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Characterization and Identification of Bacteria Associated with the Gills of Culturable African Catfish (*Clarias gariepinus*)

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ABSTRACT

Fish farming, especially the African catfish (*Clarias gariepinus*) farming plays a vital role in addressing protein demands in Nigeria. However, disease outbreaks linked to bacterial fish pathogens pose significant challenges to aquaculture productivity and public health. Therefore, this study isolated and characterized the bacteria associated with the gills of *C. gariepinus* sourced from three farms in Rumuolumeni, Port Harcourt, Rivers State, Nigeria. Nine live fish samples were collected, and the gills were aseptically processed for the isolation of bacteria associated with the gills using standard plate count on nutrient agar. Isolates were identified using morphological, biochemical, and molecular (16S rRNA) methods. Results obtained showed that, the total heterotrophic bacterial count ranged from 1.5± 1.1 to 3.3± 0.02 $\times 10^5$ CFU/g. Molecular identification revealed the presence of seven bacterial species: *Vibrio cholerae, Enterobacter aerogenes, Salmonella paratyphi, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis,* and *Staphylococcus aureus*. *Staphylococcus aureus* was the most prevalent (26.9%), followed by *S. epidermidis* (19.2%) and *Salmonella paratyphi* (15.4%). All the bacterial isolated are potential pathogens and therefore are of potential health risks to consumers of catfish. There is therefore the need by relevant authorities to have policies in place for improved aquaculture management practices to ensure fish safety and public health.

Keywords: African Catfish (*Clarias gariepinus*), Fish Gills, Fish Pathogens, Bacteria, *Vibrio cholerae,* Public Health.

Introduction

Fish are a crucial food source that provides low-cholesterol protein to approximately 60% of the world's population (Sichewo et al., 2013). Developing countries obtain approximately 30% of their annual animal protein needs from fish (Abisoye et al., 2011). Fish serve as a valuable source of animal protein for humans and their livestock, and the fishing industry supplies more than 40% of the protein in the diet for two-thirds of the global population (Omeji et al., 2010), as well as a significant amount of employment, especially in rural areas.

The fish-farm sector is a major livelihood source for over 3 million people. Fish is also one of the best sources of vitamins and minerals, which are essential nutrients needed to enrich both infant and adult diets.

In Nigeria, it has been observed that fish is consumed fresh, preserved, or processed (smoked), forming a highly valued delicacy that crosses social, economic, age, religious, and educational boundaries (Adebayo-Tayo et al., 2008). The demand for fish as a source of protein increases as the human population grows. In an attempt to increase fish supply as a protein source, there has been a tremendous increase in the development of fish farming in Nigeria (Usip et al., 2014).

The continuous increase in the human population requires more food production to meet the consequent increasing demands (Ogonna et al., 2017). However, Nigeria has a big fish requirement deficit as the country imports over 900,000 metric-tonnes of fish against its estimated domestic fish production at 450,000 metric tonnes/year (Imam & Dewu, 2010).

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Clarias gariepinus, the African catfish, is generally considered to be one of the most important tropical catfish species for aquaculture in West Africa and a very important freshwater fish in Nigeria (Amande & Nwaka, 2013). They are commonly categorized as omnivores since they feed largely on aquatic insects, fish, and higher plant debris (Ahmad, 2014); thus, they are prone to parasitic and microbial attacks (Tripathi, 2014). In Nigeria, catfish farming is proving to be a lucrative option for small-scale inland fisheries, and the consumption of its products is on the increase (Otubusin, 2011). However, farmers are constrained by massive fry and fingerling mortalities, especially in the culture system, due to the invasion of pathogens (Iyaji *et al.*, 2009).

There are a variety of organisms that cause infectious diseases of the gill. These organisms can be bacteria, viruses, fungi, etc., and each of these organisms attacks different parts of the fish's organs, which will cause different symptoms too (Nawar et al., 2024).

This study, therefore, seeks to understudy the bacteria associated with the gills of catfish obtained from fish farms in Rumuolumeni in Port Harcourt, Rivers State, Nigeria. This is to have knowledge of the types of bacteria that could compromise fish safety and public health.

