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Isolation and Characterization of Bacteria from Sub-Adult African Mud Catfish (*Clarias gariepinus*) from Selected Fish Farms in Ogun State, Nigeria

[1]F.A. Awe, [1]A.M. Hammed, [1]O.M. Olarinmoye, [1]F.G. Owodeinde, [1]O.A. Adeboyejo, [1]E.O. Clarke, [1]O.O. Whenu, [2]A.A. Akinyemi, [2]G.N.O. Ezeri, [3]M.O. Bankole and [4]A.O. Olanloye
[1]*Department of Fisheries, Faculty of Science, Lagos State University, Ojo, Lagos, Nigeria*
[2]*Department of Aquaculture and Fisheries Management,*
[3]*Department of Microbiology,*
[4]*Department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria*

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Corresponding Author:
F.A. Awe
Department of Fisheries, Faculty of Science, Lagos State University, Ojo, Lagos, Nigeria

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Abstract: The need for proper identification of bacterial organisms from catfish cannot be overemphasized, hence, it provides update information on emerging and existing organisms thereby enriching the gene bank on fish disease. Catfish samples were collected from Elegbeji, Sanni, Kunle, Johnson, Adewale and Awosanya fish Farms and taken to the Microbiology laboratory, Federal University of Agriculture, Abeokuta, Ogun State for analyses and samples were collected from flesh, gills and intestine were subjected to microbial examination for colonial, Morphological characteristic, Biochemical tests and Molecular tests. Bio Edit was used for importing and mining nucleotides sequences into Gene Bank. The results revealed the following bacterial organisms: fish farms (1 and 2) *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aeromonas veronii*, *Bacillus subtilis* were identified from the skin and Gills while *Enterococcus feacium* was also identified from the intestines. At farms (3 and 4), the Major bacterial organisms identified from skin, gills and Intestines of the fish were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aeromonas veronii*, *Bacillus subtilis* while farms (5 and 6) had *Enterococcus feacium* and *Streptococcus agalactiae*. The BLAST result is a confirmation of the bio-chemical test earlier carried out with percentage similarity ranging from 78-98% and their accession number, the bacterial organisms identified were *Aeromonas veronii*, *Enterococcus feacium*, *Pseudomonas aeruginosa*, *Bacteria subtilis*, *Staphylococcus aureus*, *Streptococcus agalactiae*. The bacterial organisms were present on the fish but not invasive and only become dangerous if conditions necessary for disease initiation are present such as susceptible host, virulent pathogen and favourable environment are

present at the same time. Therefore, preventive medicine is the solution for fish farm management but additional bacterium *Aeromonas veronii* was detected and need to be added into Nigeria Gene bank data for catfish.

INTRODUCTION

Aquaculture has the potential to address the gap between aquatic food demand and supply and to help countries achieve their economic, social and environmental goals, thus, contributing to the 2030 Agenda[1, 2].

World aquaculture production is highly predisposed to disease occurrence that might affect part or outright loss of fish and farmers income[3]. The susceptibility of fish to disease outbreak and infection may be caused by overcrowding, periodic handling, high or sudden changes in temperature, poor water quality and poor nutritional status. Poor sanitation in an intensive aquaculture may be a source of introduction of pathogens resulting in fish death[4-6].

At improving delicate and specificity of pathogen discovery, molecular methods could be used for such situation. The molecular techniques include array of Polymerase Chain Reaction (PCR), restriction enzyme digestion, probe hybridization, microarray and *in situ* hybridization. In preventing diseases outbreak, pathogens can be recognized from asymptomatic fish, therefore, molecular diagnostic methods are better because they are quick and sensitive to detect fish disease than the conventional diagnostic techniques. Since molecular diagnostic techniques are faster and more sensitive than conventional diagnostic techniques, pathogens can be detected from asymptomatic fish, so, disease outbreak could be prevented.

