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Biochemical and Molecular Characterization of *Pseudomonas putida*, *P. fluorescens* and *P. aeruginosa* Isolate from *Oreochromis niloticus* (TILAPIA) and *Clarias gariepinus* (AFRICAN CATFISH)

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ABSTRACT

Bacterial pathogens are a major cause of economic losses in aquaculture and pose serious threat to public health. This study investigates the phenotypic and the genomic characteristics of pathogenic *Pseudomonas* species in two finfish species, *Oreochromis niloticus* (Tilapia) and *Clarias gariepinus* (African catfish), sourced from the Galadima fish market in Kano metropolis, Nigeria. Twenty fish samples, including 10 Tilapia and 10 African catfish were randomly selected and tissue samples (liver, spleen, intestine and gills) were collected for analysis. *Pseudomonas* spp. were screened via culturing and isolation techniques and biochemical tests. Molecular identification was carried out based on 16S rRNA gene sequence analysis. Out of all the samples analyzed, 6 % tested positive for *Pseudomonas* spp., including *P. putida* (5 %), *P. aeruginosa* (1.25 %), and *P. fluorescens* (1.25 %). This study confirms the presence of potentially pathogenic *Pseudomonas* species in commercially important finfish species from the Galadima fish market in Kano, Nigeria. These findings highlight the need for regular monitoring and molecular surveillance of bacterial pathogens in aquaculture products to prevent economic losses and safeguard public health.

Keywords

Tilapia, African catfish, Sequencing, Bacteria

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Abbreviations

PCR: Polymerase chain reaction
NCBI: National Center for Biotechnology Information
MR: Methyl red, VP: Voges Proskauer
BLAST: Basic local alignment search tool

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Introduction

Aquaculture is currently the fastest-growing sector of food-animal production globally [1] . In 2022, global aquaculture production reached approximately 130.9 million tons, significantly contributing to food security, especially in food-insecure regions [2]. The industry also supports the livelihood of millions of people worldwide [3]. According to World Bank (2024) classification, the total share of fisheries and aquaculture harvested by non-high-income countries in recent decades has increased from about 33 % in the 1950s to 84 % in 2022, Of this total production, the upper-middle-income countries contributed 56 %, lower-middle-income countries 26 %, high-income countries 16 % and low-income countries 2 % [3]. Global fish production is estimated to reach approximately 196 million tons by 2025 accounting for about 17.3 % of the total consumption of animal protein worldwide. In 2017, fish represented about 6.8% of the total animal protein consumption, with a per capita consumption estimate of 20.3 kg. This provides about 20 % of average per capita animal protein diet for nearly 3.3 billion people, and contributes at least 10 % of such protein intake for 5.6 billion people [4].

Finfish species such as tilapia and catfish are affordable sources of animal protein, and by-products [5]. They also serves as a source of income for low-income populations in rural, developing or undeveloped areas [6]. These species are known generally to occupy various habitats (freshwater, brackish or marine) and come in diverse shapes, sizes and biological adaptations.

Despite these benefits, infectious diseases remain a major setback to the aquaculture industry. They result in great economic losses due to fish mortality, poor marketability, increased treatment cost and potential zoonotic disease transmission [7].

Among bacteria pathogens *Pseudomonas* species stand out as gram-negative opportunistic bacteria, with high adaptability to survive in various environmental conditions, including the aquaculture environment [8]. Numerous species of *Pseudomonas* are known to be pathogenic for Aquatic and other animals including humans [9]. Pseudomonadiasis is one of the most prevalent diseases in global aquaculture [10], causing severe economic losses and decreased fish farming efficiency [11]. It has been reported to be among the most common diseases of fish, causing almost 100 % mortality in some cases [12]. Although many Pseudomonas spp have been described as opportunistic pathogens, several species including *P. putida, P. fluorescence, P. aeruginosa, P. anguilliseptica, P. baetica, P. chlororaphis, P. koreensis, P. luteola, P. plecoglossicida,* and *P. pseudoalcaligenes* were identified to be the primary pathogens of several disease cases of farmed fish [8, 9]..