Materials and Methods

Study area

The study area is Rumuolumeni, located in Obio/Akpor Local Government Area of Rivers State, Nigeria. It lies between latitude 4˚ 47' 49''North and longitude 6˚ 56' 29''. The climate of the area is tropical with two seasons: rainy and dry seasons. The rainy season is from mid-March to October, with rainfall ranging from 2000 to 2500 mm in most areas, while September to February usually make up the dry season; however significant rainfall has been observed even in the month of November. The map showing the study location is presented in Fig. 1.

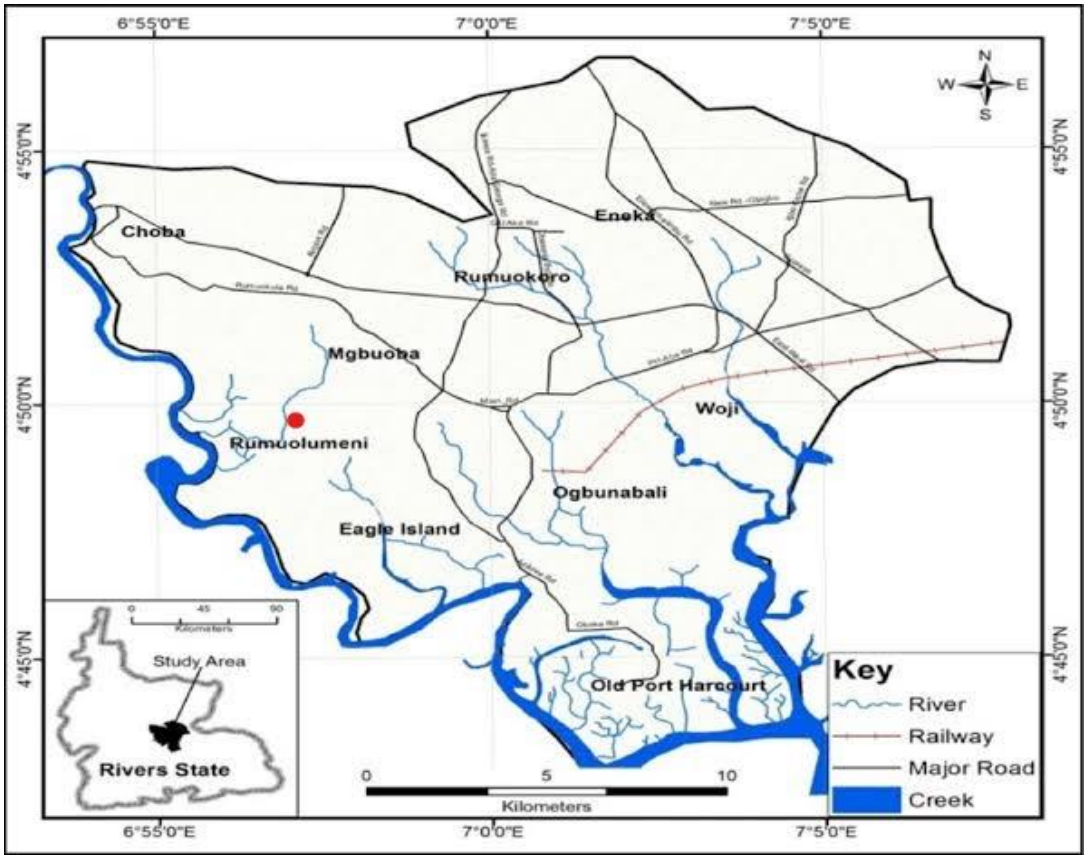

Fig. 1: Map of Port Harcourt showing Rumuolumeni, the study area

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Collection of Fish Samples

Nine (9) table-sized *Clarias gariepinus* were bought from three fish farms: farm 1 is located at coordinate 4°00' East and 4°00' North in Mgbudohia community; farm 2 is located at coordinate 4°14' East and 4°10' North at Navy junction; and farm 3 is located at coordinate 4°11' East and 4°13' North at Nkpor community in Rumuolumeni. The fish were bought between 10 A.M. and 12 noon at all study sites and transported alive in disinfected plastic containers (covered with net) to the Department of Biology Research Laboratory, Ignatius Ajuru University of Education, for the study.

