 50mM $MgCl_2$, 0.5µl of 2.5mM dNTPs, 0.5µl 5mM Forward primer, 0.5µl of 5mM Reverse primer, 0.05µl of 5units/ul Taq with 2µl of template DNA and 5.05µl of distilled water and the PCR profile used has an initial denaturation temperature of 94^{0}C for 3mins, followed by 30 cycles of 94^{0}C for 60 seconds, 56^{0}C for 60 seconds 72^{0}C for 120 seconds and the final extension temperature of 72^{0}C for 5 minutes and the 10^{0}C hold forever. The amplicon was further purified before the sequencing using 2M Sodium Acetate washing techniques. The pellet was resuspend in 5µl sterile distilled water. The PCR mix used includes 0.5µl of BigDye Terminator Mix,1µl of 5X sequencing buffer, 1µl of 16S Forward primer with 6.5µl Distilled water and 1µl of the PCR product making a total of 10µl. The PCR profile for Sequencing is a Rapid profile, the initial Rapid thermal ramp to 96^{0}C for 1min followed by 25 cycles of Rapid thermal ramp to 96^{0}C for 10 seconds Rapid thermal ramp to 50^{0}C for 5 seconds and Rapid thermal ramp to 60^{0}C for 4 minutes, then followed by Rapid thermal ramp to 4^{0}C and hold forever. The PCR sequence product was purified before the sequencing using 2M Sodium Acetate washing techniques.The pellet was re-suspend in 5 µl sterile distilled water. The combination of 9µl of Hi Di Formamide with 1µl of Purified sequence making a total of 10µl was prepared and loaded on Applied Biosystem (AB1 3130xl model) .

Antibiotics and Sensitivity

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15.2g of Mueller Hinton Agar was dissolved in 400ml of distilled water and mixed, then autoclaved at 121°C for 15 minutes. It was later brought out and allowed to cool then it was poured on the petri-dish and allowed to solidify. The plates were later labelled and the slant isolates was introduced into the prepare Mueller hinton agar for culture in which Smearing was done to widen the occurrence of the microorganism.

The antibiotics known as motor disc antibiotics was placed on the smeared media, in order to view or observe how the organisms are been sensitive or resistance to the organism using 20 samples. It was incubated at 37°C for 18-24 hours at the end of the incubation they should be enough bacteria to form visible colonies in the areas touched by the inoculating loop, around the antibiotics and read for the zone of inhibitions.

Nucleotide Sequence and Statistical Analysis

The sequences were aligned to 16S rRNA gene sequences in the Genbank DNA database and the homology of the sequences were analyzed using Basic Local Alignment Search Tool (BLAST) program of the National Centre for Biotechnology Information (NCBI) and the statistical analysis to determine the phylogenetic tree was done using CLC software while descriptive statistics was used to analyze data on morphometric and water parameter.

RESULTS and Discussions

Morphometric Characteristics of *Clarias gariepinus*

Morphometric Characteristics of *Clarias gariepinus* from Ebute Iboro Location on Yewa River has indicated in Table 1 shows that fish samples had mean value in Weight, Standard Length, Total Length and Head Length of 645g, 42.6±0.2cm, 51.6±0.2cm and 10.6±0.1cm respectively. Table 2 shows that water parameters taken has follows; Temperature, Dissolve Oxygen and pH readings of 28.6^{o}C, 5.5ppm and 8.3 respectively.

Molecular Characterization by Gene Sequencing

The size of the amplified band with 16S universal primer was 1.6Kb for the 10 samples Plate 1, this is similar to Akinyemi and Oyelakin 2014. The blasting of the sequence for the isolates shows that there are ten types of bacteria species present. The bacteria are four *Citrobacter spp,* two *Pseudomonas species,* two *Aeromonas spp,* two *Serratia spp,* and one *Eshericha coli, Shigella boydii, Pantoea anthophila, Bacillus spp, Morganella morgani* each.