In regions like Nigeria, limited information about the exact pathogen responsible for fish diseases hampers the implementation of effective preventative and control measures [7, 10]. Therefore, species-specific detection of *Pseudomonas* may help for establishing a more complete understanding of their pathological significance [13]. Molecular detection could also guide researchers to gain a clear understanding of the ecological impact of the pathogen and also overcome the deficiencies of the traditional approaches.

Given the limited literature and data on the molecular characterization of important microorganisms of fish and environmental health concerns from the study area, this study is the first of its kind in Kano, Nigeria. The present study aim to describe the phenotypic and genomic characteristics of pathogenic *Pseudomonas* species isolated from two fish species, Oreochromis niloticus and *Clarias gariepinus*, sold at the Galadima fish market in Kano metropolis, Nigeria.

Result

Pseudomonas species isolation rates

In this study, three Pseudomonas species (*P. fluorescens* P. *aeruginosa* and *P. putida*) reported to be fish pathogens were isolated and characterized. The overall isolation rate of pathogenic *Pseudomonas* from all the samples was 6 (7.5 %) after molecular characterization (5 % *P. putida*, 1.25 % *P. aeruginosa* and 1.25 % *P. fluorescens*) (Table 1).

Table 1.
Summary of the total isolation rate after Molecular characterization

Isolate	*Clarias gariepinus*	*Oreochromis niloticus*	Total (%)
P. putida	3	1	4 (5)
P. fluorescens	1	0	1 (1.25)
P. aeruginosa	1	0	1 (1.25)
Total	5	1	7.5

Pseudomonas fish species-specific isolation rates

The isolation rate from the African catfish (Clarias gariepinus) was 5 (12.5 %) while the specific isolation rates from the gills, liver, spleen and intestine were 3 (40 %), 0 %, 0 (0 %), 2 (20 %) respectively (Table 2). The isolation rate from the Tilapia (*Oreochromis niloticus*) was 1 (2.5 %) while the specific isolation rate from the gills, liver, spleen and intestine

were 0 %, 0 %, 1 (10) %, 0 % respectively (Table 2). Table 2 shows the isolation rate in different parts of *C. gariepinus* and *O. niloticus* from the market. There was significant difference in the isolation rate between *C. gariepinus* and *O. niloticus* ($p < 0.05$).

Table 2.
The percentage of Pseudomonas isolation in fish species from Galadima market, Kano Nigeria at the level of Biochemical characterization

Organs examined	*Clarias gariepinus*		*Oreochromis niloticus*	
	Isolation rate (out of 10)	%	Isolation rate (out of 10)	%
Gills	4	40	1	10
Liver	0	0	0	0
Spleen	1	10	1	10
Intestine	2	20	0	0
Total (out of 40)	7	17.5	2	5

Organ-Specific isolation rates of Pseudomonas

There were a significant difference in the isolation rate between the various body parts across both fish species ($p < 0.05$). The gills of *C. gariepinus* exhibited the highest rate of contamination, with an isolation rate of 30%. The gills and intestine are the only parts of the *C. gariepinus* contaminated, the intestine followed with a 20 % isolation rate. For the spleen, the isolation rate from the *C. gariepinus* was 0 % and 10 % from the *O. niloticus*. No *Pseudomonas* species were isolated in all the liver samples from both *C. gariepinus* and *O. niloticus*. Overall, the gills and intestines were the primary sites of *Pseudomonas* presence in *C. gariepinus*, while the spleen was the only contaminated site in O. niloticus. The gills samples from *C. gariepinus* had a higher isolation rate (30 %) than the O. niloticus (0 %). Similarly, the intestine of *C. gariepinus* had a higher isolation rate (20 %) than the *O. niloticus* (10 %). However, the spleen samples from *O. niloticus* had a higher isolation rate (10 %) than the *C. gariepinus* (%) (Table 3).