Preparation of Fish Sample for Analysis

The fish samples were cleaned with cotton wool and sterile distilled water, after which they were cut with a sterile knife. The gills of the fish samples were aseptically obtained, minced, and ground separately. Ten grams of minced gills were immersed in 90 ml of normal sterile saline solution in a 250 ml beaker to give a 1:10 stock dilution. This was homogenized by swirling gently before 1 mL was withdrawn using a sterile pipette into a test tube containing 9 mL sterile normal saline. The dilution was followed serially to obtain a dilution of 10^5 (Robinson et al., 2024).

Isolation and Enumeration of the Total Bacteria Count

The bacterial counts of the samples were determined using the standard plate count method (Abu et al., 2014) on nutrient agar. Aliquots (0.1 ml) of the dilutions of 10^{-3} were plated on nutrient agar in triplicate and were evenly spread using a sterile bent glass rod. The plates were incubated for 24 hours at 37 °C. After incubation, the plates were examined, and the number of colony-forming units (CFU) that developed was counted and recorded. Distinct colonies that appeared were subcultured into freshly prepared nutrient agar plates aseptically to obtain pure cultures of the isolates. Pure isolates of the resulting growth were stored at 4°C in nutrient agar slants.

Identification and Characterization of Bacteria

Bacterial isolates were identified based on their colonial morphology (size, shape, colour, texture, and elevation), Gram staining, biochemical tests, and Molecular methods (molecular sequencing using 16S rRNA). The biochemical tests (Catalase, Citrate, Oxidase, Methyl Red, Voges Proskauer, Indole, and Sugar Fermentations) were carried out according to standard procedures (Prescott et al., 2011). The resulting responses of the isolates were compared with standard reference organisms and with those of known taxa (Abu et al., 2014)

Molecular Sequencing

DNA Extraction (Boiling Method)

The molecular characterization of the isolates was done as described by Robinson et al. (2024). In this method, five milliliters of an overnight broth culture of the bacterial isolates in Luria Bertani (LB) were spun at 14,000 rpm for 3 minutes. The cells were resuspended in 500 µl of normal saline and heated at 95°C for 20 minutes. The heated bacterial suspension was cooled on ice and spun for 3 minutes at 14,000 rpm. The supernatant containing the DNA was transferred to a 1.5 ml microcentrifuge tube and stored at –20°C for downstream reactions.

DNA Quantification

The extracted genomic DNA was quantified using a Nanodrop 1000 spectrophotometer. The software of the equipment was launched by double-clicking the Nanodrop icon. The equipment was initialized with 2 µl of sterile distilled water and blanked using normal saline. Two microliters of the extracted DNA were loaded onto the lower pedestal, and the upper pedestal was brought down to contact the DNA on the lower pedestal. The DNA concentration was measured by clicking on the "measure" button (Robinson et al., 2024).

16S rRNA Amplification

The 16S rRNA region of the isolates was 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 µl for 35 cycles.

The PCR mix included the 2× DreamTaq Master Mix supplied by Inqaba, South Africa (containing Taq

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Polymerase, dNTPs, $MgCl_2$), primers at a concentration of 0.5 µM, and the extracted DNA as template. PCR conditions were as follows: initial denaturation at 95°C for 5 minutes; denaturation at 95°C for 30 seconds; annealing at 52°C for 30 seconds; extension at 72°C for 30 seconds for 35 cycles; and final extension at 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 130 V for 30 minutes and visualized on a blue-light transilluminator (Akinyemi et al., 2016).

DNA Sequencing

Sequencing was performed using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria, South Africa. Sequencing was carried out at a final volume of 10 µl, with components including 0.25 µl BigDye® Terminator v1.1/v3.1, 2.25 µl of 5× BigDye sequencing buffer, 10 µM PCR primer, and 2–10 ng PCR template per 100 bp. The sequencing conditions consisted of 32 cycles at 96°C for 10 s, 55°C for 5 s, and 60°C for 4 min (Akinyemi et al., 2016).

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. The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 500 replicates represented the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) was shown next to the branches.

Results

The results of the total heterotrophic bacterial count obtained from the fish gills are presented in Table 1. Results showed that the total heterotrophic bacterial load ranged from 1.5± 1.1 to 3.3± 0.02 $\times 10^5$ CFU/g. More so, despite the differences in bacterial count across the fish gills of the farms, there were no significant differences (P>0.05).

