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MOLECULAR CHARACTERIZATION, VIRULENCE PROFILING, AND ANTIMICROBIAL RESISTANCE OF *Aeromonas* ISOLATES FROM CULTURED *Clarias gariepinus*: IMPLICATIONS FOR AQUACULTURE AND PUBLIC HEALTH

[*1,2]O.M. RUBEN, [2]G.E. ADEBAYO, [2]D.O. FOLORUNSHO-ROBERTS, [3]D.A. ADAH, [5]A.G. OGUNTUASE, *AND* **[4,6]O.B. DAODU**

[1] *Landmark University SDG 2 – Zero Hunger, Landmark University, Omu-Aran 251103, Nigeria*
[2] *College of Agricultural Sciences, Landmark University, Omu-Aran 251103, Nigeria*
[3] *Department of Veterinary Medicine, Faculty of Veterinary Sciences, University of Ilorin, Ilorin 240211, Nigeria*
[4] *BioCore Diagnostics Veterinary Centre, Ilorin 240211, Nigeria*
[5] *College of Pure and Applied Sciences, Landmark University, Omu-Aran 251103, Nigeria*
[6] *Department of Veterinary Microbiology, Faculty of Veterinary Sciences, University of Ilorin, Ilorin 240211, Nigeria*
[*] *Correspondence: oghenochuko.oghenebrorhie@lmu.edu.ng +2348069843853, 0000-0002-4622-3950*

ABSTRACT

Bacterial pathogens like Aeromonas threaten aquaculture through high fish mortality and pose significant zoonotic risks to humans due to increasing multidrug resistance. This study aimed to characterize Aeromonas isolates from cultured Clarias gariepinus in Kwara State, Nigeria, examining their species diversity, virulence profiles, antimicrobial resistance, and associated public health risks. A total of 119 catfish were sampled across 13 farms; isolates were identified via biochemical tests and 16S rRNA-PCR, followed by screening for virulence (flaA, aerA/haem) and resistance (TEM, CTX, SHV) genes. Molecular characterization and phylogenetic analysis of sequenced isolates confirmed species identities with GenBank accession numbers PV364439, PV364441, PV364438, and PV364443. Findings also revealed 63 of 119 (52.9%) fish tested positive as Aeromonas hosts, with A. dhakensis (55.7%), A. hydrophila (24.05%), and A. veronii (6.33%) being the primary species identified. All isolates exhibited multidrug resistance (MAR index > 0.2), including 100% resistance to amoxicillin-clavulanate (Augmentin) and 98.73% to Amoxycillin. Genetic analysis detected the flaA virulence gene in 44.4% of selected isolates and resistance genes TEM (50%), CTX (16.7%), and SHV (11.1%). Socio-demographic data highlighted significant awareness gaps, with 60% of farmers handling fish with bare hands despite having open wounds. The study concludes that the high prevalence of virulent, multidrug-resistant Aeromonas strains poses a severe threat to food security and public health. It recommends mandatory veterinary oversight for antibiotic use and improved farmer education on zoonotic risks to ensure sustainable aquaculture

Keywords: Antimicrobial resistance, Aquaculture, Food security, *Clarias gariepinus*, Public health

INTRODUCTION

Bacterial pathogens threaten fish production, leading to high mortality rates and increased production costs in aquaculture systems (Okon *et al*., 2022; 2023). The *Aeromonas* genus encompasses over 30 species of gram-negative bacteria, many acting as opportunistic pathogens. These species have been implicated in severe mortality within fish farms, resulting in substantial economic losses (Adah *et al*., 2021; Oladele *et al*., 2021). Both fish and humans can contract infections caused by pathogenic *Aeromonas* species, raising concerns about their presence in aquaculture environments (Ahmed *et al*., 2018). The risk of infection extends to farmers and their families who work in contaminated settings, particularly in regions where aquatic animals are consumed fresh. Moreover, studies indicate that *Aeromonas* species exhibit resistance to multiple antibiotics (Igere *et al*., 2021; Saka *et al*., 2017), posing a severe public health risk. Treating infections from these resistant strains is more complex compared to non-resistant bacteria, resulting in higher healthcare expenses, extended hospitalizations, increased mortality, and reduced profitability for aquaculture operations. In the United States, *antimicrobial-resistant i*nfections are responsible for more than 2 million cases and around 23,000 deaths each year (Poddar, 2018).

The zoonotic ability and the multidrug resistance documented for members of the *Aeromonads* bacteria are serious challenges to fish farmers, influencing the sustainability of fish farming and food security in general (Bartie & Desbois, 2024; Janda & Abbott, 2010). *Aeromonas* infections in African catfish farming have far-reaching implications for food security and national development, especially in countries where farming of different species of fish is a significant contributor, such as Nigeria (Rasmussen-Ivey et al., 2016). The species, being a valuable protein source in many countries, causes disease-related losses that contribute to decreased food availability and higher prices, affecting food security at both local and national levels – (Odeyemi & Ahmad, 2017). Furthermore, the economic challenges faced by fish farmers due to *Aeromonas* infections can stifle aquaculture growth, limiting its contribution to national economic development and job creation (Piotrowska *et al*., 2017).

Additionally, the treatment of diseases caused by *Aeromonas* spp. in humans and fish, including gastrointestinal disorders, infections of the skin and soft

tissues, and in rare instances, severe systemic infections, is significantly affected by the multidrug resistance that has been documented for these species (Austin & Austin, 2016; Tomás, 2012). This highlights how crucial it is to manage and control *Aeromonas* species in aquaculture for both economic and public health reasons. Disease issues in fish production hinders the achievement of the United Nations Sustainable Development Goals (SDGs), which emphasis sustainability, food security, healthy food production, human health promotion, and water pollution reduction (SDGs 2 & 3) (Lynch *et al*., 2020).

Studies on *Aeromonas* infections in farmed catfish have been documented by various authors in Nigeria (Adah *et al.*, 2021; 2023; Saka *et al*., 2017). These studies have consistently reported the multidrug resistance of the bacteria to commonly used antibiotics such as amoxicillin and tetracyclines. Nevertheless, significant knowledge gaps persist. First, most prior Nigerian studies have relied predominantly on phenotypic identification methods, with molecular characterization of *Aeromonas* isolates to species level remaining limited. Second, the co-occurrence of virulence genes (particularly *flaA* and *aerA/haem*) alongside antimicrobial resistance genes (TEM, CTX-M, SHV) within the same isolates has not been systematically investigated in *C. gariepinus* farms in Kwara State. Third, and critically, the zoonotic awareness and biosafety practices of farmers who handle these fish daily have received little empirical attention.

Therefore, this study aimed to: (i) determine the prevalence of Aeromonas species in farmed *C. gariepinus* in Kwara State; (ii) characterize isolates to the species level using biochemical and molecular methods; (iii) assess virulence gene profiles (flaA, aerA/haem) and antimicrobial resistance genes (TEM, CTX-M, SHV); and (iv) evaluate fish farmers' awareness of zoonotic risks and biosafety practices.

MATERIALS AND METHODS

Study locations and sites

The study was conducted in Irepodun Local Government Area (LGA), Kwara State, Nigeria, located at coordinates 8° 8' 48" N and 4° 54' 47" E (Figure 1). The LGA covers an area of 737 km² and comprises nine districts: Irepodun-Oro, Omu-Arun, Ajase-Ipo, Arandun, Esie, Ipetu, Rore, Aran-Orin, and Ijan. A total of six (6) active fish farms were identified across these districts.