Advancements have been made at tailoring the sensitivity and specificity of identifying bacterial, viral and parasitic diseases of fish. First, DNA is removed from sample and further probed by DNA hybridization and analysed by Restriction Fragment Length Polymorphism (RFLP), this is how molecular techniques operates. In particular, DNA is enlarged by the Polymerase Chain Reaction (PCR) with the aid of specific primers for diagnostic sequences. Next to this is RFLP, PCR linked to hybridization with specific oligoprobes or non-specific primers used to get random amplified polymorphic DNA (RAPD)[7, 8].

Polymerase chain reaction: PCR was rst invented by Saiki *et al*.[9] Polymerase chain reaction is a technique for amplifying a specific region of DNA, defined by a set of two "primers" at which DNA synthesis is initiated by a thermostable DNA polymerase. Normally, a minimum of a million-fold increase of a specific section of a DNA molecule can be generated and the PCR product can be discovered by gel electrophoresis. The base pairs (bp) in length is normally between 150-3,000 of the regions magnified[10]. Primer design is important to obtain greatest possible sensitivity and specificity. Therefore, the primers should be sufficiently long to allow a high annealing temperature and reduce the opportunity for non specific primer annealing, but primers that are too long may facilitate nonspecific annealing even to regions of DNA that are not perfectly complementary to the primer sequence. In forming a new copies, the template DNA that may be in several forms, starting from a simple tissue lysate to purified DNA, primers, polymerase enzyme to catalyze creation. During each round of the thermocycling reaction, the template DNA is denatured, primers anneal to their complementary regions and polymerase enzyme catalyses the addition of nucleotides to the end of each primer, thus creating new copies of the target region in each round. According to ideal or assumed set of facts, the increase in quantity of product after each round will be in maximal multiplication or geometric.

The use of rRNA genes in PCR assays is enormous as it had been used for *Renibacterium salmoninarum*[11], *Aeromonas salmonicida*[12] and *Yersinia ruckeri*[13]. For important disease causing agent that are not listed in European Community legislation but. These same techniques are used in their detection and study of significant pathogens like *Vibrio anguillarum*[14], *Lactococcus garvieae*[15], *Piscirickettsia salmonis*[16], *Flexibacter*[17], *Flavobacterium*[18], *Photobacterium*[19] and *Mycobacterium*[20].

MATERIALS AND METHODS

Preparation of broth culture: A 0.8 g of nutrient broth was dissolved in 100 mL of distilled water in a conical flask and covered with foil paper. The broth was placed in an autoclave to sterilize it for 15 min at 121°C. After sterilization, the flask was allowed to cool and 5 mL of nutrient broth was aseptically pipetted into sterile labeled McCartney bottles. Each bacterium isolate was transferred into specified McCartney bottles labeled with 9ml of nutrient broth with the aid of a sterile inoculating wire loop; the broth culture was then incubated at 37°C for 24 h. The culture was diluted until the final suspension obtained was 1.0×10 cfu mL^{-1}. This was done at the Biotechnology Laboratory, Federal University of Agriculture, Abeokuta, Ogun State.

Extraction of DNA using Ctab method: Genomic DNA extraction, PCR-mediated amplification of the 16SrRNA gene fragments and sequencing of PCR products were carried out as described by Akinyemi and Oyelakin. Bacteria isolates grown overnight were transferred to eppendorf tube and it was spun down at 14.000 rpm for 2 min, the supernatant was discarded and 600 µL of 2X CTAB buffer was added to the pellet and it was incubated at 65°C for 20 min. The sample was removed from the incubator and allowed to cool to room temperature and chloroform was added, the sample was mixed by gently inversion of the tube several times. Thereafter, the sample was spun at 14.000 rpm for 15 min and the supernatant was transferred into a new eppendorf tube and equal volume of cold Isopropanol was added to precipitate the DNA. The sample was kept in the freezer for 1 h and later spun at 14.000 rpm for 10 min and the supernatant was discarded and the pellet was washed with 70% ethanol later the sample was air dried for 30 min on the bench. The pellet was resuspended in 100 µL of sterile distilled water. DNA concentration of the samples was measured on spectrophotometer at 260 and 280 nm and the genomic purity were determined. The genomic purity was between 1.22-6.47 for all the DNA samples.