Table 4. The four isolates h ad between 89%-94% homology identity with *Citrobacter freundii*, *Pseudomonas spp* (92%-94% homology), *Serratia spp* 87%-90% homology whereas others had 87% 94% homology. (Fig. 1) showed the clustering analysis for the ten strains, *Pseudomonas flluorescens, lysinibacillus fusiformis, Pseudomonas plecoglossicid and, Pseudomonas nitroreducens* are very closely related forming one sub-group. The isolates in the second sub-group are *Pseudomonas flluorescens, Pseudomonas nitroreducens* and *Pediococcus acidilactici* while *Pseudomonas putida* and two *Pseudomonas flluorescens species* stand alone.

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The Agarose gel electrophoresis has 25kg size in plate 1; The characteristic of the 20 isolates of bacteria selected were examined for studies on the basis of their sensitivity or resistivity, to the antibiotic used, the haracteristic and taxonomic identification of the 20 bacterial isolates, the PCR sequencing the PCR has 1600bs (size) but after it was observed that 16 bacteria isolate were identified taxonomically the bacteria present in **C1** (*Aeromonas hydrophila*) from the gill, **C2** (*Pantoea anthophila*) from the skin, **C3** (*Cronobacter sakazakii*) from the gill, **C4** S*erratia marcescens*)from the gill, **C5** (*Shigella boydii*)from the gut, **C6** (*Pseudomonas sp*) from the skin, **C7** (*Citrobacter freundii*) from the skin, **C8** (*Citrobacter freundii*)skin, **C9** (*Pseudomonas gessardii*) gill,**C10** (*Citrobacter freundii*) skin, **C11** S*serratia liguefaciens*)gut, **C12** (*Citrobacter freundii*)skin, **C13** (*Esherichia coli*)skin, **C14** (*Aeromonas hydrophila*) skin, **C15** (*bacillus sp*)gut, **C16** (*Morganella morganii*)from the gills as showed in table 3.

Antibiotics Sensitivity Pattern of the Bacteria Isolates Strained from *Clarias gariepinus*

The antibiotic sensitivity test was carried out to know how susceptible or resistible the identified bacteria are to the motor disc which had 8 classes of antibiotics around it, used using disc diffusion method. The sensitivity test shows that Cefixime (CXM) and CRX were 100% resistance to the identified bacteria isolates, followed by Ceftazdime (CAZ) which is 90% resistance. As for the sensitivity test, Ofloxacin (OFL), CPR and Nitrofurantoin (NIT) were 30% sensitive to the bacteria isolate, followed by Gentamicin (GEN) which is 25%. The zone of inhibition was measured with the use of a transparent ruler, when there is a round clearing of the bacteria around it, it means the organisms is sensitive to it. But when the organisms are growing on it, it means the organisms resistant to it. When they is no round clearing around it is represented as 0 (mm) which is showed in table 5.

Table 1: Water Quality Parameters

Water Parameters	Readings
pH	8.1
Temperature	29.8°
Dissolved Oxygen	5.35ppm

Table 2: Morphometric Characteristics of Ten *C. gariepinus* in Ebute-Iboro

Sample	Weight (g)	Total length (cm)	Standard length (cm)	Head length (cm)
C1	253	40.0	35.5	9.0

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C2	364	47.0	42.3	11.0
C3	939	46.0	40.0	10.5
C4	911	48.0	41.0	10.2
C5	965	48.0	41.2	10.5
C6	806	46.5	41.0	10.0
C7	926	49.0	43.0	10.5
C8	871	48.0	42.5	11.0
C9	887	52.0	46.0	11.0
C10	934	45.5	40.0	11.0

Table 3: Occurrence Rate of Bacteria Isolate obtained from the Fish Samples.

