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Molecular Characterization and Evaluation of Antibiotic Resistance Gene of Bacteria Associated with Gills of *Arius heudelotii* and *Clarias gariepinus*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study was aimed at the isolation and molecular characterization of bacteria associated with catfish from culture and wild environments. Samples of gills of Arius heudelotii and Claris gariepinus fishes of economic value from the New Calabar River Choba and earthen ponds from Aluu in Rivers State were collected and analysed. Characterization of isolates from the gills of the catfish was done using the 16S rRNA gene sequencing method to identify their relationship. The presence of a resistance gene was examined by polymerase chain reaction followed by agarose gel electrophoresis of the amplification products. The mean total heterotrophic bacterial counts was

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6.5995±0.947 LogCFU/g for the gills' samples obtained from the earthen pond Aluu. New Calabar River had 4.5041±1.054 LogCFU/g for the gills' samples. A total of 12 bacterial flora were isolated from the gills of the sampled catfish: Escherichia coli, Klebsiella spp., Enterobacter spp., Citrobacter spp., Bacillus subtilis, Proteus vulgaris, Streptococcus spp., Shigella spp. Staphylococcus spp., Micrococcus spp., Vibrio spp. and Acinetobacter spp. Vibrio azureus, Aeromonas dhakensis, Proteus vulgaris, Aeromonas hydrophila. Vibrio azureus and Providencia alcalifaciens were found to be carriers of the TEM gene, while Providencia alcalifaciens carried the CTX-M gene. SHV gene was found in Vibrio azureus, Providencia alcalifacians, Proteus vulgaris, Aeromonas hydrophila, Providencia alcalifacians. However, the Oxa gene was not identified in the bacterial genera. The high probability of transferring them to other microbes to cause antibiotic pollution has become a serious public health challenge. Microbial loads with high or low presence of some potential pathogens call for public concern.

Keywords: catfish; gills; molecular; resistance gene; antibiotic, bacteria.

1. INTRODUCTION

The catfish farming industry accounts for 70 per cent of aquaculture production, *Clarias gariepinus* is the most cultured species and has a substantial economic value [1,2,3]. Osawe [4] identified good qualities of catfish, such as its survival in different cultural systems and diverse environments. Catfish can grow fast, and the air breathes, which makes it vital to the sustainability of the aquaculture industry in Nigeria [3], [5].

The favourable environmental conditions of some of the African countries have encouraged fish farming and these conditions help the growth of catfish, especially the African catfish. Catfish can tolerate a varying range of climate changes, high stocking densities under culture conditions, fast growth rate, acceptability of artificial feed, high fecundity rate, ease of artificial breeding and high market value. However, catfishes have been identified as a reservoir of bacteria that hinders their suitability for human consumption [6]. Catfish serves as a staple food for many Nigerians and Africans at large [7]. Catfish are known today as the leading species for freshwater and saltwater production [8,9].

Arius heudelotti and *Clarias gariepinus* are examples of catfish that are always ready for human consumption. In Nigeria, African catfish is the most farmed fish. Almost everyone enjoys eating it. As earlier stated, catfish is not harshly affected by environmental conditions, also it has an economic value and is capable of surviving for several days during marketing. On the other hand, *Arius heudelotii* grows well and tolerates water that is brackish or swampy fields of the wild environment. Many people eat it as food. Both *A. heudelotii* and C. *gariepinus* are economically viable for farmers and nutritionally important for consumers [10].

Health-related diseases are a major concern for doctors and the public. The bacteria that cause this disease are found in many seafood species, such as *Clarias gariepinus* and *Arius heudelotii*. According to a recent study by Dar et al. [11] and Gong et al. [12], fish diseases are significant challenges nowadays that disrupt the production of fish for consumption in the world. Bacterial and fungal infections and other ecological factors (poor water quality) cause diseases in fish. Generally, these factors are contributors to the high rate of mortalities both in culture as well as in wild fishes. Among the seafood, Clarias gariepinus has the highest percentage of human consumption. According to a study by Ayandiran et al. [13], Clarias gariepinus in Egypt is the hope of solving human nutritional problems. In Nigeria, fish and fish products are cheap in terms of cost compared to meat and are a leading source of nutrients for the teeming population of the country [13].