Table 3.
The percentage of Pseudomonas isolation in fish species from Galadima market, Kano Nigeria after Molecular characterization

Organs examined	*Clarias gariepinus*		*Oreochromis niloticus*	
	Isolation rate	%	Isolation rate	%
Gills	3	30	0	0
Liver	0	0	0	0
Spleen	0	0	1	10
Intestine	2	20	0	0
Total	5	12.5	1	2.5

Discussion

Fish and fishery products are vital for global nutrition and food security. Nonetheless, they are highly perishable, with quality influenced by species differences, feeding habits, and environmental conditions [15]. Improperly processed or raw fish can act as vehicle for pathogenic microorganisms, posing notable public health risks [16]. Among these, *Pseudomonas* spp are recongnised as important opportunistic pathogens in fish, with the potential to cause disease in humans while also serving as indicators of fish quality and safety [9]. In this study we established to species level the occurrence of the fish pathogen (*Pseudomonas* spp) of public health importance with an overall prevalence of 6 %.

Masbouba [17] reported a higher isolation rates, 36.9 % for *P. fluorescens* and 29.1 % for *P. aureginosa*, from diseased *Clarias gariepinus*. The elevated isolation rates in that study may be attributed to sampling clinically infected fish from an outbreak on a farm. Olayemi *et al.* [18] isolated *P. aerugenosa* from the gills of *C. gariepinus* in Ile-Ife, Nigeria.

The presence of *Pseudomonas* in the spleen, intestine and gills of fish (*C. gariepinus* and *O. niloticus*) sampled from the Galadima fish market in Kano metropolis showed bacterial contamination. This cause for concern, as *P. putida* and *P. fluorescens* are known to cause septicemia in fish. Their infections often resemble motile Aeromonas septicemia especially in stressed fish stocks [19,20]. As fish serve as an affordable source of protein, such contamination poses a serious health hazard to humans and may serve as zoonosis risks to consumers [21].

Interestingly, the findings of this study differs considerably from those of a similar investigation in Khartoum, where isolation rates of Pseudomonas from gills and intestines were 63 % and 31 % respectively [22]. The differences in isolation and characterization methodologies, sample origin, hygienic practices and the different sources of water and its quality may have contributed to the differing results. Moreover, the higher isolation rates in the

previous study may be associated with clinical infections in fish populations.

Fisheries are vital to food security, livelihoods, and economic growth in many Africa nations, including Nigeria [21, 23]. The study's findings indicate that fish in Kano region are susceptible to Pseudomonas infections. These infections have the potential to degrade fish quality, higher growth rates, increase mortality, and ultimately reduce profit margins for fish farmers. Additionally, managing such infections often requires antibiotic treatment, which raises production costs [24].

Pseudomonas species are particularly concerning due to their resistance to multiple antibiotics. In Nigeria, the regulation of antibiotic use in aquaculture is weak, increasing the risk of antimicrobial resistance (AMR) [25]. This issue threatens not just local aquaculture sustainability but also feeds into the global AMR crisis. The situation becomes even more alarming when Pseudomonas infections are transmitted to humans [26]. The risk escalates when Pseudomonas infections are transmitted to humans, *P. aeruginosa* is an opportunistic human pathogen and can pose serious health risks, especially to immunocompromised individuals, through the handling or consumption of contaminated fish [27].

In conclusion, the detection of *P. putida*, *P. aeruginosa* and *P. fluorescens* in fish from Kano, Nigeria, underscores the need for improved public awareness and deeper understanding of pathogenic bacterial infections in aquaculture. Enforcing strict hygienic control protocols, implementing preventive strategies, and promoting effective biosecurity practices are essential steps toward safeguarding healthy aquaculture environments

Materials and Methods

Study Area

This study was conducted at Galadima market, located in Fagge Local Government Area of Kano state, Nigeria (Latitude 12.0127° N and longitude 8.5344° E) [14]. Galadima Market is the largest hub for fresh fish in the region, where farmers supply fresh fish for wholesale and retails from the wild and in captivity (aquaculture) for consumption.