Name of organism	No of occurrence	% Occurrence
Citrobacter spp	4	25.00
Eshericha coli	1	6.25
Pseudomonas spp	2	12.50
Shigella boydii	1	6.25
Aeromonas spp	2	12.50
Pantoea anthophila	1	6.25
Cronobacter sakaakii	1	6.25
Serratia spp	2	12.50
Bacillus spp	1	6.25

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Morganella morgani	1	6.25

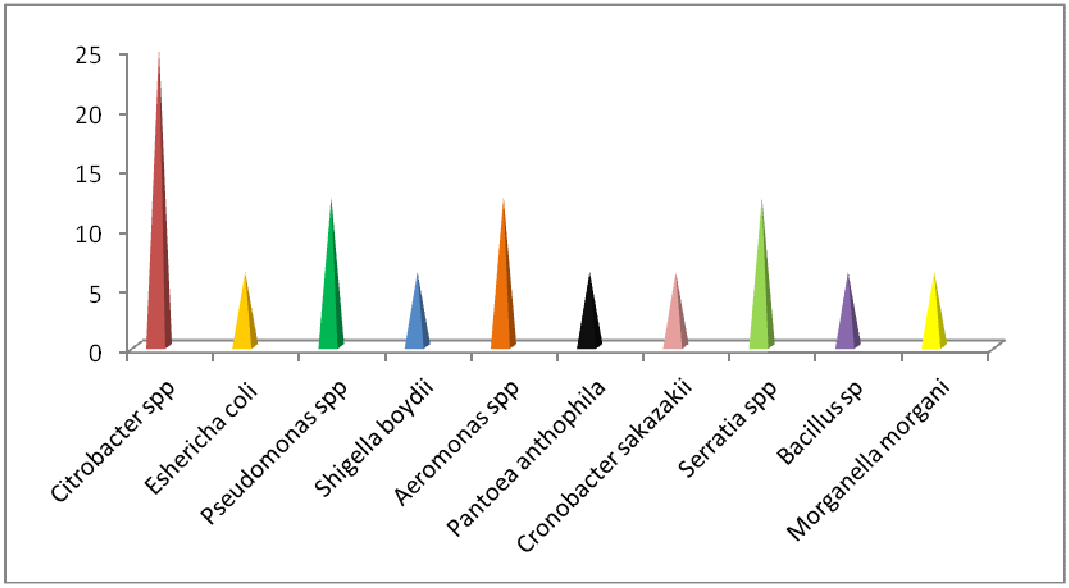

Fig 1: Percentage Occurrence rate of Bacterial Isolate obtained from *Clarias gariepinus*.

Table 4: Nearest relatives of various selected bacteria isolate

Isolates	Source	Nearest Relatives	% Homologous
C1	Gill	*Aeromonas hydrophila*	96
C2	Skin	*Pantoea anthophila*	94
C3	Skin	*Cronobacter sakazaki*	89
C4	Gill	*Serratia marcescens*	87
C5	Gut	*Shigella boydii*	87
C6	Skin	*Pseudomonas sp*	92
C7	Skin	*Citrobacter freundii*	90
C8	Skin	*Citrobacter freundii*	94