Sadly, this nutritious seafood harbours bacterial infections such as *Vibriosis, Bacillus, E. coli*, *staphylococcus* and others that can cause diseases of fish, economic loss and public health hazards [14]. Some *Vibrio* are pathogenic to humans and/or marine animals through a variety of infectious diseases. Recently, several reports indicate that the incidence of *Vibrio* infection in humans is increasing, and *Vibrio* infection in animals poses an ongoing threat to aquaculture [15,16] and pose a serious health danger to human. For example, when infected with *Vibrio cholerae*, it can cause diarrhoea, which is often fatal if left untreated, with thousands of cases and more than 100,000 deaths. Lloyd et al. [16] lamented that the rise of antimicrobial resistance (AMR) among pathogenic *Vibrio* spp. is a significant public health problem. According to the Rivers State Ministry of Health [17], the

health conditions of many individuals are not encouraging and very unacceptable. The reason for the poor health of the population is malaria, upper respiratory tract infections, diarrhoea, etc. Even though there are preventable diseases such as these diseases are responsible for passion and premature death in the state. They impact agricultural and business productivity as well as school absenteeism and time lost at work [17].

Based on this background this study is aimed at molecularly characterising bacterial and evaluating their genes associated with the gills of *Arius heudelotii* and *Clarias gariepinus*

2 MATERIALS AND METHODS

2.1 Study Area

The study was carried out in private fish farms (earthen ponds) in the Aluu community of Ikwerre Local Government and marine water (New Calabar River) in Choba community in Obi Akpor Local Government Area, all in Rivers State Nigeria. The New Calabar River is located within longitude $006^0 53^1$- $53^0 86^1$ E and latitude $04^0 53^1$ – $19^0 20\ ^1$ N in Choba, Rivers State, Nigeria. Aluu village is located about 25km from Port Harcourt and is one of the oldest rural areas in Ikwerre. The name Aluu means "to fight" and reflects the community's warrior spirit. After the planting season, some community members engage in fishing, hunting and small-scale trading to supplement family income while waiting for the crops to be harvested. At the village's main market, women build warehouses to sell the surplus food they produce and also sell [18].

2.2 Sample Collection

Samples of twenty-four (2 each month) of *Arius heudelotii* and 24 (2 each month) *Clarias gariepinus* were collected from New Calabar River and earthen pond, respectively from January – December 2022 (12 months) and transported immediately to the Department of Microbiology Laboratory, University of Port Harcourt, Nigeria, for analysis.

2.3 Isolation of the Bacteria

2.3.1 Total heterotrophic bacterial counts

The total heterotrophic bacterial counts were done using nutrient agar. The medium was prepared according to the manufacturer's instructions. Tenfold serial dilutions were carried out using normal saline as a diluent. Alignment (0.1ml) of appropriate dilutions spread plates onto the surface of NA plates and incubated for 24 hours at 37°C. The bacteria colonies were counted after the incubation. The colony-forming unit was determined with the formula below.

$$\text{THB}\left(\frac{\text{Cfu}}{\text{ml}}\right) = \frac{\text{Average number of colonies } \times 1}{\text{Dilution factor } \times 1/\text{Volume plated}}$$

2.4 Molecular Identification

The discrete colonies were molecularly identified to characterize bacteria and evaluate their genes associated with the gills of *Arius heudelotii* and *Clarias gariepinus*. Hence, it was performed through five cogent steps.

2.4.1 DNA extraction

Extraction was done using a ZR bacterial DNA mini-prep extraction kit supplied by Inqaba South Africa. Heavy growth of the pure culture of the suspected isolates was suspended in 200 microliters of isotonic buffer in a ZR Bashing Bead Lysis tube, and 750 microliters of lysis solution were added to the tube. The tubes were secured in a bead beater fitted with a 2ml tube holder assembly and processed at maximum speed for 5 minutes. The ZR bashing bead lysis tube was centrifuged at 10,000x*g* for 1 minute.