Purchase of Fish

A total of 119 sub-adult catfish (*Clarias gariepinus*) with an average live weight of 300 g were purchased from randomly selected farms across four (4) districts within the LGA. The fish were transported in separate sterile bowls containing 10 L of cold water at a temperature of 17°C to the Microbiology Laboratory and molecular laboratory of BioCORE Veterinary Diagnostic Center, Ilorin, Kwara State.

Knowledge Assessment on Fish Health and Zoonotic Bacteria

In conjunction with fish sample collection, a semi-structured questionnaire was administered to 30 fish farmers and processors to assess their knowledge regarding fish health, zoonotic diseases, and the handling/management of diseased fish. The sample size represents the total active fish farmers, farm attendants, and processors in the area.

Preparation of Fish for Microbiology

Upon arrival at the laboratory, the experimental fish were placed in ice-cold water (10°C) to immobilise them for anesthesia, as described by (Kinkel *et al*., 2010). Fish were then placed on laboratory slabs and disinfected with methylated spirit before dissection to access internal organs.

Sample Collection, Culture, and Isolation

Swabs were taken from the gills and intestines of each catfish and inoculated into alkaline peptone water. The samples were incubated for 24 hours at 37°C. Subsequently, a subculture was performed on Aeromonas Medium Base (Ryan) agar (Oxoid, CM0833, United Kingdom) and incubated for an additional 24 hours at 37°C.

Isolate Identification

Biochemical identification was conducted to characterise bacterial isolates following the World Health Organisation (WHO) manual for laboratory investigation of bacterial organisms (Abbott *et al*, 2003; Mzula *et al*., 2019). Isolates exhibiting characteristics consistent with *Aeromonas* were subjected to biochemical tests, including catalase test, oxidase test, and other sugar fermentation tests (glucose, glycerol, lactose, maltose, D-mannitol, D-sorbitol, sucrose, dulcitol, galactose). All isolates were preserved as glycerol stocks according to standard methods and stored at −20°C until further analysis (Drk *et al*., 2023). Validation of identified *Aeromonas* species was performed using the online Advanced Bacteria Identification Software (ABIS) (Sorescu & Stoica, 2021), where biochemical results were inputted to obtain a percentage probability for species identification.

Nucleic Acid Extraction

From the 79 Aeromonas isolates, 19 were selected to include representatives of all four phenotypic species groups (proportional to species distribution). The Nigerian Institute of Medical Research's (NIMR) Genomic DNA purification kit was used to extract DNA from phenotypically identified *Aeromonas* species isolated from *Clarias gariepinus*. This was done in order to validate the presumed *Aeromonas* species and the manufacturer's instructions on DNA purification from bacteria were strictly followed. 100 µl of the bacterial cells was harvested into phosphate buffer saline (PBS), and mixed vigorously using the Sybron Thermolyne Maxi Mix II Vortex (M-16715 Mixer). Using micropipette, 1 ml of the bacteria mixture was put into a 1.5 ml centrifuge tube and centrifuged using the Eppendorf Centrifuge 5420 (Eppendorf SE, Germany) at 10,000 g for 5 minutes and the supernatant discarded. 300 µl of lysis buffer was added and vortexed for 30 seconds. 10 µl of proteinase K was then added and the mixture vortexed again. The

mixture was then incubated for 10 minutes and allowed to cool. 300 µl of the binding buffer was added, vortexed, and centrifuged at 10,000 g for 1 minute. 200 µl of absolute ethanol was then added to the sample. The sample was then transferred into the spin column placed into a collection tube and centrifuged for 1 minute. The flow through in the collection tube was discarded and the opening of the collection tube blot on a tissue and reinserted into the collection tube. 500 µl of wash buffer 1 was added and centrifuged at 10,000 g for 1 minute, and the flow through discarded. This was repeated for was buffer 2. The spin column and collection tube were dry spanned at highest centrifugal speed for 2 minutes. The spin column was placed into a fresh 1.5 centrifuge tube, and the collection tube was disposed of. The eluted DNA was then used for PCR amplification after 100 µl of elution buffer was added straight to the center of the spin column, incubated for one minute at room temperature, and centrifuged for one minute at 10,000 g.

Primer selection, Gene amplification, and Gel Electrophoresis

Following the selection made by Adah *et al*. (2024) in her research, published primers for bacterial identification that target the 16S rRNA were chosen. Conventional polymerase chain reactions (PCR) were used to amplify the 16S rRNA in order to characterize the isolates of *Aeromonas* to the species level. 16S F: (5'GTGCCAGCAGCCGCGCTAA3') and 16S R: (5'AGACCCGGGAACGTATTCAC3'), which were produced at Macrogen (South Korea), were used in the study Table 1. Five microliters (10 ng/µl) of DNA template, ten and two microliters of nuclease-free water, 0.4 microliters (10 µl) of each forward and reverse primer (Macrogen, South Korea), and 4 µl of PCR master mix (FIREPol ® Master Mix Ready to Load (5x), Solis Biodyne, Estonia) were used in the 20 µl PCR reaction. The steps followed in conducting the PCR include: initial 3 minutes of denaturation at 95°C, 30 cycles of denaturation at 95°C for 30 seconds, 30 seconds of annealing at 58°C, 100 seconds of extension at 72°C, and 7 minutes of final extension at 72°C using the PCR Mastercycler ® nexus (Eppendorf, Hamburg, Germany). The PCR products of amplified genes were separated by subjecting 5 µl of the PCR products to 1.8 % agarose gel (CLS-AG 100[LE Agarose], Cleaver Scientific, UK) electrophoresis at 80 V for 45 minutes in 50x TAE buffer followed by 30 minutes stainning in ethidium bromide solution (30 µl). A 100 bp DNA ladder, Ready to Load, from Solis Biodyne in Estonia, was utilized to specify the amplicon sizes and viewed using the UVP Transilluminator (Upland, CA 91786 USA) and photographed.

Table 1: Primer sets used in this study

Gene class	Primer name	Primer sequence direction (5-3)	Amplicon size (bp)
rrs gene	16 S RNA	F: GTGCCAGCAGCCGCGCTAA R: AGACCCGGGAACGTATTCAC	1,500
Virulent genes	Fla	F: TCCAACCGTYTGACCTC R: GMYTGGTTGCGRATGGT	414
	Aer-A	F: AAACCTGGCTTACGCAACTGT R: ACCCGTCTGCAAATCATGGAT	269
Antimicrobial resistant genes	TEM-164.SE TEM-165.AS	TCGCCGCATACACTATTCTCAGAATGA ACGCTCACCGGCTCCAGATTTAT	108
	CTX-M-U1 CTX-M-U2	ATGTGCAGYACCAGTAARGTKATGGC TGGGTRAARTARGTSACCAGAAYCAGCGG	585
	Bla-SHV.SE Bla-SHV.AS	ATGCGTTATATTCGCCTGTG TGCTTTGTTATTCGGGCCAA	593

Additionally, virulent and antibiotic-resistant genes present in the PCR positive isolates selected were detected using FlaF and R, Aer-aF and R, TEM-164.SE and 165.AS, CTX-M-U1 and U2, and Bla-SHV.SE and AS. Inqaba Biotechnical Industries (Pty) Ltd (Pretoria, South Africa) manufactured all the primers. Similar steps were followed for PCR and gel electrophoresis with slight differences in the condition. The cycling conditions included 94°C denaturation for 10 minutes, 30 cycles of 94°C denaturation for 1 minute, 60°C annealing for 1 minute, 72°C extension for 1 minute, and a final extension step at 72°C for 7 minutes.