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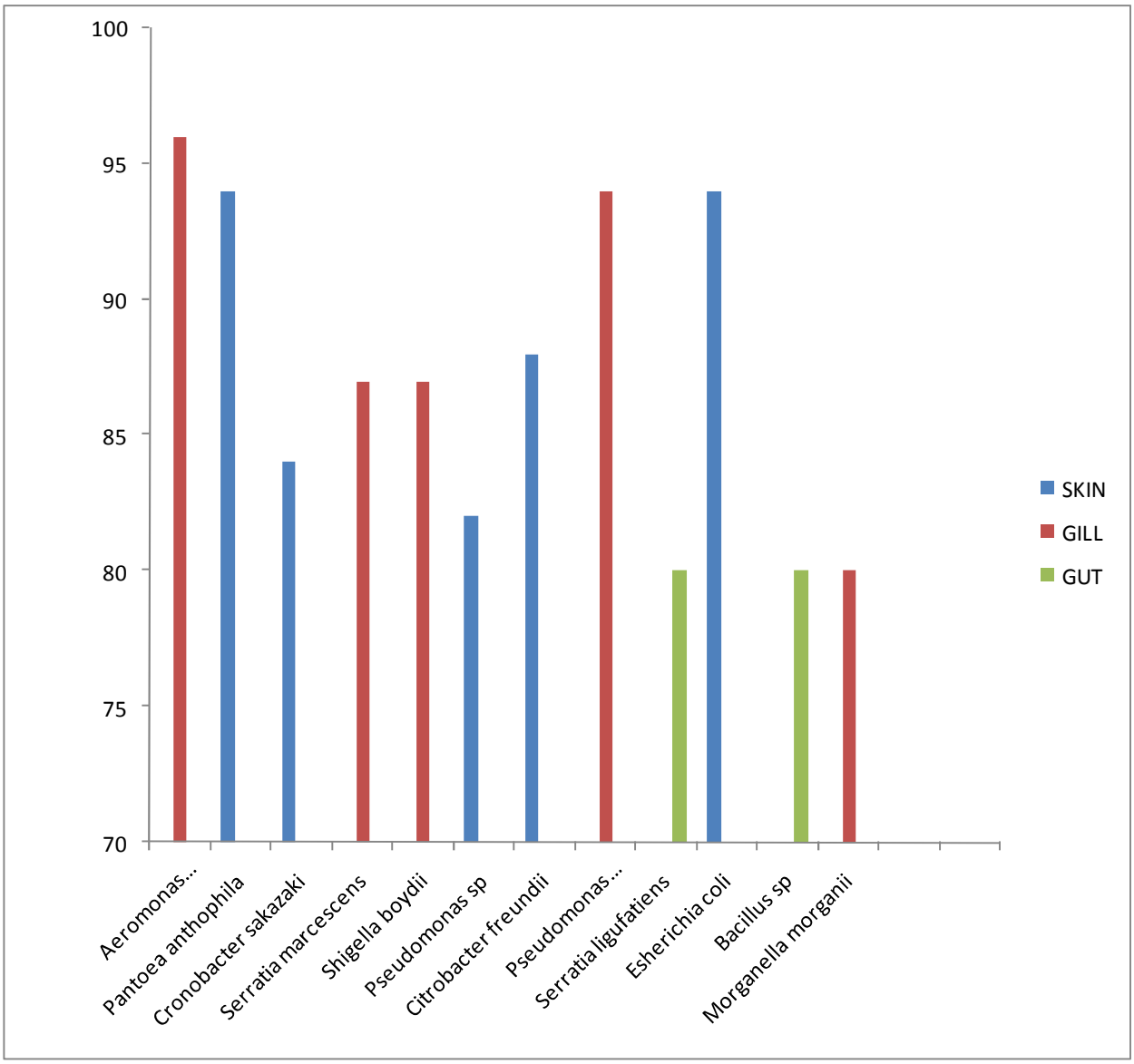
211	C9	Gill	*Pseudomonas gessardii*	94
212	C10	Skin	*Citrobacter freundii*	89
213	C11	Gut	*Serratia ligufatiens*	90
214	C12	Skin	*Citrobacter freundii*	90
215	C13	Skin	*Esherichia coli*	94
216	C14	Skin	*Citrobacter freundii*	93
217	C15	Gut	*Bacillus spp*	90
218	C16	Gill	*Morganella morganii*	90



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220 **Fig 2:** Nearest relatives of various selected bacteria isolate of the Substrate source

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222 **Table 5: Zone of inhibition of the bacteria isolate stained from *Clarias gariepinus***

bacterial isolates	OFL	AUG	NIT	CPR	CAZ	CRX	GEN	CXM
C1	S	R	R	S	I	R	R	R
C2	S	R	I	S	R	R	S	R
C3	R	R	R	R	R	R	R	R
C4	S	R	S	S	R	R	S	R
C5	I	R	S	I	R	R	R	R
C6	R	R	R	R	R	R	R	R
C7	S	R	S	S	R	R	S	R
C8	R	R	R	R	R	R	R	R
C9	R	R	R	R	R	R	R	R
C10	R	R	R	R	R	R	R	R
C11	R	R	R	R	R	R	R	R
C12	R	R	R	R	R	R	R	R
C13	R	R	R	R	R	R	R	R
C14	R	R	R	R	R	R	R	R
C15	R	R	R	R	R	R	R	R
C16	R	R	S	R	R	R	R	R
C17	R	R	I	S	R	R	I	R
C18	S	R	R	R	R	R	R	R
C19	S	S	S	S	S	R	S	R
C20	R	R	S	I	R	R	S	R