Four hundred (400) microliters of supernatant were transferred to a Zymo-Spin IV spin Filter (orange top) in a collection tube and centrifuged at 7000 x*g* for 1 minute. One thousand two hundred (1200) microliters of fungal/bacterial DNA binding buffer were added to the filtrate in the collection tubes bringing the final volume to 1600 microliters, 800 microliters were then transferred to a Zymo-Spin IIC column in a collection tube, and centrifuged at 10,000x*g* for 1 minute, the flow through was discarded from the collection tube. The remaining volume was transferred to the same Zymo-spin and spun. Two hundred (200) microliter of the DNA Pre-Was buffer was added to the Zymo-spin IIC in a new collection tube and spun at 10,000x*g* for 1 minute followed by the addition of 500 microliters of fungal/bacterial DNA Wash Buffer and centrifuged at 10,000x*g* for 1 minute.

The Zymo-spin IIC column was transferred to a clean 1.5 microliter centrifuge tube, and 100 microliters of DNA elution buffer was added to

the column matrix and centrifuged at 10,000x*g* microliter for 30 seconds to elute the DNA. The ultra-pure DNA was then stored at -20 degrees for other downstream reactions.

2.5 DNA Quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was launched by double-clicking on the Nanodrop icon. The equipment was initialized with 2 ul of sterile distilled water and blanked using normal saline. Two microliters of the extracted DNA were loaded onto the lower pedestal, the upper pedestal was brought down to contact with the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the "measure" button.

2.6 Extended Spectrum Beta- Lactamases Detection

2.6.1 Amplification of SHV genes

"SHV genes from the isolates were amplified using the SHV F: 5' CGCCTGTGTATTATCTCCCT-3' and SHV R: 5'-CGAGTAGTCCACCAGATCCT-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microliters for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M, and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 56ºC for 40 seconds; extension, 72ºC for 50 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV transilluminator for a 281bp product size" [19].

2.6.2 Amplification of TEM genes

"TEM genes from the isolates were amplified using the TEMF: 5'-ATGAGTATTCAA CATTTCCGTG-3' and TEMR: 5'-TTACCAATGCTTAATCAGTGAG-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M, and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 58ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV transilluminator for an 850bp product size" [19].

2.6.3 Amplification of OXA genes

"TEM genes from the isolates were amplified using the OXA-1F: 5'- AGCCGTTAA AATTAAGCCC-3' and OXA-1R: 5'-CTTGATTGAAGGGTTGGGCG-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microliters for 35 cycles. The PCR mix included: the X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M, and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 47ºC for 30 seconds; extension, 72ºC for 40 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV transilluminator for a 911bp product size" [19].

2.6.4 Amplification of CTX-M genes

"CTX-M genes from the isolates were amplified using the CTX-MF: 5'-CGCTTTGCGATGTGCAG-3' and CTX-MR:5'-ACCGCGATATCGTTGGT-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microliters for 35 cycles. The PCR mix included: the X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M, and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV transilluminator for a 550bp product size" [19].

2.7 16S rRNA Amplification

The 16s rRNA region of the rRNA gene of the isolates were amplified using the 16s RRNA

region of the rRNA genes of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3’ and 1492R: 5'-CGGTTACCTTGTTACGACTT-3’ primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.5uM, and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light transilluminator.

2.8 Sequencing

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25 ul BigDye® terminator v1.1/v3.1, 2.25ul of 5 x BigDye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing conditions were as follows 32 cycles of 96°C for 10s, 55°C for 5s, and 60°C for 4min.

2.9 Phylogenetic Analysis

“Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) database using BLASTN. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0” [20]. “The bootstrap consensus tree inferred from 500 replicates [21] is taken to represent the evolutionary history of the taxa analysed. The evolutionary distances were computed using the Jukes-Cantor method” [22].

3. RESULTS

Table 1 shows the level of bacterial contamination of the catfish gills sample, Fig. 1 shows the percentage of occurrence of bacteria in the gills of *Arius heudelotii*. Bacterial species isolated and their percentages of occurrence in gills of *Arius heudelotii*, were E. coli (15.56%), Shigella spp (13.33%), Klebsiella spp (11.11%), Salmonella spp (11.11%), Staphylococcus spp (11.11%), Enterobacter spp (8.89%), Proteus spp (8.89%), Citrobacter spp (8.89%), Enterococcus spp (6.67%), Bacillus spp (2.22%), and Micrococcus (2.22%).