Genomic sequencing and Phylogenetic analysis

Four (4) amplicons were selected for sequencing using stratified proportional sampling, ensuring the subsample reflected the phenotypic diversity and relative abundance of species groups present in the full isolate collection, rather than being an arbitrary subset. Sequencing was carried out at the Inqaba Biotec West Africa (IBWA) using the BigDye Terminator version 3.1 Cycle Sequencing Kit, the isolates were sequenced using an Applied Biosystems 3130xl Genetic Analyzer (Thermo Fisher Scientific, USA). Sequence was then analyzed using the BLAST and reference strains obtained from GenBank. Sequences were aligned using BioEdit v7.2.5, and a phylogenetic

tree was constructed using MEGA11 (or MEGA X) employing the Neighbor-Joining method with 1,000 bootstrap replicates. The Kimura 2-parameter model was used for distance calculation.

Statistical analysis of data

The antibiotic susceptibility data was analyzed using MS Excel and presented in tabular and graphical forms. Prevalence and diversity were presented as simple percentages.

RESULTS

Socio-Demography and Fish Farming Experiences

A total of 30 fish farmers were interviewed using a semi-structured questionnaire. Among the respondents, 60% (n = 18) were male, and their ages ranged from 19 to over 60 years. The educational background of the farmers revealed that the majority had attained tertiary education (70%, n = 21), while 6.7% (n = 2) had no formal education. Most of the interviewed farmers were married (56.7%, n = 17) and reported family sizes ranging from 2 to over 10 members.

The respondents' experience in fish farming varied significantly, with durations ranging from less than one year to 40 years. Most farmers owned their fish ponds (76.7%, n = 23), with sizes varying from 100 to 2000 m^2; conversely, 23.3% (n = 7) reported using rented fish ponds. The predominant farming system was monoculture, practiced by 70% (n = 21) of the farmers, while 26.7% (n = 8) engaged in polyculture, and only 3.3% (n = 1) utilized both monoculture and polyculture systems.

Farmers' Physical Exposure and Injury

Over 90% of the interviewed farmers reported using their bare hands to handle farmed fish, with 80% indicating that the pectoral fins of catfish had pierced them during handling. Additionally, a significant majority (86.7%) acknowledged that water from the culture environment had splashed into their mouths, eyes, and bodies while handling the fish (see Table 2).

Table 2: Farmers' exposure to cultured fish and possible injury

Exposure	Category	Frequency	Percentage
Have you used your bare hands to hold catfish?	Yes	29	96.7
	No	1	3.3
Have you been pierced by the pectoral fin before?	Yes	24	80.0
	No	6	20.0
After working with catfish, what do you do	Wash hands with water	5	16.7
	Wash with soap and water	21	70.0
	I do not do anything	4	13.3
Have you developed an abscess or wound from 3 above	Yes	10	33.3
	No	20	66.7
Have you heard of other fish workers complaining of pain from injury	Yes	26	86.7
	No	4	13.3
Has pond water splashed in your eye/mouth/body before	Yes	26	86.7
	No	4	13.3
If yes, has this caused irritation to your eyes/ body before?	Yes	14	46.7
	No	16	53.3
What do you do after water splash into your eyes	Wipe my face with a clean cloth	5	16.7
	Wash my face with water only	16	53.3
	wash my face with soap and water	6	20.0
	Both clean clothes and water	1	3.3
	Nil	2	6.7
How fast is the action taken in 4b	immediately after splash	5	16.7
	15-25 minutes	3	10.7
	After the day's work	22	73.3
	Nil	9	30.0

Farmers' awareness and knowledge of fish health and zoonosis

Figure 1 and Table 3 illustrate the practices, awareness, and knowledge of fish health and zoonosis among the interviewed farmers. Notably, over 50% of the farmers responded that they could not be infected through contact with pond water or infected fish (Figure 1). Similarly, 70% (n = 21) of the interviewed farmers reported releasing untreated pond water into the environment. Furthermore, a concerning 60% (n = 18) of the farmers handled fish with bare hands, even when they had open wounds.

Table 3 indicates that most of the interviewed farmers experienced mortality within their farms. Over 50% reported handling dead or diseased fish with their bare hands and even distributing these fish to others. These findings highlight significant gaps in awareness and practices related to fish health and zoonotic risks among the farming community.

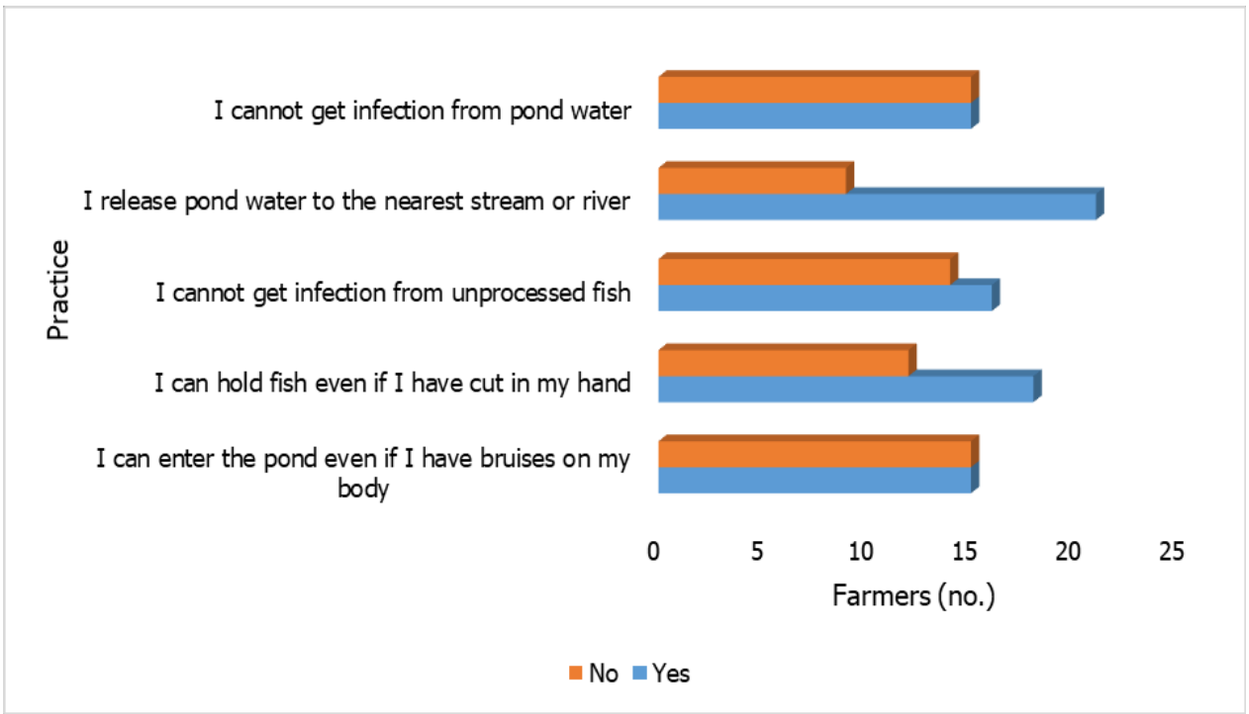

Fig 1: Farmers' awareness and knowledge of fish health and zoonosis

Table 3: Awareness and knowledge of fish health

Exposure	Category	Frequency	Percentage
What do you do when you notice your fish are showing signs of disease	Isolate fish with bare hands	3	10.0
	Throw diseased fish away with bare hands	7	23.3
	Process with bare hands	2	6.7
	Give out diseased fish	7	23.3
	I do not notice	11	36.7
On a scale of 1-5, how many times have you handled sick fish in the farm/ processing unit	Never	5	16.7
	Rarely	14	46.7
	Always	2	6.7
	Frequently	2	6.7
	Very frequently	4	13.3
Have you experienced fish mortality in your farm	Yes	20	66.7
	No	8	26.7
	No response	2	6.7
If yes, what do you do with dead fish on the farm?	Process it for sale	11	55.0
	Eat it	2	10.0
	Throw it away in the nearest bush/ river	7	35.0
	Others	0	0.0