223 **S = Sensitivity 16 and above, I= intermediate 9-15, R= Resistance 0-8**

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NOTE: A motor disc antibiotic has 8 different classes of antibiotics incorporated with it. Listed below are the antibiotics:
Ceftazdime(CAZ)30mg,Cefuroxime(CRX)30mg,Gentamicin(GEN)10mg,Cefixime(CXM)5mg,Ofloxacin(OFL)5mg,Augumentin(AUG)30mg,Nitrofurantoin(NIT)300mg,Ciprofloxacin(CPR)mg

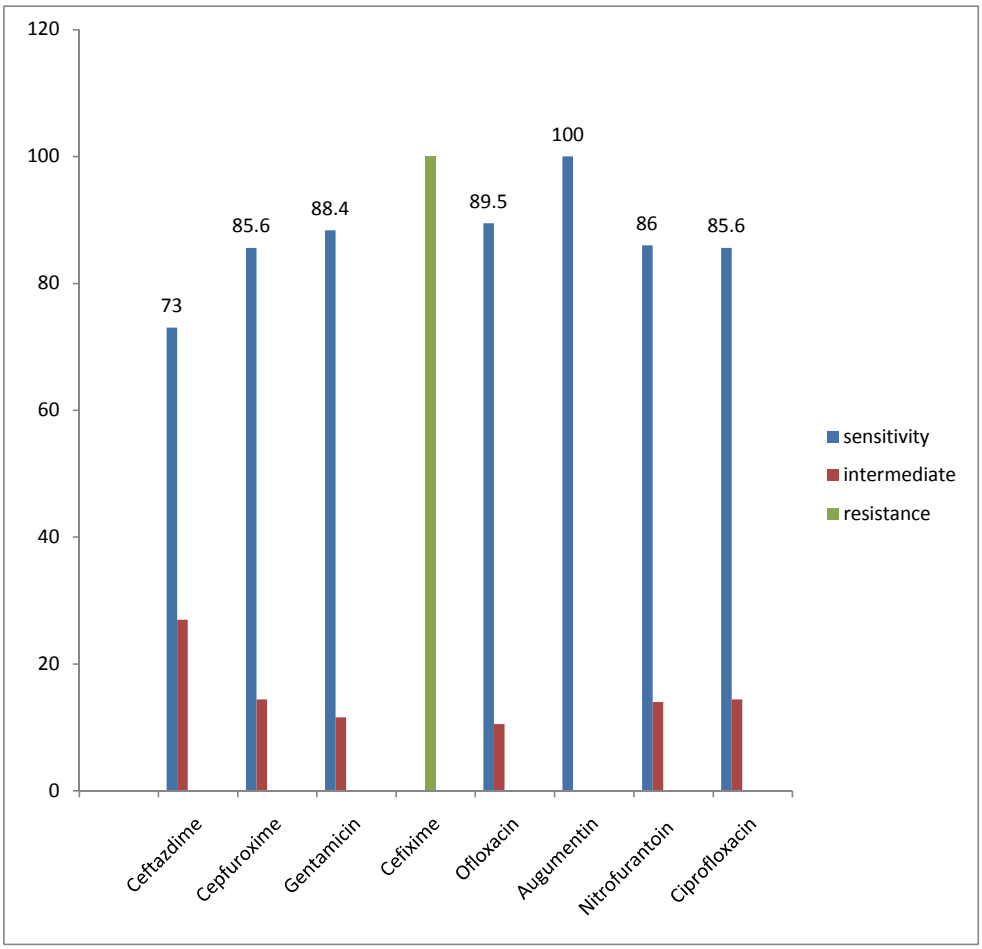

Fig 3: Sensitivity and Zone of inhibition of the bacteria isolate stained from *Clarias gariepinus*

Table 6: Anti microbial percentage sensitivity and resistance of the isolate to antibiotics

