integrity Research Journals

Global Journal of Fisheries Science
Volume 8(5), pages 121-131, October 2026
Article Number: 5C4207912
ISSN: 2782-750X
https://doi.org/10.31248/GJFS2026.114
https://integrityresjournals.org/journal/GJFS

Full Length Research

Prevalence, multidrug resistance, and multiple antibiotic resistance index of *Vibrio cholerae* and *Vibrio parahaemolyticus* in wild-caught fishes from two major dams in Ibadan, Nigeria

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Received Date: 16 August 2026 | Accepted Date: 06 October 2026 | Published Date: 30 October 2026

ABSTRACT: The emergence of AMR in aquatic environments poses significant public health risks, particularly through foodborne transmission pathways. This study investigated the prevalence, antimicrobial resistance patterns, and multiple antibiotic resistance (MAR) indices of *Vibrio cholerae* and *Vibrio parahaemolyticus* isolated from wild-caught fish obtained from Eleyele and Asejire dams in Ibadan, Nigeria. A total of 83 wild-caught fish were sampled, and tissues (muscle, liver, and intestine) were aseptically collected. Presumptive identification of *Vibrio* species was performed using Thiosulfate Citrate Bilesalts Sucrose (TCBS) agar (Millipore®, Germany) and biochemical characterization. Real-time PCR was used to characterise the isolates (final volume of 15 µL) using the Norgen NZTtech kit with cycling conditions: activation at 95°C for 1 min, denaturation at 95°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 30s, and a final hold at 4°C. Antibiotic susceptibility testing was conducted using the Kirby–Bauer disk diffusion method following CLSI guidelines. Water quality parameters of the two dams were measured using water quality test kits. The water quality of both Asejire and Eleyele dams showed high temperature, pH, dissolved oxygen, suspended solids, ammonia, nitrite, and BOD levels, while salinity remained below critical limits. *Vibrio cholerae* prevalence in fish from Eleyele Dam was highest in the muscle (72.3%), followed by the liver (63.6%) and intestine (48.5%), while *V. parahaemolyticus* showed comparatively lower but notable prevalence across the tissues. In fish sampled from Asejire Dam, *V. cholerae* prevalence was exceptionally high, with detection rates of 92%, 82%, and 92% in the muscle, liver, and kidney, respectively. In contrast, *V. parahaemolyticus* showed substantially lower prevalence in the corresponding tissues, at 20%, 22%, and 12%, respectively. Isolates exhibited high levels of multidrug resistance against the antibiotic panel. MARI values ranged from 0.4–1.0 for *V. cholerae* and 0.6–0.9 for *V. parahaemolyticus*. The findings highlight the potential role of wild-caught fish as reservoirs of multidrug-resistant *Vibrios* and underscore the need for continuous surveillance, as well as improved antimicrobial stewardship.

Keywords: Antimicrobial resistance, MAR index, multidrug resistance, Nigeria, *Vibrio* species, wild-caught fish.

INTRODUCTION

Wild-caught fish represent an important source of protein globally and may serve as reservoirs for pathogenic microorganisms, including *Vibrio* species (Dutta *et al*., 2021; Ma *et al*., 2023). These bacteria are naturally present in aquatic environments and are associated with foodborne infections in humans, particularly through the

consumption of contaminated or undercooked seafood (Hossain *et al*., 2018; Dutta *et al*., 2021). *Vibrio cholerae*, the causative agent of cholera, remains a major public health concern in developing countries, including Nigeria, where recurrent outbreaks have been reported (Salako *et al*., 2021; Eneh *et al*., 2024). Similarly, *Vibrio parahaemolyticus* is a leading cause of seafood-associated gastroenteritis worldwide (Martinez-Urtaza and Baker-Austin, 2020). The persistence of these organisms in aquatic environments, combined with increasing antimicrobial resistance, poses a significant One Health challenge (Velazquez-Meza *et al.,* 2022; Tang *et al*., 2023).

Antimicrobial resistance (AMR) has emerged as a critical global health issue, driven largely by the misuse and overuse of antibiotics in human medicine, agriculture, and aquaculture (Tang *et al.,* 2023; Sabour *et al*., 2024). Aquatic ecosystems are particularly vulnerable due to contamination from effluents, agricultural runoff, and improper waste disposal, which may promote the selection and spread of resistant bacteria (Deng *et al.,* 2020; Moyo *et al.,* 2023). Despite increasing concerns, there is limited data on the prevalence and resistance patterns of *Vibrio* species in wild-caught fish from freshwater systems in Nigeria (Agboola *et al*., 2022).