Fish Farmers' Knowledge of Preventive Practices

A majority of the interviewed farmers, specifically 66.7% (n = 20), acknowledged that wearing gloves while working with fish would be beneficial. However, 50% (n = 15) expressed the belief that handling catfish while wearing gloves would be challenging (see Table 4). This indicates a recognition of the importance of preventive practices, yet also highlights concerns regarding the practicality of implementing such measures in their daily operations

Table 4: Fish farmers knowledge of preventive practices

Exposure	Category	Frequency	Percentage
What is the source of water for your fish pond	River	12	40.0
	Stream	13	43.3
	Borehole	1	3.3
	Well	4	13.3
How often do you exchange your pond water	Weekly	3	10.0
	Monthly	5	16.7
	Twice per cropping season	10	33.3
	Once per cropping season	12	40.0
Will it be good to wear hand gloves while working with catfish?	Yes	20	66.7
	No	8	26.7
If yes, which of the gloves do you think will be good to wear?	No response	2	6.7
	Latex gloves	7	35.0
	Nylon gloves	2	10.0
	Engineers' gloves	11	55.0
	Leather gloves	0	
	None		
Is it easy to handle fish while wearing gloves?	Yes	13	43.3
	No	15	50.0

Prevalence of Aeromonas species in the study area

All isolates were gram-negative, short rod, and motile with characteristics pointing them to be *A. hydrophila*, *A. dhakensis, A. veronii* and an unidentified *Aeromonas* spp. A total of 79 *Aeromonas* spp. were isolated from fish samples with presumed *A. dhakensis* being the most prevalent at 55.7%, and least prevalent was the presumed *A. veronii* with 6.33% Table 5. Dominance of *Aeromonas* spp. in the gills and intestine of sampled fish seen in Figure 2 revealed a higher prevalence in the gills than intestine. In addition, 63 (52.9%) were identified of the 119 *C. gariepinus* sampled to be host to *Aeromonas* spp. with the highest prevalence observed in Ajase 2 with 85.2% of the sampled fish positive for *Aeromonas* spp. Table 6. Phenotypically identified *Aeromonas* spp. (isolates) were randomly selected and afterward 18 were confirmed using 16S rRNA-PCR.

Table 5: Prevalence of presumed *Aeromonas* by species in the study area

S/n	*Aeromonas* species	Frequency	Percentage
1	*A. dhakensis*	44	55.70
2	*A. hydrophila*	19	24.05
3	*A. veronii*	5	6.33
4	*Aeromonas* sp.	11	13.92
	Total	79	100

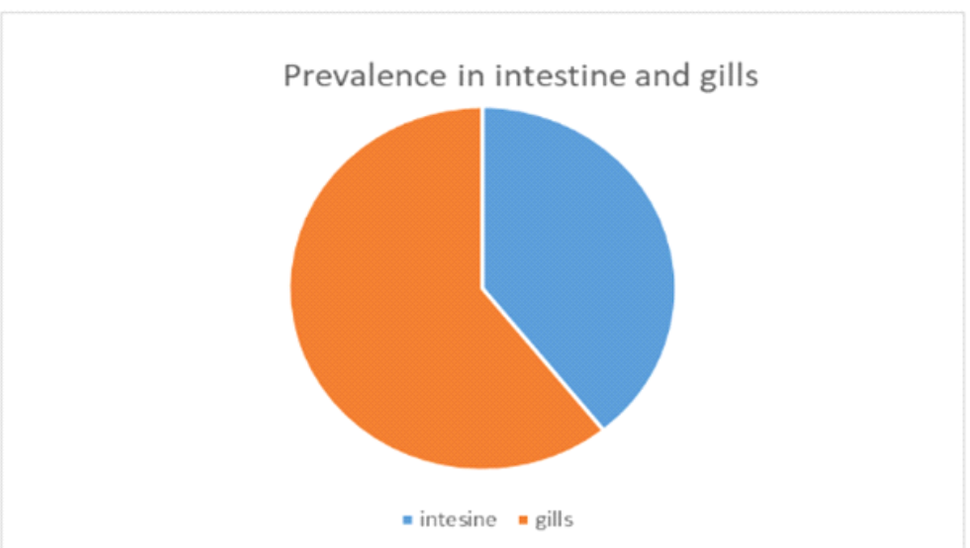

Fig 2: *Aeromonas* prevalence in gills and intestines

Table 6: Farm level prevalence of *Aeromonas*-positive fish by sampling location

Location	No. of fish sampled	Aeromonas positive host	Percentage
Ajase 2	27	23	85.2
Aran Orin	15	3	20
Oro	23	10	43.5
Omu-aran 1	15	9	60
Omu-aran 2	29	11	37.9
Ajase 1	15	7	46.7
Total	119	63	52.9

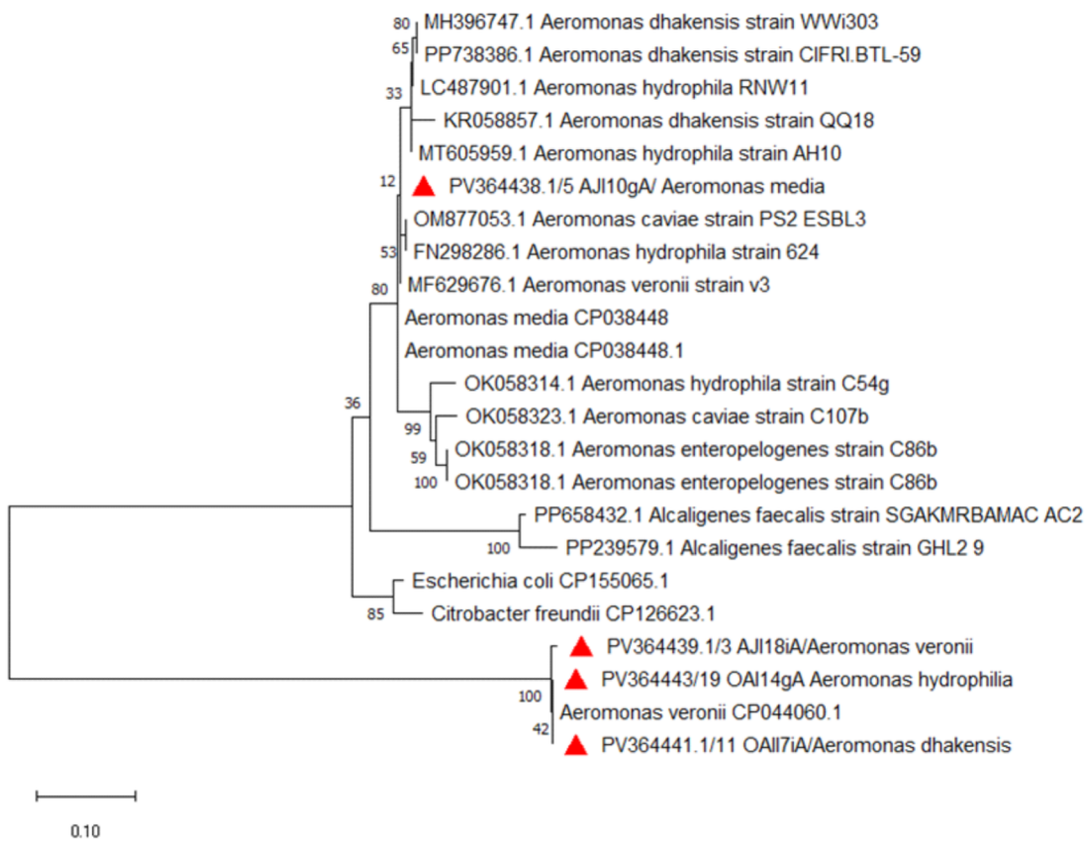

Fig 3: Phylogenetic analysis of selected *Aeromonas* isolate from fish samples (Red triangles indicate sequenced isolates from this study).