DNA electrophoresis: Agarose gel electrophoresis was used to determine the quality and integrity of the DNA by size fractionation on 1.0% agarose gels. Agarose gels were prepared by dissolving and boiling 1.0 g agarose in 100 mL 0.5×TBE buffer solutions. The gels were allowed to cool down to about 45°C and 10 µL of 5 mg mL^{-1} ethidium bromide was added, mixed together before pouring it into an electrophoresis chamber set with the combs inserted. After the gel has solidified, 3 µL of the DNA with 5 µL sterile distilled water and 2 µL of 6X loading dye was mixed together and loaded in the well created. Electrophoresis was done at 80 V for 2 h. The integrity of the DNA was visualized and photographed on UV light source.

Dilution of DNA and primer screening: About 10 µL of each DNA was taken into eppendorf tube and 990 µL sterile distilled water was added to make 1000 µL. The final concentration became 20-50 ng μL^{-1}. Seven DNA samples were selected randomly for primer screening. Twenty RAPD primers were used for the screening. Ten polymorphic primers were later used for the whole samples.

PCR analysis using I6S primer: PCR analysis was run with a universal primer for bacteria called 16S. The PCR mix comprises of 1 µL of 10X PCR buffer, 1.0 µL of 25 mM $MgCl_{2,}$ 0.8 µL of 2.5 mMdNTPs, 0.5 µL 5p Mol Forward primer, 0.5 µL of 5 pMol Reverse primer, 0.1 µL of 5 units/µL Taq with 2 µL of 10 ng μL^{-1} template DNA and 3.1 μL^{-1} of distilled water to make-up 10 µL reaction mix.

The 16sF is 27F and the 16sR is 1525R: The PCR profile used is initial denaturation temperature of 94°C for 5 min, followed by 36 cycles of 94°C for 30 sec, 56°C for 30 sec, 72°C for 45 sec and the final extension temperature of 72°C for 7 min and the 10°C hold forever The amplicon from the reaction above was loaded on 1.5% agarose gel and the gel picture is attached as PCR. The PCR was purified with the following protocol.

Purification of PCR products: The amplicon is further purified before the sequencing using 2M Sodium Acetate wash techniques. To about 10 µL of the PCR product, add 1 µL 2M NaAct pH 5.2, followed by 20 µL Absolute Ethanol, incubated at room temperature for 15 min, spin at 10,000 rpm for 15 min, then wash with 2 vol (40 uL) 70% ethanol and air dried. Re-suspended in 10 uL of ultrapure water and keep at 4°C for sequencing.

PCR for sequencing: The primer used for the reaction was forward I6S. The PCR mix used includes 2.5 µL of BigDye Terminator Mix, 2 µL of 5X sequencing buffer, 3.2 pMol forward primer with 20 µL distilled water and 1 µL of the PCR product making a total of 20 µL, The sequence for the forward primer was 27F: AGAGTTTGATCMTGGCTCAG and reverse primer was 1525R: AAGGAGGTGWTCCARCCGCA. The PCR profile for Sequencing is a Rapid profile, the initial Rapid thermal ramp to 96°C for 1min followed by 25 cycles of Rapid thermal ramp to 96°C for 10 sec Rapid thermal ramp to 50°C for 5 sec and Rapid thermal ramp to 60°C for 4 min, then followed by Rapid thermal ramp to 4°C and hold forever.

Purification of PCR sequencing products: The PCR sequence product was also purified before the sequencing running using 2M Sodium Acetate wash techniques. To 10 µL of the PCR product were added 1 µL 2M NaAct pH 5.2, then another 20 µL Absolute Ethanol was added and kept at room temperature for 15 min, spin at 10,000 rpm for 15 min, then wash with 2 vol (40 uL) 70% ethanol and air dried. It was re-suspended in 10 uL of ultrapure water and kept at 4°C for sequencing running.