Bacterial diseases pose significant challenges in both wild and ornamental fish populations, and common pathogens include *Aeromonas*, *Vibrio*, and *Streptococcus* species, which are opportunistic infections (Hadfield, 2021). Pathogenic *Vibrio* species, including *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*, pose a significant health threat in aquatic environments through contaminated seafood consumption, particularly as seawater temperatures rise (Diner *et al.,* 2021). A global meta-analysis has revealed prevalence rates of *V. cholerae* (9.56%), *V. parahaemolyticus* (24.77%), and *V. vulnificus* (5.29%) in fish (Ma *et al*., 2023). Abdelaziz Gobarah *et al*. (2022) have also identified virulence genes in *Vibrio* isolates from fish, including *tdh* and *trh* in *V. parahaemolyticus*, and *ctx* in *V. cholerae*.

Vibrio cholerae is a Gram-negative aquatic bacterium that causes cholera, a waterborne infectious disease in humans (Hurst, 2019). Studies have shown that *V. cholerae* can be isolated from the intestines and skin of fish, indicating that these aquatic animals may serve as carriers of the pathogen, potentially leading to human exposure through the consumption of contaminated seafood (Hossain *et al*., 2018). Numerous cholera outbreaks have occurred in Nigeria since 1970, with notable incidents in 1991, 2010, 2018, and 2024 (Eneh *et al*., 2024). The World Health Organisation estimates that cholera affects millions of people annually, resulting in substantial morbidity and mortality, particularly in regions lacking adequate sanitation and clean drinking water (Bagheri-Josheghani and Bakhshi, 2022).

Vibrio parahaemolyticus, also a Gram-negative halophilic bacterium, is found in marine environments and has been reported as a leading cause of seafood-borne gastroenteritis worldwide (Martinez-Urtaza and Baker-Austin, 2020). It has been recognised as a significant foodborne pathogen, particularly associated with the consumption of raw or undercooked seafood. The pathogenic potential of *V. parahaemolyticus* is attributed to its ability to produce various virulence factors, including thermostable direct hemolysin (*tdh*) and TDH-related hemolysin (*trh*), which play crucial roles in its pathogenicity (De Menezes *et al*., 2020).

Antimicrobial resistance is a critical global health issue, with projections of 10 million annual deaths by 2050 (Alarape and Adeyemo, 2017; Tang *et al*., 2023). The primary cause is the overuse and misuse of antimicrobials, particularly antibiotics (Tang *et al*., 2023). Antimicrobial resistance affects humans, animals, and the environment, exemplifying the One Health approach (Velazquez-Meza *et al*., 2022). The World Health Organisation has identified antimicrobial resistance as one of the top ten global public health threats facing humanity, emphasising the urgent need for coordinated action across various sectors to combat this issue (Sabour *et al*., 2024). Multidrug-resistant organisms have been defined as those resistant to at least one agent in three or more antimicrobial categories (Aurich *et al*., 2022; Pitiriga *et al*., 2022). The overuse and misuse of antibiotics in aquaculture have resulted in the selection of resistant bacterial strains, which can transfer resistance genes to human pathogens, complicating treatment options for infections in humans and animals (Mawardi, 2023). Studies have shown that *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio alginolyticus* exhibit high levels of resistance to commonly used antibiotics (Dewi *et al*., 2022). According to Dewi *et al*. (2022), findings from the Malaysia study showed that *V. vulnificus* demonstrated a 100% resistance rate to ampicillin, while *V. cholerae* showed an 83.3% resistance rate to the same antibiotic. Antibiotic resistance has also been established in *V. parahaemolyticus* infections; various studies have reported resistance to multiple antibiotics, complicating treatment options for infected individuals (Lee *et al*., 2018; Mawardi, 2023).

Antibiotic resistance in *Vibrio* species is an increasing global concern, and antimicrobial resistance poses a significant threat in Africa, particularly in sub-Saharan regions (Moyo *et al*., 2023). Due to the dearth of information on the prevalence of these *Vibrio* species in cultured Catfish in Nigeria, coupled with reported cholera outbreaks in Nigeria, especially in Ibadan, and the increasing reports of antibiotic resistance, this study focused on two major dams that serve as sources of wild-caught fish and water for Oyo State. The objectives of this study were to determine the prevalence of *Vibrio cholerae*

and *Vibrio parahaemolyticus* and to evaluate their antibiotic resistance profiles in wild-caught fish from Eleyele and Asejire dams in Ibadan, Nigeria.

MATERIALS AND METHODS

Ethical consideration

The research protocol was approved by the University of Ibadan Animal Care and Use in Research Ethics Committee (UI-ACUREC), under approval number UI-ACUREC/19/0141.