Antibiotic susceptibility profile and Multiple antibiotic resistance (MAR) index of Aeromonas species

The antibiogram profile of the isolated and identified *Aeromonas spp.* screened against six (6) different antibiotic groups (Cephalosporin, Carbapenems, Aminoglycosides, Tetracyclines, Fluoroquinolones, and Aminopenicillins) is shown in Table 7. The highest sensitivity profile (73.42%) of *Aeromonas* species from identified isolates was observed for gentamicin, and the highest resistance profile (100%) was observed for amoxicillin-clavulanate. Table 8 shows that the highest MAR index of 0.88 was recorded for *A. veronii* against Oxytetracycline, Gentamicin, Amoxycillin, Norfloxacine, Ciprofloxacin, amoxicillin-clavulanate, Meropenem antibiotics. In contrast, the lowest MAR index, 0.5, was detected for *A. hydrophila* against Oxytetracycline, Meropenem, Amoxycillin and amoxicillin-clavulanate. Antimicrobial resistant genes were profiled following the PCR extraction, and three antimicrobial genes (TEM, CTX, and SHV) of *Aeromonas* sp. were found (Table 9). Eight (8) isolates included the TEM gene, three (3) contained the CTX gene, and two (2) contained the SHV gene.

Table 7: Antibiogram profile of *Aeromonas* species

	***Aeromonas* species isolates (79)**		
Antibiotics (Disk load)	**Susceptible**	**Intermediate**	**Resistant**
Oxytetracycline (30)	20 (25.32%)	10 (12.65%)	49 (62.03%)
Cefuroxime (30)	35 (44.30%)	29 (36.71%)	15 (18.99%)
Gentamicin (10)	58 (73.42%)	5 (6.33%)	16 (20.25%)
Amoxycillin (25)	0 (0%)	1 (1.27%)	78 (98.73%)
Norfloxacin (5)	27 (34.18%)	11 (13.92%)	41 (51.90%)
Ciprofloxacin (5)	28 (35.44%)	28 (35.44%)	23 (29.11%)
Amoxicillin-clavulanate (30)	0 (0%)	0 (0%)	79 (100%)
Meropenem (10)	7 (8.86%)	10 (12.66%)	62 (78.48 %)

Table 8: Multiple antibiotic resistance (MAR) index of *Aeromonas* species

S/n	***Aeromonas* species**	**No of resistant antibiotics**	**Resistance profile**	**MAR index**	**Stance level**
1	*A. hydrophila*	4	OXY, AMX, AUG, MEM	0.5	MDR
2	*A. dhakensis*	6	OXY, CFX, AMX, NOR, AUG, MEM	0.75	MDR
3	*A. veronii*	7	OXY, GEN, AMX, NOR, CIP, AUG, MEM	0.88	MDR
4	*Aeromonas* spp.	6	OXY, GEN, AMX, NOR, AUG, MEM	0.75	MDR

Table 9: Antimicrobial resistant genes in the *Aeromonas* spp.

Extraction Code	**R16 Primer**	**RESULTS**		
		TEM	**CTX**	**SHV**
1	+	-	-	-
2	+	-	-	-
3	+	+	-	-
4	+	+	-	-
5	+	+	-	-
6	+	+	-	-
7	+	+	-	-
8	+	-	-	+
9	+	+	+	-
10	+	+	+	-
11	-	-	-	-
12	+	-	+	-
13	+	+	-	-
14	+	-	-	+
15	+	-	-	-
16	+	-	-	-
17	+	-	-	-
18	+	-	-	-
19	+	-	-	-

Detection of virulence genes

Following PCR extraction, the flaA gene was detected in 44.4% of isolates while aerA was not detected in any isolate (Table 10).

Distribution of virulent and antimicrobial resistant genes in the selected isolates

Among *Aeromonas* species selected, *A. veronii* was the highest (n= 7; 38.9%) while the least was *A. media* (n= 3; 16.7%) (Table 11). Also, TEM gene was the most common among the selected isolates (8; 50%) while SHV was the least (n= 2; 11.1%). Among the *Aeromonas* species selected, *A. media* had the highest isolates with TEM gene while none was found among *A. hydrophilia*. Also, two (2) out of seven *A. veronii* isolates selected had CTX gene (28.6%) while one (1) of four (4) *A. dhakensis* selected had it (25.0%). Only *A. veronii* had SHV gene.

None of the selected *Aeromonas* has aerolysin gene while at least one of the isolates had FLA gene. Both *A. hydrophilia* and *A. media* had the highest.

Table 10. Virulence genes in the *Aeromonas* spp.

Extraction Code	R16 primer	RESULTS	
		aerA/haem	flaA
1	+	-	-
2	+	-	-
3	+	-	+
4	+	-	-
5	+	-	+
6	+	-	+
7	+	-	-
8	+	-	-
9	+	-	+
10	+	-	-
11	-	-	-
12	+	-	-
13	+	-	+
14	+	-	-
15	+	-	+
16	+	-	-
17	+	-	-
18	+	-	-
19	+	-	+

Table 11: Distribution of virulent and antimicrobial resistant genes in the selected isolates

Aeromonas species	No. of isolate (%)	Antibiotic resistant gene			Virulence gene	
		TEM (%)	CTX (%)	SHV (%)	AerA (%)	Fla (%)
A. hydrophila	4 (22.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2(50.0)
A. veronii	7 (38.9)	4 (57.1)	2(28.6)	2 (28.6)	0 (0.0)	2(28.6)
A. media	3 (16.7)	3(100.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (66.7)
A. dhakensis	4 (22.2)	1 (25.0)	1(25.0)	0 (0.0)	0 (0.0)	1(25.0)
TOTAL	**18**	**8 (44.4)**	**3(16.7)**	**2 (11.1)**	**0 (0.0)**	**7(38.9)**

Key:
β-lactamases- TEM, CTX, SHV, Hemolytic toxins- AerA, Fla

Gel electrophoresis

In figure 4, the 16S rRNA gene of the 18 isolates were visible at a base pair of 1500bp. In figure 5, nine (9) TEM gene were visible at a base pair of 108bp, while 3 CTX genes were visible at a base pair of 585bp and 2 SHV genes were visible at a base pair of 593bp. In figure 6, only the Fla gene was present at a 414bp.