Study design

The aim of this research was to isolate *Pseudomonas* species of medical importance from two major fish species freshly brought for sale at the Galadima fish market in the Kano metropolis. A total of 80 tissue samples were collected, comprising the gills, liver, spleen, and intestine, from 20 fish specimens (10 *Clarias gariepinus* and 10 Oreochromis niloticus). Their morphometric measurements were also recorded (Table 4). Samples were cultured on Nutrient agar and and bacterial isolates were subjected to biochemical characterization followed by molecular identification through DNA sequencing.

Culture and Bacterial isolation

Culture, isolation and identification of bacteria were carried out based on the method described by [8]. Each sample was inoculated on Nutrient agar and incubated at 37°C for 24 hours. The growths were subjected to Gram staining and biochemical test for further identification.

The inoculation Media was prepared based on the manufacturer's instructions. A sterilized spatula was used to cauterize the surface of the sample. The cauterized surface was then cut using sterilized scissors. A swab stick was then inserted deep into the cut tissue sample to collect samples for primary smear preparation. Secondary and tertiary smear were made using a sterilized wire loop. The samples were incubated at 37oc for 24 hours. Plates were examined for bacterial growth, subcultured and re-incubated at 37°C for another 24 hours. Colonial morphology was then studied (Figure I). All isolates were preserved and later sent to South Africa for DNA sequencing.

Biochemical characterisation

Biochemical tests were conducted after the colony morphology assessment, assessment to further characterize the isolates (Figure 2). Standard conventional biochemical assays, included Gram staining, urease, citrate, catalase activities and oxidase, Indole, Methyl red (MR), Voges Proskauer (VP) and motility tests were carried out [8]. The test tubes containing the test media were labeled and arranged properly in a test tube rack, each was inoculated with an inoculum of the isolates and incubated at 37oc for 24 hours. After incubation, reagents such as Kovac's, VP1 and VP2 were added to the incubated peptone water for both Indole and MRVP tests respectively Reaction were recorded accordingly (Table 5) and DNA was extracted from confirmed isolates for sequencing.

Table 4.
Morphometric measurements of the Oreochromis niloticus and Clarias gariepinus collected from Galadima Fish Market, Kano State, Nigeria.

S/N	Fish Species	Total Length (cm) ± SD	Total Weight (g) ± SD	Standard Length (cm) ± SD
1	Oreochromis niloticus (range)	39.07 ± 5.52 (26.1 - 49.6)	589.23 ± 233.9 (140 - 1150)	36.27 ± 5.23 (22.15 - 47.2)
2	Clarias gariepinus (range)	41.29 ± 3.44 (35.0 - 48.2)	658.94 ± 174.2 (353.2 - 1020.5)	39.0 ± 3.45 (33.3 - 46.0)