Study areas and fish samples

Fish samples were collected from Eleyele Dam (7°20′–7°25′ N; 3°51′–3°56′ E) and Asejire Dam (7°21′45″ N; 4°08′00″ E) in Ibadan, Oyo State, Nigeria. Eighty-three adult wild-caught fish (Eleyele Dam (n=50); Asejire Dam (n=33)) were collected over a period of 6 weeks. The water samples from the dams were also collected during the sampling periods. Water parameters measured were temperature (ºC), dissolved oxygen (DO), salinity, pH, total suspended solids (TSS), ammonia (NH_3), nitrite (NO_2^-), and biological oxygen demand (BOD).

Bacterial isolation

Each fish was pithed, and muscle, liver, and intestines were aseptically harvested and kept in separate sterile nylon sample bags. The collected fish organs were separately pre-enriched in buffered peptone water and incubated for 24 hours at 30°C. The pre-enriched samples were then streaked on Thiosulfate Citrate-Bile salts Sucrose (TCBS) Agar (HIMEDIA®) and incubated at 30°C for 24 hours, while the resultant colony growths were then subjected to Gram staining, biochemical, and antibiotic sensitivity tests.

Gram staining

Gram staining was performed by preparing a smear of the test organism on a clean, grease-free microscope slide. The smear was heat-fixed by passing the slide through the flame of a Bunsen burner. The smear was flooded with crystal violet and allowed to stand for 60 seconds, after which it was rinsed with water. Gram's iodine was then applied and allowed to react for 60 seconds before the slide was rinsed with water. The smear was decolourized with 70% ethanol for 30 seconds and subsequently rinsed with water. It was then counterstained with safranin for 30 seconds, rinsed, and blot-dried. The stained preparation was examined microscopically under the ×100 objective using immersion oil.

Catalase test

One drop of 3% hydrogen peroxide was placed on a clean glass slide. A sterile wire loop was used to pick a colony from the culture plate and emulsify it in the hydrogen peroxide drop. The immediate production of gas bubbles indicated a positive catalase reaction, whereas the absence of bubbles indicated a negative reaction.

Oxidase test

A piece of filter paper was moistened with oxidase reagent containing 1% tetramethyl-p-phenylenediamine dihydrochloride. Using a sterile loop, a colony from the agar plate was smeared onto the moistened filter paper. The development of a purple colour within 10 seconds was interpreted as a positive oxidase reaction.

Sugar fermentation tests

These were performed using glucose, lactose, maltose, and sucrose. One gram of each sugar and 1.5 g of peptone were dissolved separately in 100 mL of distilled water. Subsequently, 0.3 mL of bromocresol purple indicator was added to each preparation, and the contents were thoroughly mixed. The media were dispensed into test tubes containing inverted Durham tubes for the detection of gas production. The preparations were sterilised at 121°C and 15 psi for 15 minutes. After sterilisation, the test tubes were inoculated with the respective test isolates and incubated at 37°C for 48 hours. Gas production was indicated by the accumulation of gas in the Durham tube, while acid production was indicated by a colour change of the bromocresol purple indicator from purple-blue to yellow.

Characterisation of Presumptive *Vibrio cholerae* and *Vibrio parahaemolyticus* Using Real-Time PCR

Twenty grams of Nutrient Agar was dissolved in 1000 mLs of distilled water. The solution was heated in boiling water, agitated frequently until the medium was completely dissolved. The medium was then autoclaved at 121°C for 15 minutes. After cooling, the medium was poured into sterile Petri dishes and allowed to cool and solidify. The thawed Vibrio isolates were streaked on the prepared agar and later incubated overnight for 18 h at 30°C. Five mLs of Luria Broth was aliquoted into test tubes, and a loopful of isolate was suspended in the broth and corked. Broths containing each isolate were incubated in a shaker at 30°C

and 120rpm overnight. In determining the bacterial density, 100 µL of *Vibrio* isolates were aspirated into the 96-well microtitre plate and placed into a microplate reader for optical density measurement at 600 nm (an optical density (OD600 of 1 is approximately equivalent to 109 bacterial cells).