Table. 1. Bacterial isolates and their percentage occurrence in the gills of catfish

Bacterial Isolates	Gills
E. Coli	13 (34.2)
Klebsiella spp	11 (31.4)
Enterobacter spp	9 (28.1)
Citrobacter spp	8 (30.0)
Bacillus spp	4 (16.0)
Proteus spp	8 (30.8)
Shigella spp	12 (33.3)
Enterococcus spp	7 (31.8)
Salmonella spp	9 (25.0)
Staphylococcus spp	6 (21.4)
Micrococcus spp	2 (11.1)
Acinetobacter spp	2 (18.2)
Total	**91 (28.0)**

Fig. 2, shows the percentage of occurrence of bacteria in the gills of *Clarias gariepinus*, Klebsiella spp (13%), Shigella spp (13%), E. coli (13%), the most percentage frequently distributed bacteria isolate in the gills of C. gar, Salmonella spp (8.8%), Proteus spp (8.8%), Bacillus spp (6.5%), Enterobacter spp (10.9%), Enterococcus spp (8.88%), Staphylococcus spp (2.2%), Citrobacter spp (8.8%), Acinetobacter spp (4%), and Micrococcus (2.2%).

The obtained 16s rRNA sequence from the isolates produced an exact match during the mega blast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of the isolates showed a percentage similarity to other species at 100%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolates within the Proteus, Providencia, Vibrio, and Aeromonas sp and revealed a close relatedness to Proteus vulgaris, Providencia alcalifaciens, Vibrio azureus, Aeromonas hydrophila and Aeromonas dhakensis (Fig. 3).

Table 2 shows the isolates coded with H1, H2, H3, H4, H5, H6, H7, H8 H9, and H10 were identified as *Vibrio azureus, Providencia alcalifacians, Aeromonas dhakensis, Aeromonas dhakensis, Proteus vulgaris, Proteus vulgaris, Proteus vulgaris, Vibrio azureus, Aeromonas*

hydrophila, and *Providencia alcalifacians*, respectively with their accession numbers.

Plate 1-4 shows the resistant gene of isolates in the primers used. For Plate 1, the TEM gene were identified in H2 and H8 (*Providencia alcalifaciens and Vibrio azureus*). Plate 2 identified the CTX-M gene in H2 (*Providencia alcalifaciens*), Plate 3 identified the SHV gene H1, H2, H5, H9 and H10 (Vibrio azureus, Providencia alcalifacians, Proteus vulgaris, Aeromonas hydrophila, Providencia alcalifacians). While in (Plate 4) the Oxa gene was not identified in the bacterial genera.

Table 2. Molecularly identified isolates with their Accession numbers

Isolate Code	Molecular Identification	Accession Number
H1	Vibrio azureus	MZ049675
H2	Providencia alcalifacians	ON459767
H3	Aeromonas dhakensis	ON375398
H4	Aeromonas dhakensis	ON375398
H5	Proteus vulgaris	LC654887
H6	Proteus vulgaris	LC654887
H7	Proteus vulgaris	KY494852
H8	Vibrio azureus	MZ049675
H9	Aeromonas hydrophila	ON203020
H10	Providencia alcalifacians	ON459767

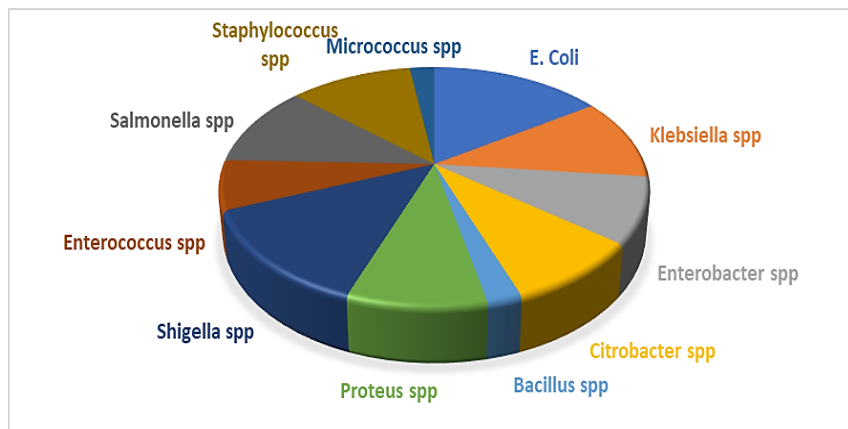

Fig. 1. Percentage frequency of occurrence of bacterial isolates from gills of *Arius heudelotii* during a months study period.