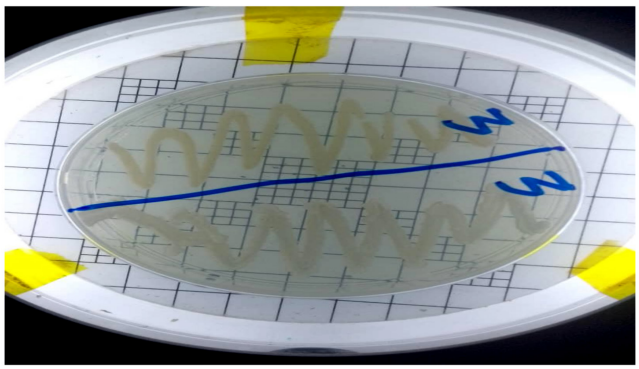

Figure 1.
Pseudomonas on nutrient agar

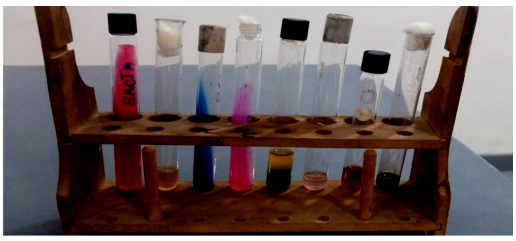

Figure 2.
Biochemical identification of Pseudomonas species

Table 5.
Biochemical tests and reactions

S/N	Test	Pseudomonas spp
1	Gram reaction	-
2	Oxidase	+
3	Indole	-
4	Methyl red	-
4	Voges-proskauer	-
5	Citrate	+
6	Catalase	+
7	Urease	-
8	Motility	+

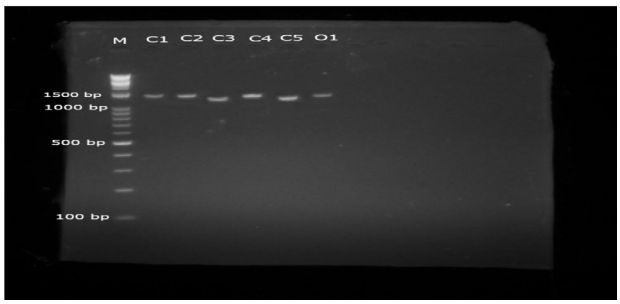

Figure 3.
Gel image showing position and base pairs for the different Pseudomonas species. Keys: C= *Clarias gariepinus*; O= *Oreochromis niloticus*; M= *Molecular ladder*: C1 = *P. putida*, C2= *P. putida*, C3= *P. fluorescen*, C4= *P. putida*, C5= *P. aeruginosa*, O1 = *P. putida*.

Extraction of genomic DNA, amplification by PCR and Sequencing

The confirmed isolates were sent to Inqaba Biotec™ (Pretoria, South Africa) for PCR, agarose gel electrophoresis and Sanger sequencing. Genomic DNA was extracted using the QIAamp DNA kit (Qiagen, Germany). DNA concentration and purity were assessed before molecular analysis. Primer selection, PCR amplification, purification of the amplified products, DNA sequencing and the sequence analysis were performed as described by Duman et al., 2021 [8].

PCR amplication targeted universal primer 16S rRNA (small subunit ribosomal RNA) gene using the primers listed in Table 6.

Figure 3 is a gel image showing position and base pairs for the different *Pseudomonas* species.

Species level identification was performed using sequencing of DNA dependent RNA polymerase subunits. The sequences obtained from the sequencing were analyzed and aligned. By BLAST the homologous searches were done from the results of the sequencing. The nucleotide sequence of the PCR product showed 99.29, 99.22, 94.81 % and 94.72 *Pseudomonas* putida, 96.52 % *Pseudomonas* aeruginosa and 94.72 % *Pseudomonas* fluorescens respectively. The results revealed similarity levels to published *Pseudomonas* species sequences in the NCBI database.

Authors' Contributions

A, B, and C conceived, designed and supervised the experiment
A, B, D, E, H and I collected and prepared the samples for the experiment
C, D, E, F, G, H and I conducted the experiment
D, G and F analysed and interpreted the results
A, B, C, F and E led the writing of the manuscript.

All authors contributed significantly to the success of the experiment, analysis and manuscript writing.

Table 6.
Universal Primers

Universal primer set used for PCR amplification	Target	Sequence (5' to 3')
16S-27F	16S rDNA sequence	AGAGTTTGATCMTGGCTCAG
16S-1492R	16S rDNA sequence	CGGTTACCTTGTTACGACTT

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Competing Interests

The authors declare that there is no conflict of interest.

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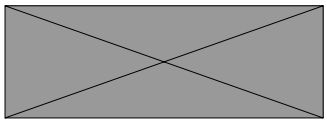

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