The DNA extraction was done by incubating Luria Broth (LB) culture overnight and later transferred into well-labelled 15 mL centrifuge tubes and spun down at 4000 rpm for 5 min to retrieve the pellet, then discarded the supernatant. The retrieved pellets were again suspended in 250µL Norgen bacterial genomic NZTtech DNA extraction kit re-suspension buffer (pH 7.5, 50 mM Tris, 1 M sorbitol, 10 mM EDTA, 1 unit/µL Lyticase, and 0.1% β-mercaptoethanol). The total mixture was treated with 3 µL of RNase A per sample to remove contaminating RNA. Afterwards, 25 0µL of lysis buffer P was added to the mix, with the addition of 12µL of Proteinase K solution. The mixture was mildly vortexed and incubated for 30 min at 55°C. After the period of incubation, 500µL of solution BX was added and the mixture was vortexed, followed by the addition of 500µL of absolute ethanol. The resulting solution was transferred into spin columns and centrifuged at 8000 rpm for 1 min. Spin columns were washed with 500µL of wash solution A twice at 14000rpm for 1min. Elution of DNA was performed by adding 200 µL of the elution buffer to the spin columns, centrifuged at 6000 rpm for 1 min, and then re-centrifuged at 14000 rpm for 2 min to recover genomic DNA (gDNA). The gDNA samples were then stored at -20°C until further analysis. Real-Time PCR Assay: The master mix was separately prepared for the Vibrio DNA, positive, and negative controls. 7.5 µL of qPCR Master Mix (Universal) was added to the Primer (0.3 µL), Probes, and Internal Control universal mix, PCR grade Water (3.7 µL), and DNA Sample (3.5 µL) (in case of positive control Master mix preparation, 3.5 µL of Vibrio parahaemolyticus Positive Control and 3.5 µL of Vibrio parahaemolyticus Negative Control for the negative control Master mix preparation) to have a total volume of 15 µL. The cyclic settings for the qPCR included one cycle of activation step which lasted for 1 min at 95°C. The denaturation process was for 30 seconds at 95°C, annealing for 30 seconds at 58°C, and extension for 30 seconds at 72°C, while the final step or hold was set at 4°C. The Norgen-type *V. parahaemolyticus* real-time PCR protocol specified Ct ≤35 as positive, while results around 35–40 should be repeated, and if the repeat remains in that range, it is interpreted as negative.

Antibiotic sensitivity test

The antibiotic susceptibility test was performed using the agar disc diffusion method as described by Bauer *et al.* (1966). The 16 antibiotic discs used contain the following antibiotics: Ceftriaxone sulbactam (CRO (45µg)), Amplicox (ACX (10µg)), Cefexime (ZEM (5µg)), Levofloxacin (LBC (5µg)), Amoxicillin Clavulanate (AUG (30µg)), Cefotaxime (CTX (25µg)), Imipenem/Cilastatin (IMP (10/10µg)), Ofloxacin (OFX (5µg)), Gentamycin (GN (10µg)), Nalidixic Acid (NA (30µg)), Nitrofurantoin (NF (300µg)), Cefuroxime (CXM (30µg)), Chloramphenicol (C (30µg)), Ciprofloxacin (CIP (5µg)), Erythromycin (E (15µg)), and Trimethoprim (W (5µg)). The fresh overnight cultures of confirmed *Vibrio cholerae* and *Vibrio parahaemolyticus* isolates were suspended in sterile physiological saline and adjusted to a 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU/mL). The standardized suspensions were uniformly inoculated onto Mueller–Hinton agar plates using sterile swabs. Selected antibiotic discs were aseptically placed on the inoculated plates using sterile forceps, and the plates were incubated under the appropriate conditions. Following incubation, the diameters of the inhibition zones were measured in millimetres and interpreted as susceptible, intermediate, or resistant according to CLSI M100, 34th edition (2024). Escherichia coli ATCC 25922 was used as the quality-control strain.

Multiple antibiotic resistance index

The MAR index for each isolate was calculated as the ratio of the number of antibiotics to which the isolate was resistant to the total number of antibiotics tested.

Statistical analysis

Data were analysed descriptively using frequencies and bar charts. The chi-square test was used to determine statistical differences between the distribution of the Vibrio isolates among different analysed fish tissues. Differences in prevalence between dams were not statistically tested due to study limitations.

RESULTS

The average water quality parameters values for both Asejire and Eleyele dams were 30°C and 28°C (temperature), 5mg/L and 5.5mg/L (dissolved oxygen), 7.8 and 7.5 (pH), 150mg/L and 110mg/L (total suspended solids), 0.4 mg/L and 0.5 mg/L (ammonia and nitrite), 33mg/L and 31mg/L (biochemical oxygen demand), and 250 mg/L and 180mg/L (salinity concentration) respectively (Figure 1).