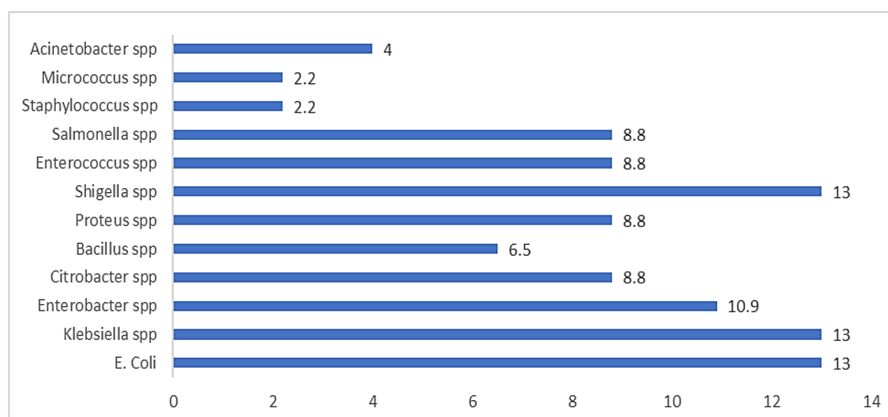

Fig. 2. Percentage frequency of occurrence of bacterial isolates from gills of *Clarias gariepinus* during a months study period

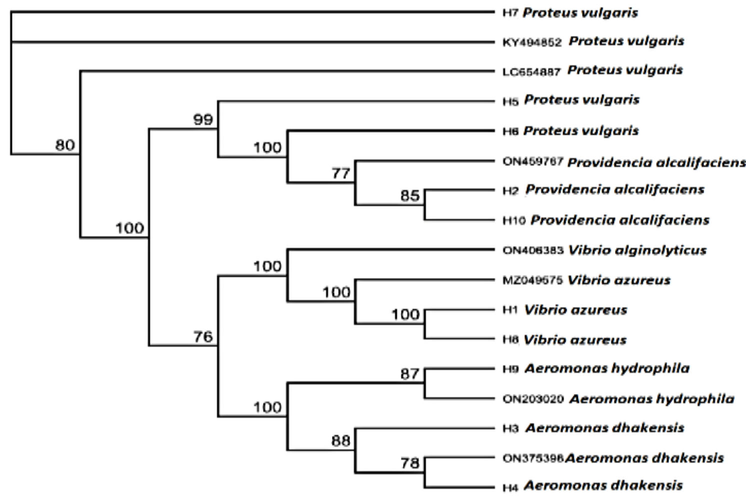

Fig. 3. Phylogenetic tree showing the evolutionary distance between the bacterial isolates

Plate (1 to 4): resistant gene of isolates in the primers

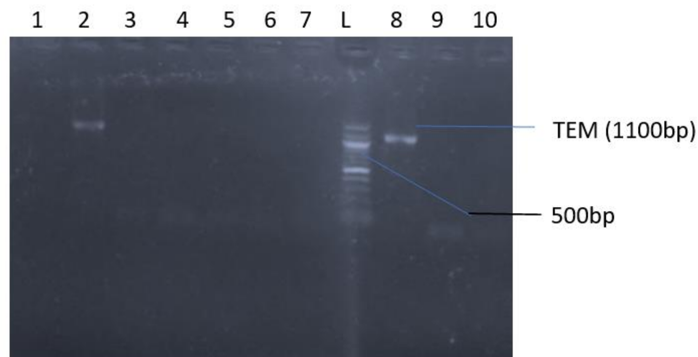

Plate 1. Agarose gel electrophoresis showing the amplified TEM gene. Lanes 2 and 8 show the TEM genes at 1100bp while lane L represents the 100bp molecular ladder