Preparation of sample for Gene Sequencer (ABI 3130xl machine): The Cocktail mix is a combination of 9 µL of Hi Di Formide with 1 µL of Purified sequence to make a total of 10 µL. The samples were loaded on the machine and the data in form A, C, T and G were released.

Statistical analysis: The analysis of data was done following One Way Analysis of Variance (ANOVA) using SPSS Version 21 and Duncan's Multiple Range Test. Bio Edit used for importing and mining nucleotides sequences into Gene Bank, blasting of the sequences were carried out on NCBI website.

RESULTS

Suspected bacterial organisms from biochemical reactions on isolates from *Clarias gariepinus* from different fish farms: The Major bacterial organisms identified from flesh, gills and intestines of the fish were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aeromonas veronii*, *Bacillus subtilis*, *Enterococcus feacium* and *Streptococcus agalactiae* from fish farms 1 and 2 (Table 1), this was after reactions from biochemical tests of catalase, starch hydrolysis, coagulase, citrate utilization, indole, oxidase and sugar fermentation. Staphylococci were catalase, gelatin hydrolysis, coagulase and sugar fermentation positive. Both *Enterococcus feacium* and *Staphylococcus aureus* were non motile; *Pseudomonas aeruginosa*, *Aeromonas veronii*, *Enterococcus feacium* were Gram negative bacteria while *Staphylococcus aureus*, *Streptococcus agalactiae*, *Bacillus subtilis* were Gram positive bacteria. The following suspected bacterial organisms, *Staphylococcus aureus, Pseudomonas aeruginosa, Aeromonas veronii* and *Enterococcus feacium*, *Bacillus subtilis* were identified from the flesh and Gills while *Enterococcus feacium* was also identified from the intestines of the fish in addition to bacterial from flesh and gills from Farms 3 and 4 (Table 2).

In addition, the Major bacterial organisms identified from flesh, gills and Intestines of the fish were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aeromonas veronii*, *Bacillus subtilis*, *Enterococcus feacium* and *Streptococcus agalactiae* from farms 5 and 6 (Table 3).

Blast results of bacteria isolated from catfish *Clarias gariepinus* from the selected farms: The BLAST results confirmed the bio-chemical test earlier carried out on percentage similarity ranging from 78-98% and their accession number, the bacteria identified from different parts of the sampled fish were *Aeromonas veronii*, *Enterococcus feacium*, *Pseudomonas aeruginosa*, *Bacteria subtilis*, *Staphylococcus aureus*, *Streptococcus agalactiae* (Table 4 and Fig. 1-3).

Table 1: Biochemical reaction of bacterial isolates from *Clarias gariepinus* from fish farms 1 and 2