Vibrio cholerae formed yellowish colonies on TCBS agar,

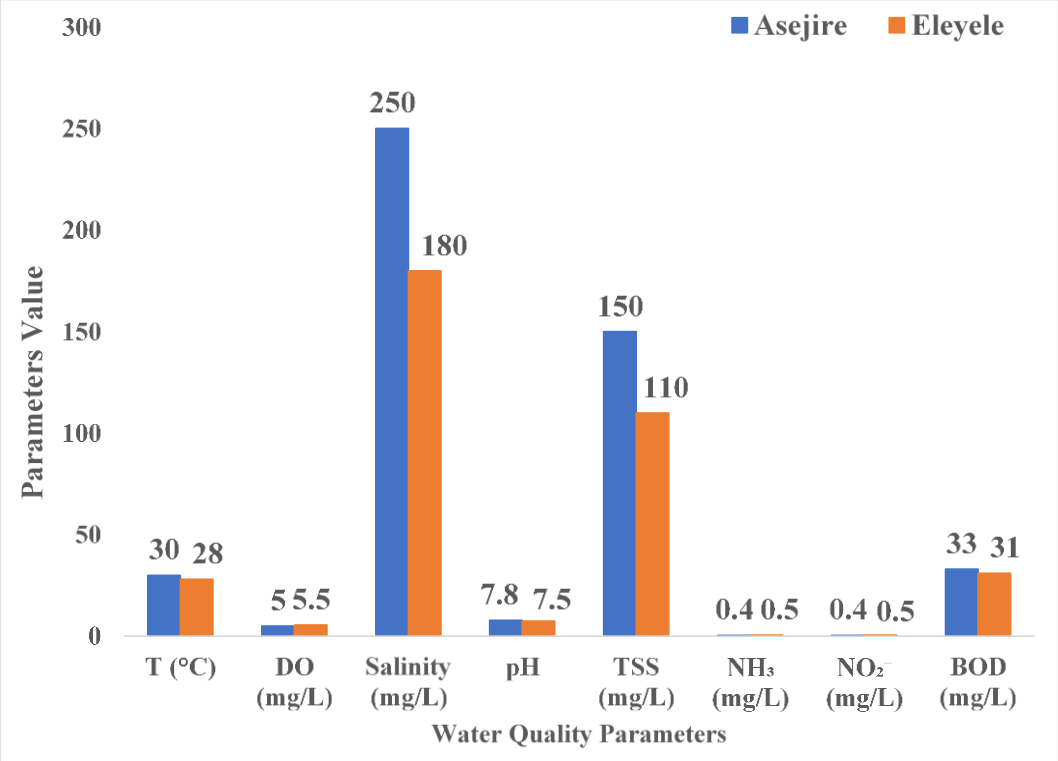


Figure 1. Water quality parameters of Asejire and Eleyele Dams.

tested positive for oxidase, catalase, sucrose, glucose, lactose, and maltose, and had a pinkish, curved-rod (comma) shape. *Vibrio parahaemolyticus* showed greenish colony growth, was positive to oxidase, catalase, glucose, and maltose tests, but negative to sucrose and lactose, with a pinkish curved-rod (comma) shape. The real-time PCR results showed a classic sigmoid curve, which indicates a successful and exponential amplification of the *Vibrio parahaemolyticus* target gene (Figure 2) at a Ct value between 18 and ≤ 35, while the isolates with Ct values above 35 were confirmed to be *Vibrio cholerae*.

The average percentage prevalence of *V. cholerae* in the tissues (muscle, liver, and intestines) of the wild-caught fish from Asejire Dam was 72.3%, 63.6%, and 48.5%, respectively, while that of *V. parahaemolyticus* was 39.4%, 39.4%, and 33.3%, respectively (Figure 3). The average percentage prevalence of *V. cholerae* obtained in muscles, liver, and intestines from fish tissues from Eleyele Dam was 92%, 82%, and 92%, respectively, while that of *V. parahaemolyticus* was 20%, 22%, and 12%, respectively (Figure 4).

A total of 194 *Vibrio cholerae* and 64 *Vibrio parahaemolyticus* isolates were recovered from fish sampled from Eleyele and Asejire dams. Generally, the prevalence of *V. cholerae* was significantly higher than that of *V. parahaemolyticus* ($p < 0.0001$). Among the fish organs examined, muscle recorded the highest occurrence of *V. cholerae* (79.5%), followed by liver (71.1%) and intestine (65.1%); however, these differences were not statistically significant ($p = 0.141$). Similarly, no significant difference was observed in the distribution of *V. parahaemolyticus* among muscle, liver, and intestine ($p = 0.575$). Reservoir-specific analysis revealed significant differences in the occurrence of both species. Asejire dam recorded a significantly higher prevalence of *V. cholerae* than Eleyele dam ($p<0.0001$), whereas *V. parahaemolyticus* was significantly more prevalent in Eleyele dam than Asejire dam ($p<0.001$).