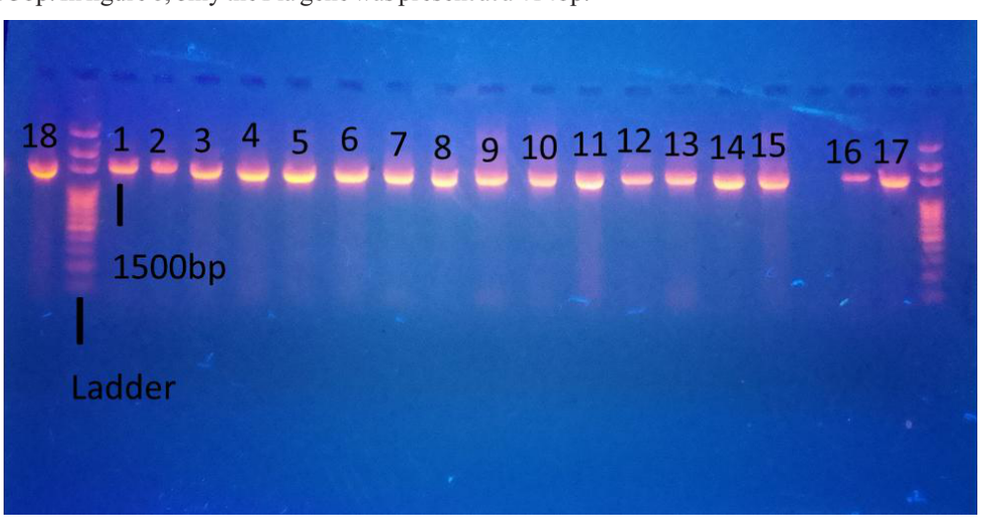

Fig 4: Gel electrophoresis result showing the 16srRNA at 1500bp

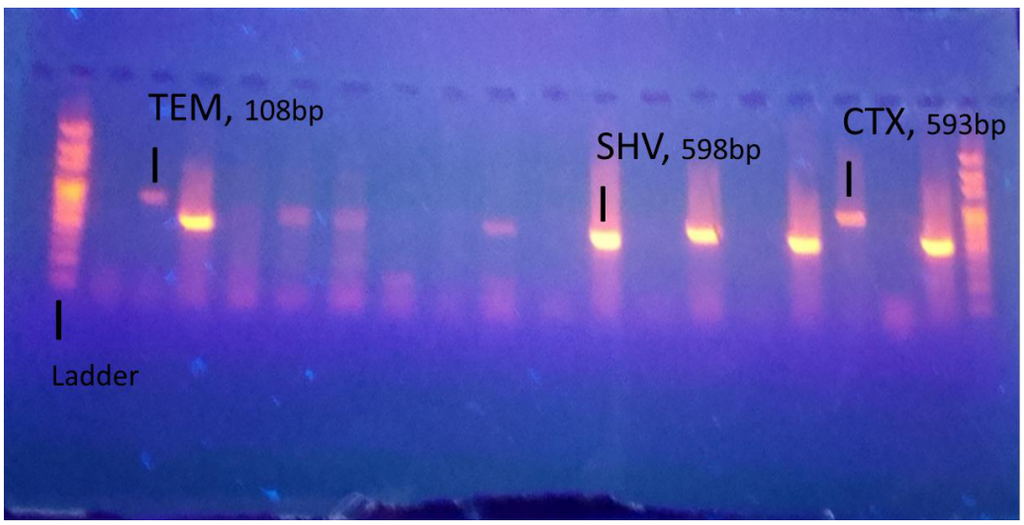

Fig 5: Gel electrophoresis result showing the antibiotic resistant genes TEM, CTX and SHV at 108bp, 585bp and 593bp respectively.

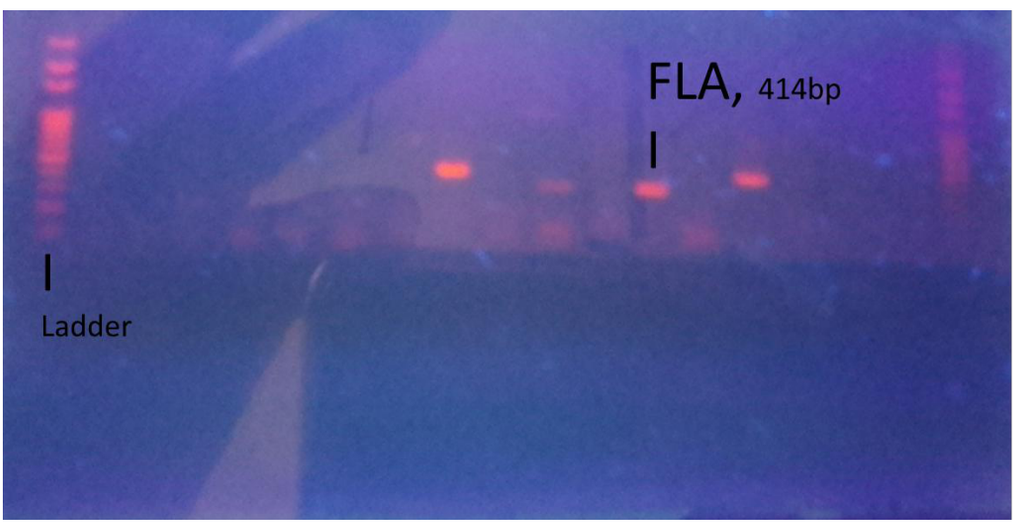

Fig 6: Gel electrophoresis results showing the virulent gene FLA at 414bp

DISCUSSION

The sociodemographic study of fish farmers and processors interviewed in the study area indicates a lack of awareness regarding zoonotic organisms, leading to practices that could jeopardise their health and that of others. Their frequent exposure to diseased fish evidences this, as does the careless release of fish pond water into the environment and the infrequent exchange of impounded fish pond water throughout the cropping season. These findings align with those of (Mzula *et al*. 2019), who reported that many fish farmers have previously experienced fish mortality on their farms.

The prevalence of *Aeromonas* species in the study area can be attributed to the bacteria's waterborne nature (Eluma *et al*., 2023) and the poor management practices adopted by local fish farmers, which facilitate the spread of these pathogens. Variations in prevalence across different sampling areas may result from the interplay between environmental factors, host conditions, and pathogen characteristics (Adah *et al*., 2021). Notably, *A. dhakensis* was identified as the most prevalent *Aeromonas* species isolated from experimental fish, contrasting with Mailafia (2007) findings, which indicated that *A. hydrophila* was the dominant species, while *A. salmonicida* exhibited the lowest frequency. These discrepancies may arise from differences in fish species, isolation methods, geographical locations, and water quality.

The high occurrence of *Aeromonas* spp. in the experimental fish underscores the species' dominance in the aquatic ecosystem. Given the socio-economic significance of fish farming, this elevated prevalence poses potential public health risks, as *Aeromonas* spp. are known to cause various human illnesses (Janda &Abbott., 2010). Although these bacteria are typically killed at temperatures below 100 °C (Isonhood & Drake, 2002), exposure to contaminated water and improper fish processing can lead to infections. The overall prevalence rate of 52.9% for *Aeromonas* spp. recorded in this study exceeds the 21% reported by Mailafia (2007) in Zaria,

Nigeria, yet is comparable to Kishk *et al*. (2020) findings of a 50.67% prevalence from 75 fish samples.
Furthermore, a narrow diversity of species was observed in this study; among the identified isolates (79), from the genus Aeromonas, 4 different species were recognized. This aligns with Fauzi *et al*. (2021) report of only four species from 61 isolates. Similarly, Adah *et al*., (2023) documented *A. caviae, A. hydrophila, A. veronii biovar sobria,* and *A. veronii biovar veronii* from a total of 55 isolates obtained from *Clarias gariepinus* reared in concrete tanks in Kwara State. Mzula *et al*. (2019) noted only two *Aeromonas* spp. in their study on farmed Nile tilapia from Tanzania; however, Igbinosa *et al.* (2012), identified 24 *Aeromonas* spp. in their research.

High percentage of *Aeromonas* isolate susceptibility to Gentamicin (first generation) followed by cefuroxime (second generation) suggest that these antibiotics could effectively treat infections caused by these bacteria and corroborate Almeida et al.'s findings (Almeida *et al*., 2023). Gentamicin might be recommended for empirical therapy of *Aeromonas*-associated bacteremia, as also seen in the work of –Sun et al., (2021) in China. However, the 20.25% resistance rate and the risk of developing multi-drug resistant strains necessitate antibiotic susceptibility testing prior to treatment. The 100% multidrug resistance (MDR) recorded for the 4 species evaluated aligns with Adah *et al*. (2021) who reported the habitual use of these antibiotics without appropriate veterinary oversight or regulation to safeguard consumer health.