Isolate Organs	Gram	Shape and arrangement	Catalase production	Gelatin hydrolysis	Starch hydrolysis	Coagulase production	Motility	Citrate utilization	Indole production	Oxidase production	Sugar fermentation test					Suspected organism
											Glucose	Sucrose	Mannitol	Maltose	Lactose	
FL 1	+	Cocci; clustered	+	+	-	+	-	-	-	-	+	+	-	-	-	*Staphylococcus aureus*
FL 2	+	Cocci; clustered	+	+	-	+	-	-	-	-	+	+	-	-	-	*Staphylococcus aureus*
FL 3	-	Bacilli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
FL 4	-	Bacilli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
FL 5	-	Bacilli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
FL 6	-	Bacilli	+	+	-	-	+	+	+	+	+	+	+	+	-	*Aeromonas veronii*
FL 7	+	Bacilli	+	+	+	-	+	+	-	-	+	-	+	+	-	*Bacillus subtilis*
FL 8	-	Bacilli	+	+	-	-	+	+	+	+	+	+	+	+	-	*Aeromonas veronii*
FL 9	-	Bacilli	+	+	-	-	+	+	+	+	+	+	+	+	-	*Aeromonas veronii*
G1	+	Bacilli	+	+	+	-	+	+	-	-	+	-	+	+	-	*Bacillus subtilis*
2	+	Cocci	+	+	-	+	-	-	-	-	+	+	-	-	-	*Staphylococcus aureus*
3	+	Bacilli	+	+	+	-	+	+	-	-	+	-	+	+	-	*Bacillus subtilis*
4	+	Cocci; clustered	+	+	-	+	-	-	-	-	+	+	-	-	-	*Staphylococcus aureus*
5	-	Bacilli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
6	-	Bacilli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
7	-	Bacilli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
8	+	Cocci; clustered	+	+	-	+	-	-	-	-	+	+	-	-	-	*Staphylococcus aureus*
Int1	-	Bacilli	+	+	-	-	+	+	+	+	+	+	+	+	-	*Aeromonas veronii*
2	+	Cocci; clustered	+	+	-	+	-	-	-	-	+	+	-	-	-	*Staphylococcus aureus*
3	-	Bacilli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
4	+	Cocci	-	+	+	-	-	-	-	-	+	-	-	+	+	*Enterococcus feacium*
5	-	Bacilli	+	+	-	-	+	+	+	+	+	+	+	+	-	*Aeromonas veronii*
6	+	Cocci	-	+	+	-	-	-	-	-	+	-	-	+	+	*Enterococcus feacium*
7	+	Bacilli	+	+	+	-	+	+	-	-	+	-	+	+	-	*Bacillus subtilis*
8	-	Bacilli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
9	-	Bacilli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
10	-	Bacilli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
11	-	Bacilli	+	+	-	-	+	+	+	+	+	+	+	+	-	*Aeromonas veronii*
12	-	Bacilli	+	+	-	-	+	+	+	+	+	+	+	+	-	*Aeromonas veronii*
13	0	Cocci	-	+	+	-	-	-	-	-	+	-	-	+	+	*Enterococcus feacium*
14	-	Bacilli	+	+	-	-	+	+	+	+	+	+	+	+	-	*Aeromonas veronii*

Table 2: Biochemical reaction of bacterial isolates from *Clarias gariepinus* from fish farms 3 and 4

Isolate No	Gram	Shape and arrangement	Catalase production	Gelatin hydrolysis	Starch hydrolysis	Coagulase production	Motility	Citrate utilization	Indole production	Oxidase production	Sugar fermentation test					Suspected organism
											Glucose	Sucrose	Mannitol	Maltose	Lactose	
2 FL 1	-	Bacilli	+	+	-	-	+	+	+	+	+	+	+	+	-	*Aeromonas veronii*
2	-	Bacilli	+	+	-	-	+	+	+	+	+	+	+	+	-	*Aeromonas veronii*
3	-	Baccli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
4	-	Bacilli	+	+	-	-	+	+	+	+	+	+	+	+	-	*Aeromonas veronii*
5	+	Bacilli	+	+	+	-	+	+	-	-	+	-	+	+	-	*Baccillus subtilis*
6	+	Cocci; clustered	+	+	-	+	-	-	-	-	+	+	-	-	-	*Staphylococcus aureus*
7	-	Baccli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
2Gl1	-	Bacilli	+	+	-	-	+	+	+	+	+	+	+	+	-	*Aeromonas veronii*
2	-	Baccli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
3	-	Baccli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
4	+	Cocci; clustered	+	+	-	+	-	-	-	-	+	+	-	-	-	*Staphylococcus* aureus
5	-	Baccli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
6	+	Bacilli	+	+	+	-	+	+	-	-	+	-	+	+	-	*Baccillus subtilis*
2Is1	+	Cocci	-	-	-	-	-	-	-	-	+	-	-	+	+	*Streptococcus agalactiae*
2	+	Cocci	+	+	-	+	-	-	-	-	+	+	-	-	-	*Staphylococcus aureus*
3	-	Bacilli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
4	-	Bacilli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
5	-	Baccli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
6	+	Cocci	-	+	+	-	-	-	-	-	+	-	-	+	+	*Enterococcus feacium*
7	-	Bacilli	+	+	-	-	+	+	+	+	+	+	+	+	-	*Aeromonas veronii*
8	+	Cocci	-	+	+	-	-	-	-	-	+	-	-	+	+	*Enterococcus feacium*