Antibiotic sensitivity test

The *Vibrio cholerae* from the two dams were 100% resistant to Cefotaxime (CTX), Ampiclox (ACX), Cefuroxime (CXM), Imipenem/Cilastin (IMP) and Amoxicillin Clavulanate (AUG), 93.3% resistant to Ceftriaxone Sulbactam (CRO) and Cefexime (ZEM), 86.7% resistant to Gentamycin (GN), Erythromycin (E) and Trimethoprim (W), 80% resistant to Nalidixic acid (NA) and Nitrofurantoin (NF), 66.7% resistant to Chloramphenicol

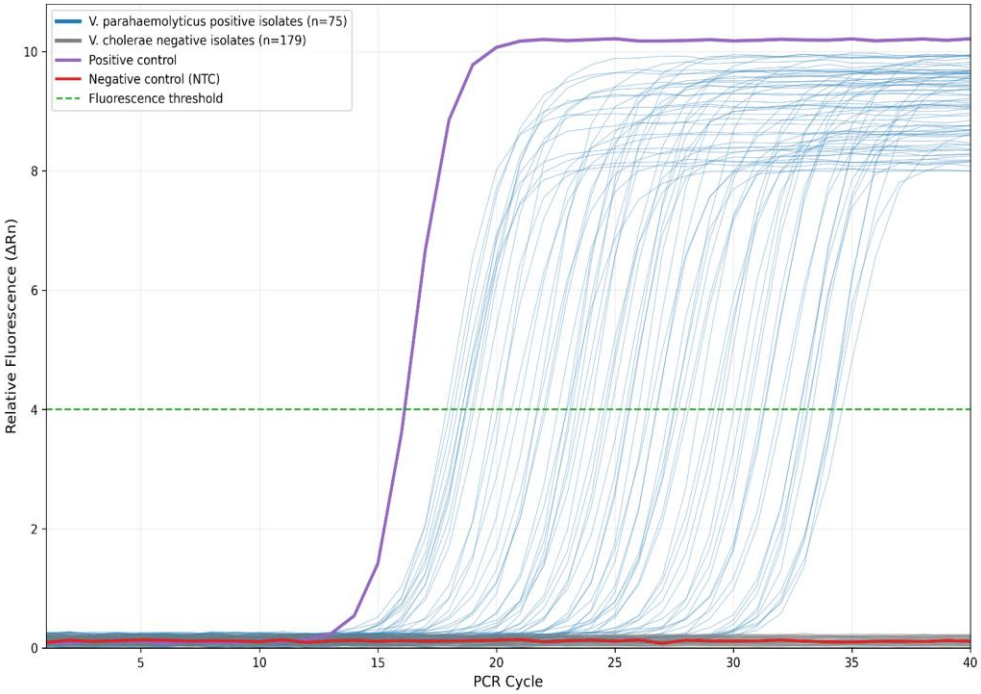


Figure 2. RT-qPCR Amplification Curves for *Vibrio* Isolates Retrieved from Wild-caught Fishes from Asejire and Eleyele Dams.

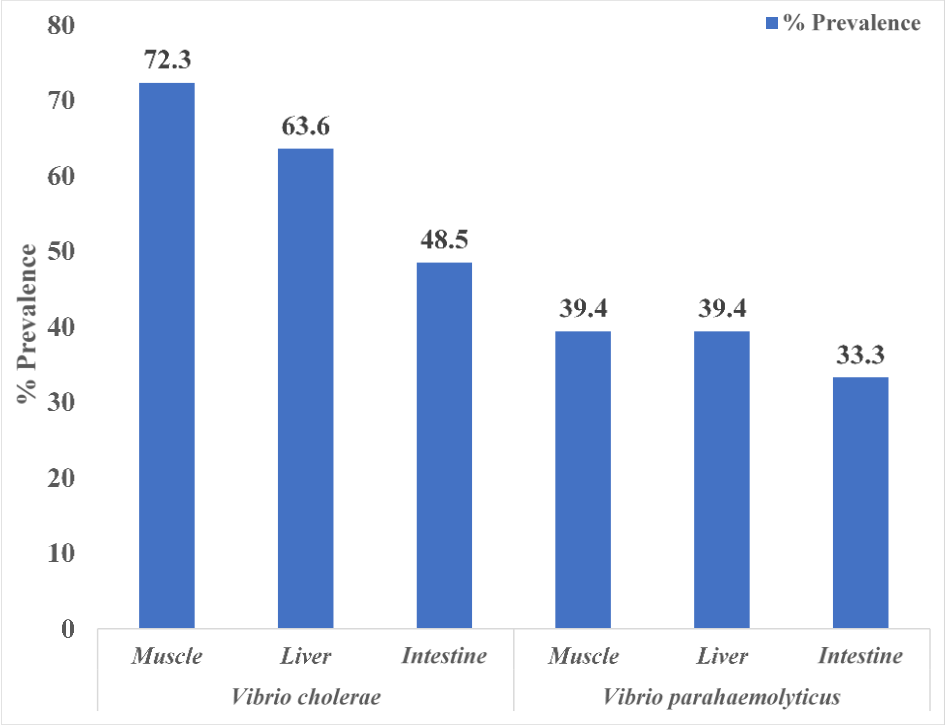


Figure 3. Percentage Prevalence of *Vibrio cholerae* and *Vibrio parahaemolyticus* isolates in sampled tissues from Wild-caught fish obtained from Asejire Dam.