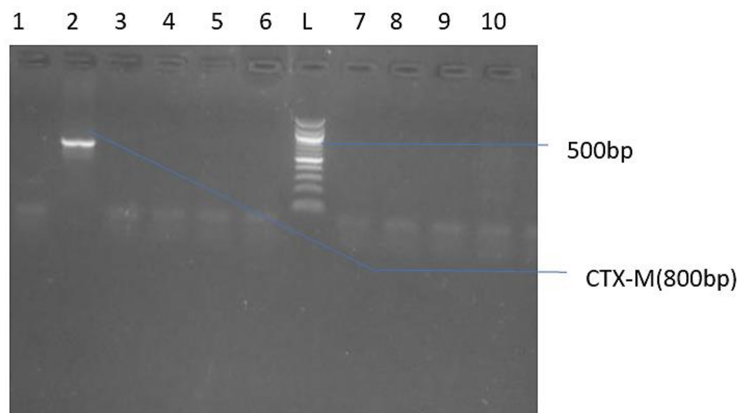

Plate 2. Agarose gel electrophoresis showing the amplified CTX-M gene. Lane 2 shows the CTX_M gene band at 800bp while lane L represents the 100bp molecular ladder

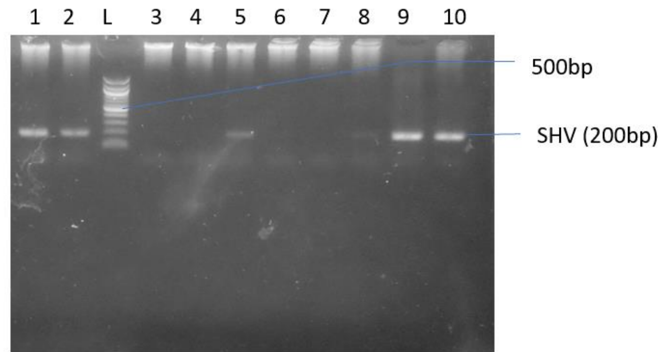

Plate 3. Agarose gel electrophoresis showing the amplified SHV gene. Lanes 1, 2, 5, 9, 10 show the SHV gene bands at 200bp while lane L represents the 100bp molecular ladder

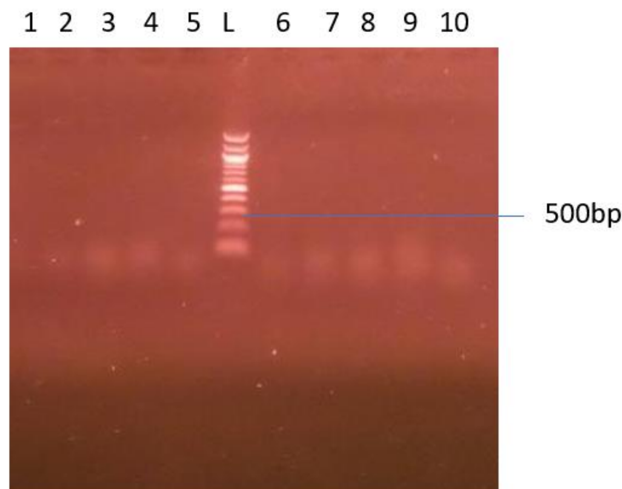

Plate 4. Agarose gel electrophoresis of the Oxa gene showing no bands. Lane L represents the 100bp molecular ladder

4. DISCUSSION

The study has shown that pathogenic bacteria are present in *C. gariepinus* from a cultured environment (earthen pond) and *A. heudelotii* from a wild environment (New Calabar River). The microbiological characteristics revealed that Gram-negative bacteria were dominant in the bacteria isolated from the earthen pond and New Calabar River. Bacterial infection has been a major challenge militating aquaculture and is responsible for high pond mortality, and it has been attributed as the main cause of economic losses [23,24]. Domitila et al. [25], reported that based on Gram staining reaction, the majority of the bacterial isolates in their study were Gram-negative. On the other hand, Gram-positive bacteria were also isolated in this study. The finding is in tandem with Al-Reza et al. [26], who reported that Gram-positive bacteria in the genera Clostridium, Bacillus, and Micrococcus have been isolated from different fish species.