	Sensitivity		Intermediate		Resistance	
	N	%	N	%	N	%

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OFL	6	30.0	1	5.0	13	65.0
AUG	1	5.0	0	0.0	19	95.0
NIT	6	30.0	2	10.0	12	60.0
CPR	6	30.0	2	10.0	12	60.0
CAZ	1	5.0	1	5.0	18	90.0
CRX	0	0.0	0	0.00	20	100.0
GEN	5	25.0	1	5.0	14	70.0
CXM	0	0.00	0	0.00	20	100.0

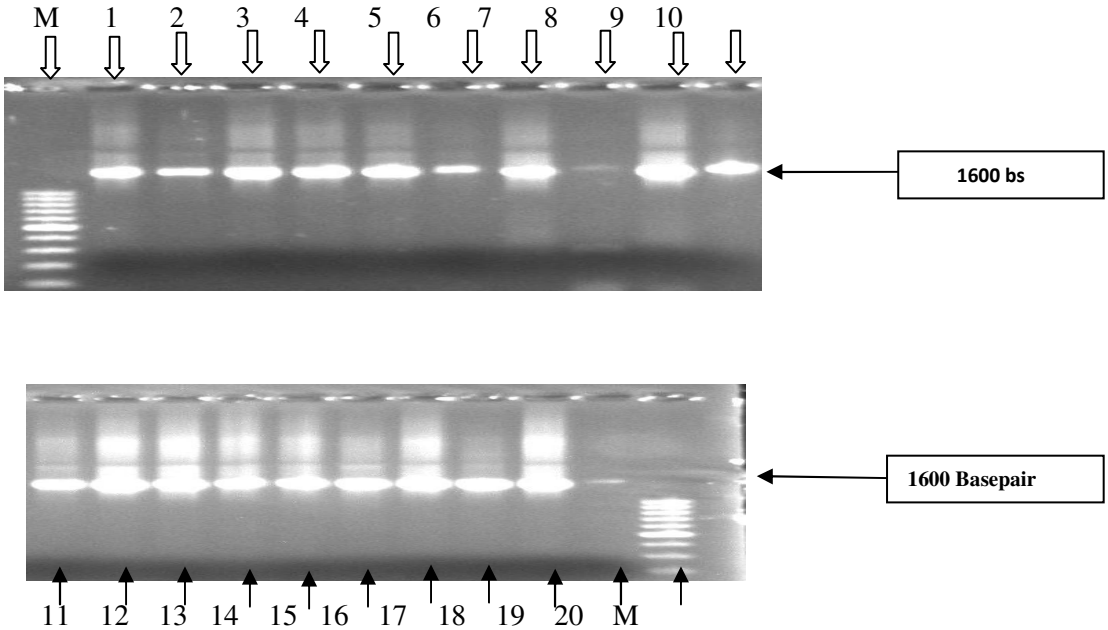

Plate 1: Gel electrophoresis for the 16S primer

Discussion

The various water quality parameters recorded at the river in which it is favorable for fish growth and culture except the pH which is read 8.1 which is little bit high and still favourable for fish culture and growth and the parameters fell within the standard range that has already been documented. The temperature of 29.8^{O}C was

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recorded and with this range it was supported and recorded by Hassan (1974) , Ugwumba (1993) and Boyd (1979) for the range of temperature.

The pH value which is recorded in this is high due to the location that is near to brackish water but the fish were able to survive and breed in this environment in which there would have been mixture during the process, pH value of 6.5 to 9.0 is documented and agreed upon by Swingle (1961) and Boyd(1985) as value most suitable for fish production and maximum growth.

Table 2 shows the morphometric characteristics of the 10 *clarias gariepinus* gotten from Ebute Igbo-oro River, the total length falls within the range of 40-52cm and the standard length falls within the range of 35.5-46cm while the head length falls within the range of 9.0-11.00. The weight in grams ranges from 252-965. Due to the bacterial infections the head length varies in the smaller fish compare to the bigger one.