This study identifies genes that confer virulence (7 isolates) and resistance to antibiotics (12 isolates) of *Aeromonas* spp., highlighting their pathogenic potential and resistance to antibiotics. Among the virulence genes analyzed, *flaA* (flagellin A) was detected in seven (7) isolates (38.9%), while *aerA/haem* was absent in all isolates. The presence of *flaA* correlates with motility and host invasion, a hallmark of pathogenicity in aquatic environments, as supported by studies demonstrating its essential role in adhesion and colonization (Tomás, 2012). The absence of *aerA/haem*, typically associated with cytotoxic and haemolytic activity, suggests strain-specific virulence variability, a common characteristic in environmental pathogens like *Aeromonas* (Janda and Abbott, 2010).

The TEM, CTX, and SHV β-lactamase genes were examined in relation to antimicrobial resistance. TEM was the most common (44.4%), followed by CTX (16.7%), and SHV (11.1%). These results are in line with reports that aquatic pathogens have TEM, which gives them resistance to β-lactam antibiotics (Piotrowska *et al.*, 2017). Less common but significant were CTX and SHV, which are linked to the production of extended-spectrum β-lactamase (ESBL) and provide resistance to third-generation cephalosporins. Different species have different resistance profiles for example, *A. media* had the highest TEM presence (100%), while *A. veronii* and *A. dhakensis* had varying distributions of CTX. These genes were detected at the anticipated molecular weights of 108 bp for TEM, 585 bp for CTX, and 593 bp for SHV, as confirmed by gel electrophoresis.
According to Rasmussen-Ivey *et al*. (2016), the phylogenetic analysis showed a variety of species. Among the 18 isolates selected for molecular analysis, *A. veronii* was the most represented (38.9%), compared to its lower overall prevalence (6.33%) in the full isolate pool (Table 5). This is in line with its known pathogenicity in humans and fish. Interspecies variability in resistance profiles was highlighted by the notable 100% prevalence of TEM in *A. media*. *Aeromonas* species' genetic diversity is further supported by the clustering of isolates in the phylogenetic tree, which shows how they have evolved to adapt to aquatic environments.

Conclusion
This study established a high prevalence of multidrug-resistant *Aeromonas* spp. (52.9%) in farmed *Clarias gariepinus* in Irepodun LGA, Kwara State, with *A. dhakensis* as the dominant species, all isolates carrying MAR indices above 0.2, and the *flaA* virulence gene detected in 44.4% of the molecular subset; the concurrent absence of *aerS* in all isolates points to pathotypic diversity warranting further investigation. The co-occurrence of resistance genes (TEM, CTX-M, SHV) with virulence determinants, compounded by a documented lack of zoonotic awareness among 50% of sampled farmers, poses a significant public health and food security risk for communities reliant on catfish as a primary protein source. Accordingly, we recommend that the Nigerian Federal Department of Fisheries and Aquaculture Development (FDFAD) enforce veterinary prescription requirements for antibiotic use in aquaculture, that state extension services implement targeted biosafety training emphasising the consistent use of personal protective equipment and that routine antimicrobial resistance surveillance be established across aquaculture environments in Kwara State. Future work should expand sampling across multiple states, incorporate MIC testing and whole-genome sequencing of MDR isolates, screen a broader panel of virulence genes, and conduct longitudinal studies to evaluate the impact of biosafety interventions on resistance gene prevalence and farmer knowledge over time.

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Authors contribution
OMR: Conceiving and designing the study, reviewing first draft, and approval of final draft. GEM and DOF: Conducting experiments, analysing data, writing of first draft and approval of final draft. DAA, AGO, and OBD: Conducting experiments, analysing data and approval of final draft.

Reference
Abbott, S.L., Cheung, W.K.W., and Janda, J.M. (2003). The genus Aeromonas: Biochemical characteristics, atypical reactions, and phenotypic identification schemes. *Journal of Clinical*

Microbiology, *41*(6):2348–2357. https://doi.org/10.1128/JCM.41.6.2348-2357.2003

Adah, D.A., Saidu, L., Oniye, S.J., Kazeem, H.M., and Adah, A. (2021). Prevalence and Risk Factors Associated with *Aeromonas hydrophila* infection in *Clarias gariepinus* and Pond Water from Fish Farms in Kaduna State, Nigeria. *Jordan Journal of Biological Sciences*, *14*(3):477–484. https://doi.org/10.54319/jjbs/140313

Adah, D.A., Sa'idu, L., Oniye, S.J., Raji, M.A., and Adah, A.S. (2023). Occurrence of *Aeromonas* spp. Resistant to Some Selected Antibiotics Isolated from Farmed *Clarias gariepinus*. *Journal of Applied Veterinary Science and Technology*, 4(1):24–29. https://doi.org/10.20473/javest.v4.i1.2023.24-29 https://doi.org/10.47191/ijpbms/v3-i5-04

Adah, D. A., Saidu, L., Oniye, S. J., Adah, A. S., Daoudu, O. B., and Ola-Fadunsin, S. D. (2024). Molecular characterization and antibiotics resistance of *Aeromonas* species isolated from farmed African catfish Clarias gariepinus Burchell, 1822. BMC Veterinary Research. 20: 16. https://doi.org/10.1186/s12917-023-03860-5

Ahmed, H.A., Mohamed, M.E.M., Rezk, M.M., Gharieb, R.M.A., and Abdel-Maksoud, S.A. (2018). *Aeromonas hydrophila* in fish and humans; prevalence, virulotyping and antimicrobial resistance. *Slovenian Veterinary Research,* 20:113-124. https://doi.org/10.26873/SVR-636-2018

Almeida, A., Nerone, J., Helen, L., Leimann, C., Larissa, T., Furtado, D.J., and Ritter, D.O. (2023). Aeromonas sp. in freshwater fish and antimicrobial resistance: emerging pathogen. merging pathogen. Ciência Rural, Santa Maria 53:7, e20220088. http://doi.org/10.1590/0103-8478cr20220088

Austin, B., and Austin, D. A. (2016). *Bacterial Fish Pathogens: Disease of Farmed and Wild Fish, Sixth Edition*. Springer. 732 pp. https://doi.org/10.1007/978-3-319-32674-0

Bartie, K. L., and Desbois, A. P. (2024). *Aeromonas dhakensis:* A Zoonotic Bacterium of Increasing Importance in Aquaculture. *Pathogens* (Basel, Switzerland), 13(6), 465. https://doi.org/10.3390/pathogens13060465

Drk, S., Puljko, A., Dželalija, M., and Udiković-Kolić, N. (2023). Characterization of Third Generation Cephalosporin- and Carbapenem-Resistant Aeromonas Isolates from Municipal and Hospital Wastewater. *Antibiotics,* 12(3):513. https://doi.org/10.3390/antibiotics12030513

Ehlers, M. M., Veldsman, C., Makgotlho, E. P., Dove, M. G., Hoosen, A. A., and Kock, M. M. (2009). Detection of blaSHV, blaTEM and blaCTX-M antibiotic resistance genes in randomly selected bacterial pathogens from the Steve Biko Academic Hospital. In *FEMS Immunology & Medical Microbiology*. 56(3):191-6. https://doi.org/10.1111/j.1574-695X.2009.00564.x

Eluma, M., Itelima, J.U., Onwuliri, F.C., Darda, F.O., and Moses, J.D. (2023). The Prevalence of *Aeromonas* species in Salad Vegetables Sourced from Four Local Government Areas of Plateau State. *International Journal of Pharmaceutical and Bio-Medical Science,* 3(5):191-197. https://doi.org/10.47191/ijpbms/v3-i5-04

Fauzi, N.N.F.N.M., Hamdan, R.H., Mohamed, M., Ismail, A., Auzureen, A., Zin, M., Faten, N., and Mohamad, A. (2021). Prevalence, antibiotic susceptibility, and presence of drug resistance genes in *Aeromonas* spp. isolated from freshwater fish in Kelantan and Terengganu states, Malaysia. *Veterinary World* 14(8):2064-2072. doi: 10.14202/vetworld.2021.2064-2072. 14.