Bacillus sp. was also isolated in this study. Our finding agrees with the findings of Domitila et al. [25]. According to Setlow [27], the predominance of the Bacillus species can be attributed to their ubiquitous nature and the ability to produce endospores which allows them to survive in fish and fish processing conditions. The bacteria contribute to fast food spoilage because they do not produce extracellular enzymes and toxins [8]. The presence of Bacillus spp. in the organs of fish poses a considerable health concern. Akani & Obire [14] pointed out that Bacillus, E. coli, Staphylococcus and others can cause disease of fish, economic loss and public health hazards, Hence, Bacillus has the potential of causing infections and food poisoning.

The bacteria in the samples include *E. coli, Shigella* spp*, Salmonella* spp*, Klebsiella* spp, *Enterobacter* spp*, Staphylococcus aureus*, *Proteus* spp*, Bacillus* spp*, Enterococcus* spp*, Citrobacter* spp*, Micrococcus* spp*, and Acinetobacter* spp. The finding in this study confirmed the view of Lazado et al. [28], who stated that fish products have been documented to harbour diverse bacteria, including pathogenic bacteria and other microorganisms. Our study agrees with the findings of Njoku et al. [29], who reported the presence of *Escherichia coli, Salmonella spp., Staphylococcus aureus, Proteus* spp*, Klebsiella* spp*,* and *Enterobacter* in some fish pond water within the Niger Delta region of Nigeria. The result also agrees with Uchechukwu & Okoli [30], The bacteria: *Staphylococcus aureus* and *Escherichia coli* in the catfish samples are the commonest bacteria found in the fish samples. *Escherichia coli* has been identified as a potential cause of diarrhoea and kidney damage as well as uncomplicated community-acquired urinary tract infections [31]. Currently, the productivity, development, and expansion of the aquaculture sector are under threat because of bacterial diseases [32]. The prevalence of bacteria may be attributed to changes or deterioration of the aquatic environment [33].

Specifically, Salmonella spp. was highly present in the catfish. Salmonella spp has been reported by Porto et al. [34] as a pathogen that causes salmonellosis, a dangerous infection characterized by enterocolitis. This salmonellosis infection is a foodborne disease (FDA) that is generating public health worries in various countries worldwide. In this present study, salmonella was found in the gills. Other past studies have found Salmonella spp in the gills [35,36,37,38].

Bacteria were found in the gills of *C. gariepinus* and *Arius heudelotii*. The higher bacteria load in the C. gariepinus from the earthen pond may be due to improper hygiene of the fish pond. Whereas loads of bacteria associated with *Arius heudelotii* from natural habitat may be due to contamination as a result of indiscriminate disposal of waste materials into the river through runoffs, animal excreta and other environmental wastes, free-roaming animals and pets such as dogs also contribute to faecal contamination of the river. The variations in the bacterial load of the gills of the fish samples existed. The results from the earthen pond (Cultured fish habitat) and New Calabar River (Wild environment), the bacterial load reported in the gills (Tables 1) was high in both *Clarias gariepinus* and *A. heudelotii.* This finding agrees with the findings of Ogbukagu et al. [39], who recovered more bacteria in the gills of the catfish. Our study also reechoed the same concern expressed by Nwankwo & Akani [9], that the presence of these organisms could constitute a public health risk and calls for adequate preventive measures.

5. CONCLUSION

The study has shown that gills of *Clarias gariepinus* and *Arius heudelotii* samples from the two sample sites (Earthen Pond Aluu and New Calabar River Choba) harbour bacteria. It confirmed the presence of microorganisms and contamination of the gills. Therefore, catfish may serve as a reservoir for pathogens thereby acting as a mechanism for cross-contamination. Importantly, in this study, it can be concluded that bacteria were present in catfish, which was identified using biochemical tests and molecular analysis. The organisms isolated in this study are all pathogenic organisms and some are resistant to some commonly used antibiotics. The study specifically reported the presence of *Vibrio azureus*, a novel species of *Vibri*o that has a hundred per cent similarity to *Vibrio alginolyticus.* They are members of the *V. harveyi* clade that share a high degree of genetic and phenotypic similarity, which can often lead to the misidentification of species within the clade. The study also revealed the presence of other organisms, and antibiotic-resistance genes present in some of the bacterial isolates that could constitute a public health risk because of its capacity to spread and pose serious antibiotic-resistance pollution to human and animal health. This calls for concern to health management and other stakeholders and needs adequate preventive measures.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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