The bacteria isolate (16 isolates*) Citrobacter freundii* (4 *isolate), Pseudomomonas sp* (2 *isolate*),*Aeromonas hydrophila*(2 *isolate*), *Serratiasp* (2 *isolate*) while the rest *Pantoeanthopilla, Cronobacter sakazakii, Sigella boydii,Esherichia coli, Bacillus sp, and Morganella marganii* has single occurrence on the isolate. It indicates that bacteria belong to *Citrobacter* and *Pseudomonas* group were more prevalent in healthy *C.gariepinus* of the water body. This finding was in line with those of *vershuere et a.,l* (2000), Balcazar *et al.,(*2006) and Geovanny *et al.,(*2007) in which several bacteria were identified and can be considered as bacillus, *Pseudomonas, Morganella morganii*, in which the dommance of all is *Cirtrobacter freudii*

The result after BLAST on NCBI reveals that the selected isolate of bacteria has their homology(%),C1 from the gill has *Aeromonas hydrophilla* (KJ806459.1) with 96%, C2 from the skin *Pantoea anthphila* (JX993784.1) with 94%, C3 from the skin *Cronobacter sakazakii*(HQ880374.1)89%. C4 from the gill *Serratia marcescens* (JQ315926.1)87%. C5 from the gut *Shigella boydii* (JQ315926.1) 87%. C6 from the skin *Pseudomonas sp*(KJ534480.1)82%. C7 from the skin *Citrobacter freundii* (DQ294287.1) 90%.C8 from the skin *Citrobacter freundii*(AB680434.1) 94%.C9 from the gill *Pseudomonas gessardii* (KJ726608.1) 94%. C10 from the skin *Citrobacter freundii* (JQ7958145.1) 89%

C11 from the gut *Serratia liguefaciense* (Gu586145.1) 80%. C12 from the skin *Citrobacter freundii* (KC2108467.1) 80%.C13 from the skin *Eshericha coli* (KC165787.1) 94%. C14 from the skin *Aeromonas hydrophila* (AB473035.1). C15 from the gut *Bacillus sp* (AY626828.1) 80%. C16 from the gill *Morganella morganii* (JX875917.1) 82%

The finding was in line with that of Tubagus *et al.,* (2009) where the 17 bacteria selected belong to Bacillus spp and their homolegy(%) ranges from 96-99% with their various accession numbers

In the study, it was observed that ofloxacin(5mg), Nitrofurantion (300mg) ciprofloxacin(5mg) are antibiotics on which the bacteria isolate present on the skin associated with *Clarias gariepenus* are highly sensitive and can be used to combart or treat them.

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It was observed that C1 *Aeromonas hydrophila,* C2 *Pantoae anthophila*, C4 *Serratia marcescens*, C7 *Citrobacter freundii*, is 30.0% Sensitive to the antibiotics oflxacin -5mg and C4 *Serratia marcescens*,C5 *Shigella boydii*, C7 *Citrobacter freundii*, C16*Morganellamorganii* are also 30% sensitive to nitrofurantion -300mg antibiotic used, C1 *Aeromonas hydrophila,* C2 pantoea anthophila, C4 *serratia marcescens*,C7 *citrobacter freundii* arise also 30% sensitive to ciprofloxacin(5mg) antibiotics used. Followed by gentamicin long antibiotics which are partially sensitive to C2 *Pantoea anthophila*,C4 *Serratia marcescens,* C7 *Citrobacter freundii*. None of the bacteria isolate were observed to be sensitive to CXM cefixine(5mg) and cefuroxime (30mg) which means they are highly resistible to it and its percentage resistance is 100%

All the bacteria isolate were observed from table- to be resistance to (CXM) and (CFR) at 100% followed by Augmentin which is 95% resistance which means the above antibiotics cannot be used to supress the bacteria