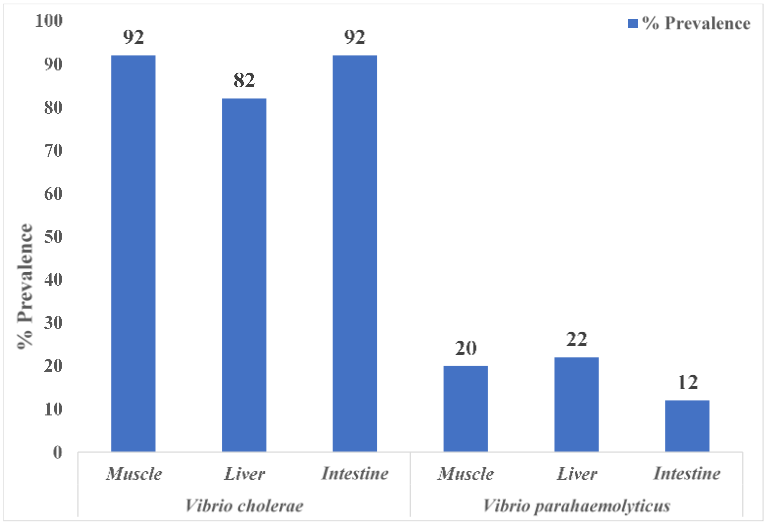


Figure 4. Percentage prevalence of *Vibrio cholerae* and *Vibrio parahaemolyticus* isolates in in sampled tissues from Wild-caught fish obtained from Eleyele Dam.

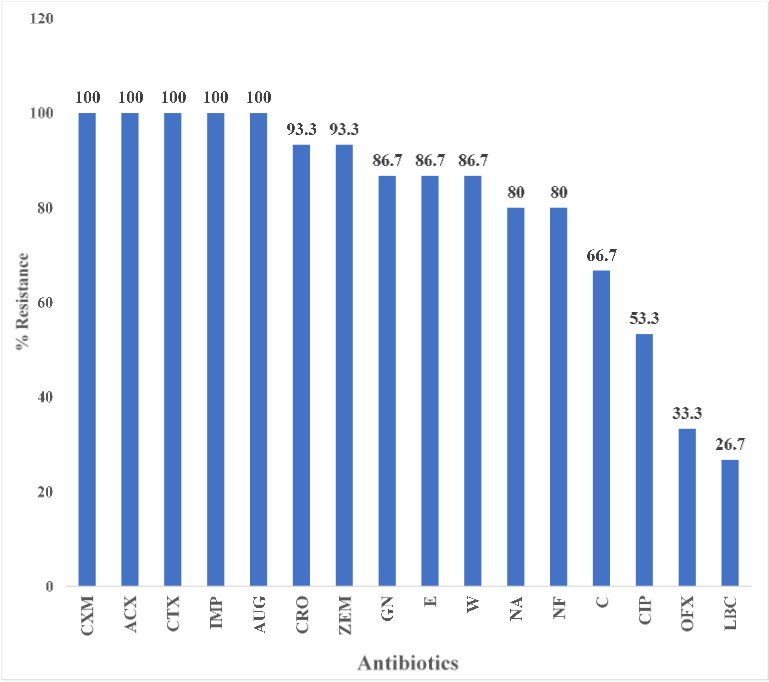


Figure 5. Antibiotic Resistance Pattern of *Vibrio cholerae* isolated from Wild-caught fish obtained from Asejire and Eleyele Dams in Ibadan, Oyo State.

(C), and 53.3% resistant to Ciprofloxacin (CIP). The *Vibrio cholerae* were 66.7% and 73.3% sensitive to Ofloxacin (OFX) and Levofloxacin (LBC), respectively (Figure 5). The multiple antibiotic resistance index of the isolated *Vibrio cholerae* ranges between 0.4 and 1 (Table 1). The *Vibrio parahaemolyticus* isolated from the two dams were

Table 1. Multiple Antibiotic Resistance Indices of *Vibrio cholerae* isolated from Wild-caught fishes obtained from Asejire and Eleyele Dams in Ibadan, Oyo State.