Table 3: Biochemical reaction of bacterial isolates from *Clarias gariepinus* from fish farms 5 and 6

Isolate No	Gram	Shape and arrangement	Catalase production	Gelatin hydrolysis	Starch hydrolysis	Coagulase production	Motility	Citrate utilization	Indole production	Oxidase production	Sugar fermentation test					Suspected organism
											Glucose	Sucrose	Mannitol	Maltose	Lactose	
3 FL 1	-	Bacilli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
2	+	Cocci	+	+	-	+	-	-	-	-	+	+	-	-	-	*Staphylococcus aureus*
3	-	Bacilli	+	+	-	-	+	+	+	+	+	+	+	+	-	*Aeromonas veroni*3Gl1
	-	Bacilli	+	+	-	-	+	+	+	+	+	+	+	+	-	*Aeromonas veronii*
2	-	Bacilli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
3	+	Bacilli	+	+	+	-	+	+	-	-	+	-	+	+	-	*Baccillus subtilis*
4	+	Cocci	+	+	-	+	-	-	-	-	+	+	-	-	-	*Staphylococcus aureus*
5	+	Bacilli	+	+	+	-	+	+	-	-	+	-	+	+	-	*Baccillus subtilis*
6	-	Baccli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
7	+	Cocci	+	+	-	+	-	-	-	-	+	+	-	-	-	*Staphylococcus aureus*
8	-	Baccli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
9	+	Cocci	+	+	-	+	-	-	-	-	+	+	-	-	-	*Staphylococcus aureus*
10	-	Baccli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
11	+	Bacilli	+	+	+	-	+	+	-	-	+	-	+	+	-	*Baccillus subtilis*12
	-	Baccli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
13	+	Cocci; clustered	+	+	-	+	-	-	-	-	+	+	-	-	-	*Staphylococcus aureus*
14	+	Cocci; clustered	+	+	-	+	-	-	-	-	+	+	-	-	-	*Staphylococcus aureus*
15	-	Baccli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
3Is1	-	Baccli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
2	-	Bacilli	+	+	-	-	+	+	+	+	+	+	+	+	-	*Aeromonas veronii*3
	-	Baccli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
4	+	Cocci	-	+	+	-	-	-	-	-	+	-	-	+	+	*Enterococcus feacium*
5	+	Cocci; clustered	+	+	-	+	-	-	-	-	+	+	-	-	-	*Staphylococcus aureus*
6	-	Baccli	-	+	-	-	+	+	-	+	+	-	+	-	-	*Pseudomonas aeruginosa*
7	+	Cocci	-	+	+	-	-	-	-	-	+	-	-	+	+	*Enterococcus feacium*
8	-	Bacilli	+	+	-	-	+	+	+	+	+	+	+	+	-	Aeromonas veronii

Table 4: BLAST results of bacteria isolated from catfish *Clarias gariepinus* from the selected farms