CONCLUSION

This work provided information on the BLASTING of gene sequence of the bacteria isolate from the gill, gut and skin of samples swabbed from a raised *clarias gariepius* in Ebute-igbo-oro landing site of Yewa river. Hence these studies confirm the existence of different species levels of bacteria identified from the skin, gills and gut of ten (16) bacteria isolate. After BLASTING to the species level using genetic method to identify it, the finding confirm C1 from the gill has *Aeromonas hydrophilla,*C2 from the skin *Pantoea anthphila*, C3 from the skin *Cronobacter sakazakii*, C4 from the gill *Serratia marcescens*, C5 from the gut *Shigella boydii*, C6 from the skin *Pseudomonas sp*, C7 from the skin *Citrobacter freundii*, C8 from the skin *Citrobacter freundii* , C9 from the gill *Pseudomonas gessardii*, C10 from the *skin Citrobacter freundii*,

C11 from the gut *serratia liguefaciense*, C12 from the skin *Citrobacter freundii*, C13 from the skin *Eshericha coli*,C14 from the skin *Aeromona hydrophila*, C15 from the gut *Bacillus sp*, C16 from the gill *Morganella morganii* only from samples swabbed from a *raised clarias gariepinus* at Ebute-igbo-oro landing site of Yewa river of which may be different in another place due to the filter effect of the gill, feeding habbit or the slime layer of the skin and its sensitivity.

REFERENCES

Adeleye, O.A (1992): Conservation needs of fisheries resources for sustainable captive practices. Proceedings of the 10thannualcomference of the Fisheries Society of Nigeria. Pp. 230-234.

Akinyemi, A. A. and O. O. Oyelakin, (2014). Molecular Characterization of Bacteria Isolates from Farm-Raised Catfish *Clarias gariepinus* (Burchell,1882)Department of Aquaculture and Fisheries Management, Biotechnology Centre, Federal University of Agriculture, Abeokuta, Nigeria.

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Balcazar J.L., Blas I de, Zarzuela I.R., Cunningham D., Vendrell D., Muszquiz J.L. (2006).The role of Probiotics in Aquaculture.*Vet Microbial* 144:173-86.

Boyd; C.E (1979).Water Quality in warm water fish ponds.Anburn University, Alabama. 359p.

Boyd; C.E. and Lichtkoppler .F.R (1995). Water Quality Management in pond fish culture Res.Dev.Ser.: 22 Auburn University Auburn Alabama.

Ezeri, G.N.O., (2001). Haematological response of clariasgariepinus to bacteria infection and prophylactic treatment. With antibiotcs*, journal of Aquartic Science*; 16:22-24.

GeovanyGR,Luis BJ, Shen MA. (2007). Probiotics as control agents in Aquaculture.A review. J Ocean University haina 6: 76-9.

Hassan, A.T. (1974). Studies on the ecology behaviour and life history of Libelliulinedradonflies Ph.D. Thesis, University of Ibadan, Ibadan.Pp.122.

Ladipo, O; Fabiyi and Fatula, G.T. (1981), Marketing and distribution of fish in Nigeria. Report submitted to the federal development of fisheries, Lagos. Pg 35

Leisner. J.J., Vancanneyt, M., Rusul, G., Pot, B. Lefebvre, K., Fresi, A. and Tee, L.T (2001). Identification of lactic acid bacteria constituting the predom, nating microflora in an acid feamentedcondiment (tempoyak) popular in Malaysia. *International Journal of food microbio* 63: 147-157.

Swingle, H.S (1961). Method of analysis for waters organic matter and pond bottom soils used in Fisheries Research. Auburn University, Auburn Ala 119p.

Tubagas H.R., Indrawati G., Etty R., Lin S.D and Wellyzar S. (2009). Identification and phylogenetic analysis of bacterial isolates from *Litopenaeusvannamei*shrimp culture system and gut environmental based on 16S rRNA gene sequencing data. 3(2): 56-60.

Ugwumba, A.O. and Ugwumba, A.A (1993).A study of the physcio-chemical hydrology and plankton of Awba Lake in Ibadan, Nigeria.FishAcadbiz. Comm.1 (1-4): 20-39.

Verschurere L, Rombaut G, Sorgeloos P, Verstraete W. (2000). Probiotics bacteria as biologica control agents in Aquaculture. *Microbiol MolBiol Rev*. 646:55-71.