Igbinosa, I.H., Igumbor, E.U., Aghdasi, F., Tom, M., and Okoh, A.I. (2012). Emerging Aeromonas species infections and their significance in public health. *Science World Journal,* 2012(2012):625023. https://doi.org/10.1100/2012/625023

Igere, B.E., Igolukumo, B.B., Eduamodu, C., and Odjadjare, E.O. (2021). Multi-drug resistant *Aeromonas* species in Annelida: An evidence of pathogen harbouring leech in recreation water nexus of Oghara Nigeria environs. *Scientia Africana* 20(2):145-166. https://doi.org/10.4314/sa.v20i2.13

Isonhood, J.H., and Drake, M. (2002). Aeromonas species in foods. *Journal of Food Protection,* 65(3):575-82. https://doi.org/10.4315/0362-028X-65.3.575

Janda, J. M., and Abbott, S. L. (2010). The genus Aeromonas: Taxonomy, pathogenicity, and infection. In *Clinical Microbiology Reviews* 23(1):35-73. https://doi.org/10.1128/CMR.00039-09

Kinkel, M.D., Eames, S.C., Philipson, L.H., and Prince, V.E. (2010). Intraperitoneal injection into adult zebrafish. *Journal of Visualized Experiments,* 42:2126. https://doi.org/10.3791/2126

Kishk, D.M., Moustafa, N.Y., and Kirrella, G.A.K. (2020). Prevalence and virulence characteristics of *Aeromonas* species isolated from fish farms in Egypt. *Kafrelsheikh Veterinary Medical Journal,* 18(2):5–8. https://doi.org/10.21608/kvmj.2020.115274

Lynch, A.J., Elliott, V., Phang, S.C., Claussen, J.E., Harrison, I., Murchie, K.J., Steel, E.A., and Stokes, G.L. (2020). Inland fish and fisheries integral to achieving the Sustainable Development Goals. In *Nature Sustainability* 3:579–587. https://doi.org/10.1038/s41893-020-0517-6

Mailafia, S. (2007). Epidemiology and Molecular Characterization of Aeromonas Species Isolated From Fishes , Poultry and Diarrhoeic Patients in Zaria , Nigeria. *Vom Journal of Veterinary Science,* 5:27-30.

Mzula, A., Wambura, P.N., Mdegela, R.H., and Shirima, G.M. (2019). Phenotypic and molecular detection of Aeromonads infection in farmed Nile tilapia in Southern highland and Northern Tanzania. *Heliyon,* 5(8):e02220. https://doi.org/10.1016/j.heliyon.2019.e02220

Odeyemi, O. A., and Ahmad, A. (2017). Antibiotic resistance profiling and phenotyping of Aeromonas species isolated from aquatic sources. *Saudi*

Journal of Biological Sciences, 24(1):65-70. https://doi.org/10.1016/j.sjbs.2015.09.016

Okon, E.M., Okocha, R.C., Adesina, B.T., Ehigie, J.O., Alabi, O.O., Bolanle, A.M., Matekwe, N., Falana, B.M., Tiamiyu, A.M., Olatoye, I.O. and Adedeji, O.B. (2022). Antimicrobial resistance in fish and poultry: Public health implications for animal source food production in Nigeria, Egypt, and South Africa. *Frontiers in Antibiotics*, 1:1043302. https://doi.org/10.3389/frabi.2022.1043302

Okon, E.M., Okocha, R.C., Taiwo, A.B., Michael, F.B., and Bolanle, A.M. (2023). Dynamics of co-infection in fish: A review of pathogen-host interaction and clinical outcome. *Fish and Shellfish Immunology,* 4(2023):00096. Reports https://doi.org/10.1016/j.fsirep.2023.100096

Oladele, O.O., Ameji, N.O., Gurumyen, G.Y., Adanu, W.A., Kolade, T.T., Agbato, O.A., and Lombin, L.H. (2021). Mortality of *Clarias gariepinus* caused by *Aeromonas* caviae and nitrite toxicity in a fish farm. *Sokoto Journal of Veterinary Sciences,* 19(2):138-144. https://doi.org/10.4314/sokjvs.v19i2.10

Piotrowska, M., Przygodzińska, D., Matyjewicz, K., and Popowska, M. (2017). Occurrence and Variety of β-Lactamase Genes among *Aeromonas* spp. Isolated from Urban Wastewater Treatment Plant. *Frontiers Microbiology,* 8:863. https://doi.org/10.3389/fmicb.2017.00863

Poddar, B. (2018). Antibiotic Resistance: A Global Threat. Critical Care Update 2017 Edition 1/e 9pages. Jaypee Brothers Medical Publishers (P) Ltd. New Delhi, India. https://doi.org/10.5005/jp/books/13063_68

Rasmussen-Ivey, C. R., Figueras, M. J., McGarey, D., and Liles, M. R. (2016). Virulence factors of *Aeromonas hydrophila:* In the wake of reclassification. In *Frontiers Microbiology,* 25:7:1337. https://doi.org/10.3389/fmicb.2016.01337

Saka, B.A., Adeyemo, O.K., and Odeseye, A.O. (2017). Multiple antibiotic resistance indices of Aeromonas hydrophila isolates of muscle of catfish (Clarias gariepinus, Burchell 1822) from selected markets in Ibadan, Nigeria. *African Journal of Clinical and Experimental Microbiology,* 18(2):73-78. https://doi.org/10.4314/ajcem.v18i2.3

Sorescu, I., and Stoica, C. (2021). Online Advanced Bacterial Identification Software, an Original Tool for Phenotypic Bacterial Identification. *Romanian Biotechnological Letters,* 26(6):3047-3053. https://doi.org/10.25083/rbl/26.6/3047-3053

Sun, Y., Zhao, Y., Xu, W., Fang, R., Wu, Q., He, H., Xu, C., Zhou, C., Cao, J., Chen, L., and Zhou, T. (2021). Taxonomy, virulence determinants and antimicrobial susceptibility of Aeromonas spp. isolated from bacteremia in southeastern China. *Antimicrobial Resistance and Infection Control,* 10(1):1–9. https://doi.org/10.1186/s13756-021-00911-0.

Tarchouna, M., Ferjani, A., Ben-Selma, W., and Boukadida, J. (2013). Distribution of uropathogenic virulence genes in Escherichia coli isolated from patients with urinary tract infection. *The International Journal of Infectious Diseases,* 17(6):e450-3. https://doi.org/10.1016/j.ijid.2013.01.025

Tomás, J. M. (2012). The Main *Aeromonas* Pathogenic Factors. *ISRN Microbiology,* 4:2012:256261. https://doi.org/10.5402/2012/256261

Youssef, H. A., Ayoub, H. F., Soror, E. I., and Matter, A. F. (2023). Virulence genes contributing to *Aeromonas* veronii pathogenicity in Nile tilapia (Oreochromis niloticus): approaching the development of live and inactivated vaccines. *Aquaculture International* 31(3):1253-1267. https://doi.org/10.1007/s10499-022-01023-1