Site of infection	Name of bacteria	Description	Accession no	Similarity (%)
Flesh S9	AV	G18	KF853564.1	81
Gills 3G5	BS	MJ4	KF933349.1	93
Intestine I10	PA	Strain MBL	KF811604.1	80
Flesh 3S2	SS	38MP	FR719724.1	89
Intestine 2I5	PA	MBL	KF811604.1	83
Flesh S2	SS	38MP	FR719724.1	90
Flesh S7	BS	Strain TO-AJPC	CP011882.1	87
Gills G2	SA			
Intestine 2I1	STA	15-92MP	EU075070.1	98
Intestine I6	EF	Strain E1	HG798651.1	90
Gills 3G5	BS	Isolate B-20091009	AM110937.1	85
Intestine I10	PA	Strain 3.5.2	HM192785.1	93
Intestine I7	BS	Gene 1631	HE 612877.1	95
Intestine I9	PA	Strain MBL	KF811604.1	80
Intestine I14	AV	Strain R9	KF853563.1	79
Intestine I13	EF			
Intestine I8	PA	Strain MBL	KF811604.1	81
Intestine 2I3	PS	CfO-4	JN836274.1	97
Gills 2G1	AV	Strain G8	KF853564.1	81
Flesh S4	PA	NCTC	LN831024.1	86
Flesh 2S1	AV	Strain G18	KF853564.1	79
Flesh 2S2	AV	Strain G18	KF853564.1	78
Flesh 2S4	AV	Strain R9	KF853563.1	80
Intestine 2I2	SA			
Intestine 2I4	PA	NCTC 10332	LN831024.1	96
Gills 3G2	PA	NCTC 10332	LN831024.1	97
Gills 3G6	PA	NCTC 10332	LN831024.1	94
Gills 3G8	PA	NCTC 10332	LN831024.1	95
Gills 3G10	PA	NCTC 10332	LN831024.1	93
Gills 3G15	PA	NCTC 10332	LN831024.1	97
Intestine 3I10	PA	NCTC 10332	LN831024.1	94
Flesh 3S1	PA	NCTC 10332	LN831024.1	96

AV = (*Aeromonas veronii*); BS = (*Bacillus subtilis*); PA = (*Pseudomonas aeruginosa*); SS = (*Staphylococcus* sp.); SA = (*Staphylococcus aureus*); STA = (*Streptococcus agalactiae*); EF = (*Enterococcus faecium*) = PS (*Pseudomonas* sp.)

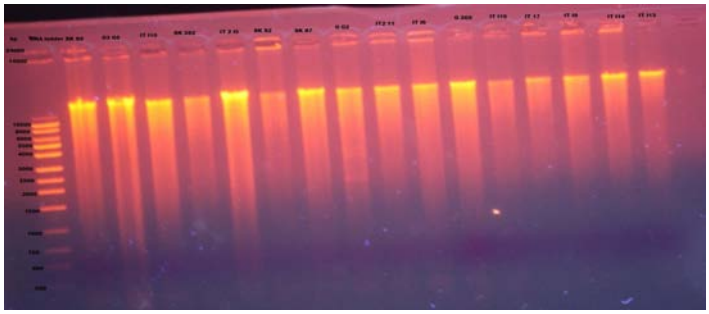

Fig. 1: Agarose gel showing DNA bands of bacterial organisms collected from *Clarias gariepi*nus viewed under the ultraviolet light for first 16 samples showing Flesh S9- INT I13 bp = DNA base pair

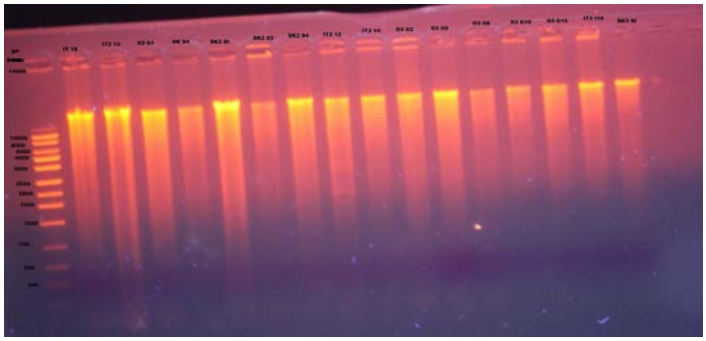

Fig. 2: Agarose gel showing DNA bands of bacterial organisms collected from *Clarias gariepi*nus viewed under the ultraviolet light for last 16 samples showing intestine I8-Flesh 3S1 bp = DNA base pair