Table 1: Total heterotrophic bacterial count (CFU/g) in the gills of culturable catfish

Fish Farms	Total heterotrophic bacterial Count (Mean± SD $\times 10^5$)
Farm 1	2.4± 1.3
Farm 2	3.3± 0.02
Farm 3	1.5± 1.1
P-value	0.871

Identification of bacteria species associated with the gills of cultured catfish using molecular characterization

Table 2 presents the bacterial species isolated from the gills of culturable catfish as identified through 16S rRNA gene sequencing. Seven bacterial strains were detected, each showing varying degrees of sequence similarity with known reference strains in the NCBI database. The isolates include *Vibrio cholerae*, *Enterobacter aerogenes*, *Salmonella paratyphi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. The similarity scores, ranging from 87% to 98.9%, indicate moderate genetic relatedness between the isolates and the reference strains, suggesting possible strain variation or genetic divergence from the typical species sequences. Among the isolates, *Salmonella paratyphi* and *E. coli* recorded the highest similarity, implying closer alignment to their reference sequences, while *Staphylococcus enterica* and *S. aureus* showed the lowest similarity.

The Phylogenetic tree of isolated bacteria is presented in Fig. 2. The phylogenetic tree illustrates the evolutionary relationship among seven bacterial isolates based on their 16S rRNA gene sequence similarities. The branching pattern indicates genetic closeness, where organisms sharing a common node are more closely related evolutionarily.

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Table 2: Bacteria species isolated from the gills of culturable catfish

S/N	Accession No	Sequence Identity	Similarity score (%)
1	NZ_BLTD01000042.1	*Vibrio cholerae*	98.9
2	M62745.1	*Enterobacter aerogenes*	88
3	EU882390.1	*Salmonella paratyphi*	97.8
4	NZ_JBTITI010000120.1	*Escherichia coli*	97.8
5	NZ_JAHXZX010000564.1	*Pseudomonas aeruginosa*	87
6	PYYZ01001450.1	*Staphylococcus epidermidis*	99.5
7	NZ_SULE01000085.1	*Staphylococcus aureus*	98.8