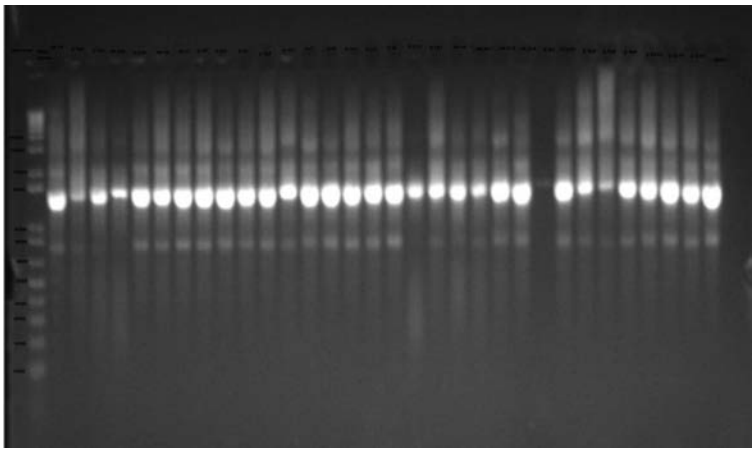

Fig. 3: Agarose gel showing the Genomic DNA bands of bacterial organisms isolated from catfish *Clarias gariepinus* used for sequencing for 32 samples, Flesh S9- Flesh 3SI bp = DN Abase pair

DISCUSSION

The isolation of bacteria from catfish were taken from different parts of the flesh, intestine and gills. The process involved weighing of fish samples followed by preparation of culture media, the actual bacteria isolation by streaking on agar plates, bacteria count and purification up to identification using colonial characteristics, morphological characteristics, biochemical and molecular characteristics. This procedure was in line with Fawole and Oso[21] on characterization of bacteria. The cultural characteristic revealed colours of bacteria from yellow, dull cream, creamy, creamy-green pigmentation and white while the surface texture had smooth, smooth and glistening and the forms were circular and irregular. On elevation some were raised, others were flat, the margin were entire or undulate while the optical characteristics were either transluscent or transparent. On morphological characteristics, Gram staining procedures were used and motility test. The same procedure was used by Fawole and Oso[21] on characterization of bacteria and Holt *et al*.[22] manual on bacteriology. Molecular tests involved DNA extraction, sequencing of the nucleotides on the National Centre for Biotechnology information data base and their level of relatedness and the ascension number written. This was used to confirm the actual identity of the bacteria and compared with the results from the biochemical tests. The molecular test thereafter confirmed the following bacterial organisms; *Aeromonas veronii*, *Enterococcus feacium*, *Pseudomonas aeruginosa*, *Bacteria subtilis*, *Staphylococcus aureus*, *Streptococcus agalactiae* after isolation from the flesh, intestine and gills parts of the fish.

This is similar to previous work by researchers where the rRNA genes have been used in PCR assays for *Renibacterium salmoninarum*[11], *Aeromonas salmonicida*[12] and *Yersinia ruckeri*[13]. The same methods are employed in the detection and study of significant pathogens that are not included in European Community legislation. These include *Vibrio anguillarum*[14], *Lactococcus garvieae*[15], *Piscirickettsia salmonis*[16], *Flexibacter*[17], *Flavobacterium*[18], *Photobacterium*[19] and *Mycobacterium*[20]. 16S rRNA gene sequencing were used to identify *E. faecalis* by Hardi, etc. In addition, the first report on molecular identification and herbal control of fish pathogenic on *E. faecalis* in Bangladesh was carried out by Rahman *et al*.[23]. Diagnostic tests for identification of fish disease include conventional microbiological, immunoserological and molecular methods[24].

CONCLUSION

A new strain of bacterium is gotten from this research which is *Aeromonas veronii* and has added to the strains found in Nigeria.

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