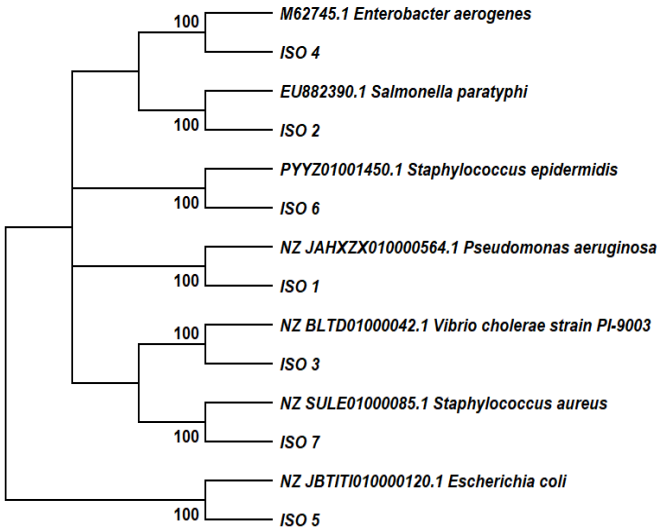

Fig. 2: Phylogenetic tree of bacteria isolated from gills of cultured catfish

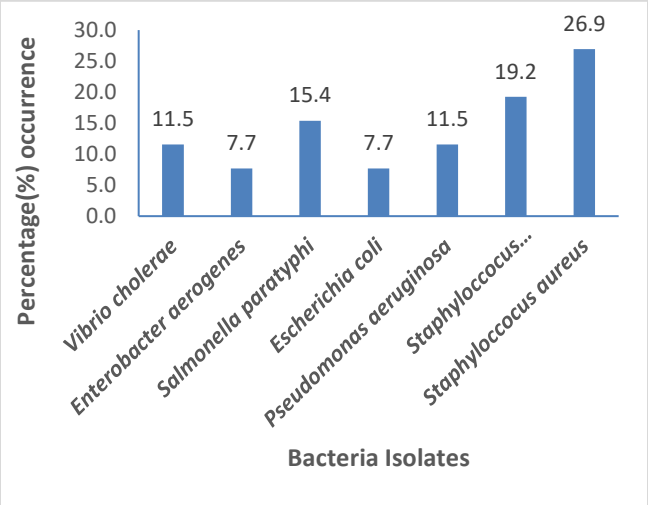

Fig. 3: Prevalence of bacterial species in catfish gills

The result of the prevalence of bacterial species isolated from the gills of catfish is presented in Fig. 3 showed that *Staphylococcus aureus* exhibited the highest frequency (26.9%), followed by *Staphylococcus epidermidis* (19.2%), *Salmonella paratyphi* was the third most prevalent (15.4%), while *Vibrio cholerae* and *Pseudomonas aeruginosa* showed equal occurrence levels (11.5%).

Discussion

Catfish (*Clarias gariepinus*) is one of the most commonly raised species in Nigeria, and aquaculture has grown in importance as a source of protein for human consumption (Ogbukagu et al., 2021). However, the microbial communities found in the tissues of farmed fish, especially in organs like the gills that are constantly in contact with the aquatic environment, have an impact on the quality and safety of the fish.

The study's total heterotrophic bacterial counts (THBC) revealed discernible variations in the microbial load among the catfish farms that were tested. Farm 3 had the lowest THBC ($1.5\times10^5\pm1.1$ CFU/g), while Farm 2 had the highest (3.3×10^5 ±0.02 CFU/g), followed by Farm 1 ($2.4 \times 10^5 \pm 1.3$ CFU/g). These discrepancies most likely resulted from variances in feeding schedules, water management, stocking density, and cleanliness practices, all of which have a major impact on the growth of bacteria on fish gills. The overall mean THBC ($2.4\times10^5 \pm 0.81$ CFU/g) indicates a moderate level of microbial contamination, typical of cultured fish exposed to organic matter and fluctuating environmental conditions.

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The lower values in the current study may indicate comparatively better aquaculture conditions or less environmental contamination when compared to previous reports by Abu & Uwadirioha (2016), Egbebi et al. (2016), and Ajayi (2012), who reported significantly higher counts in catfish gills ranging from $4.5x10^2$ to 2.25×10^7 CFU/ml.

The bacterial species identified through molecular analysis comprised a diverse microbial community, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Enterobacter aerogenes*, *Vibrio cholerae*, and *Escherichia coli*. The high percentage occurrence of *S. aureus* could imply contamination originating from human handling or contact with surfaces and equipment, which are common routes for the introduction of Gram-positive cocci into aquaculture system (El-Gendy et al., 2024). In a previous study, *Proteus* sp was reported as the most prevalent bacterial isolate in the gills of *C. gariepinus* (Akinyemi et al., 2016). *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* were also dominant, reflecting their known ecological adaptability, metabolic versatility, and ability to persist under nutrient-rich or poorly managed water conditions. The relatively low occurrence of *Vibrio cholerae* and *E. coli* could imply minimal faecal contamination, although their presence still raises important public health concerns due to their pathogenic potential. More so, *Vibrio cholerae* and *Pseudomonas aeruginosa* are known to be moderately distributed in the gills, consistent with their known roles as common aquatic bacteria capable of surviving in diverse water conditions (Uddin and Al-Harbi et al., 2012). The overall bacterial profile aligns with the findings of previous studies from Nigerian aquaculture environments, including Abu & Uwadirioha (2016), Egbebi et al. (2016), and Effiong & Isaac (2019), who similarly reported the presence of *Staphylococcus*, *Pseudomonas*, *Salmonella*, and *E. coli* in fish tissues and surrounding water.

This study has molecularly revealed the presence of the bacterial species; *Vibrio cholerae, Enterobacter aerogenes, Salmonella paratyphi, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis,* and *Staphylococcus aureus*, all of which are potential pathogens and therefore are of potential health risks to fish safety consumers of catfish.

Conclusion

The total heterotrophic bacterial counts were moderate and did not differ significantly across farms. The molecular identification showed the presence of both environmental and clinically significant bacteria, including *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella paratyphi, Escherichia coli,* and *Vibrio cholerae.* Thus, the consumption of improperly cooked fish could lead to infections associated with these pathogens. There is therefore the need by relevant authorities to have policies in place for improved aquaculture management practices to ensure fish safety and public health.

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