***Vibrio* Strains**	**No. of Isolates**	**Antibiotic Resistance Pattern**	**MARI**
Vibrio cholerae	10	CRO; CXM; ACX; CTX; IMP; OFX; ZEM; NA; GN; AUG; NF; LBC; CIP; C; E; W	1
	12	CRO; CXM; ACX; CTX; IMP; ZEM; NA; GN; AUG; NF; CIP; C; E; W	0.9
	19	CRO; CXM; ACX; CTX; IMP; ZEM; NA; GN; AUG; NF; CIP; C; E; W	0.9
	10	CXM; ACX; CTX; IMP; AUG; E; W	0.4
	11	CRO; CXM; ACX; CTX; IMP; ZEM; NA; GN; AUG; NF; CIP; C; W	0.8
	10	CRO; CXM; ACX; CTX; IMP; ZEM; NA; GN; AUG; NF; C; E; W	0.8
	12	CRO; CXM; ACX; CTX; IMP; ZEM; GN; AUG	0.5
	9	CRO; CXM; ACX; CTX; IMP; OFX; ZEM; NA; GN; AUG; NF; LBC; CIP; C; E; W	1
	14	CRO; CXM; ACX; CTX; IMP; ZEM; NA; GN; AUG; NF; CIP; C; E; W	0.9
	13	CRO; CXM; ACX; CTX; IMP; OFX; ZEM; NA; GN; AUG; NF; LBC; CIP; E; W	0.9
	9	CRO; CXM; ACX; CTX; IMP; ZEM; AUG; NF; LBC; E; W	0.7
	10	CRO; CXM; ACX; CTX; IMP; OFX; ZEM; NA; GN; AUG; NF; CIP; C; E; W	0.9
	17	CRO; CXM; ACX; CTX; IMP; ZEM; NA; GN; AUG; NF; C; E; W	0.8
	15	CRO; CXM; ACX; CTX; IMP; ZEM; NA; GN; AUG; NF; C; E	0.8
	11	CRO; CXM; ACX; CTX; IMP; ZEM; NA; GN; AUG; C; E	0.7
	12	CRO; CXM; ACX; CTX; IMP; OFX; ZEM; NA; GN; AUG; NF; E; W	0.8

NOTE: CRO: Ceftriaxone Sulbactam, CXM: Cefuroxime, ACX: Ampiclox, CTX: Cefotaxime, IMP: Imipenem/Cilastin, OFX: Ofloxacin, ZEM: Cefexime, NA: Nalidixic acid, GN: Gentamycin, AUG: Amoxicillin-Clavulanate, NF: Nitrofurantoin, LBC: Levofloxacin, CIP: Ciprofloxacin, C: Chloramphenicol, E: Erythromycin, W: Trimethoprim.

Table 2. Multiple Antibiotic resistance indices of *Vibrio parahaemolyticus* isolated from Wild-caught fishes obtained from Asejire and Eleyele Dams in Ibadan, Oyo State.

***Vibrio* Strains**	**No. of Isolates**	**Antibiotic Resistance Pattern**	**MARI**
Vibrio parahaemolyticus	5	CRO; CXM; ACX; CTX; IMP; ZEM; NA; GN; AUG; NF; C; E; W	0.8
	5	CRO; CXM; ACX; CTX; IMP; OFX; ZEM; NA; GN; AUG; NF; CIP; C; E; W	0.9
	9	CRO; CXM; ACX; CTX; IMP; OFX; ZEM; NA; GN; AUG; NF; C; E; W	0.9
	7	CRO; CXM; ACX; CTX; IMP; OFX; ZEM; NA; GN; AUG; NF; C; E; W	0.9
	5	CRO; CXM; ACX; CTX; IMP; ZEM; GN; AUG; NF; CIP; C; E; W	0.8
	7	CRO; CXM; ACX; CTX; IMP; ZEM; NA; GN; AUG; NF	0.6
	4	CRO; CXM; ACX; CTX; IMP; ZEM; GN; AUG; NF; E; W	0.7
	4	CRO; CXM; ACX; CTX; IMP; OFX; ZEM; NA; GN; AUG; CIP; E; W	0.8
	3	CRO; CXM; ACX; CTX; IMP; OFX; ZEM; NA; GN; AUG; NF; C; E; W	0.9
	4	CRO; CXM; ACX; CTX; IMP; ZEM; NA; GN; AUG; NF; LBC; C; E; W	0.9
	3	CRO; CXM; ACX; CTX; IMP; OFX; ZEM; NA; GN; AUG; NF; C; E; W	0.9
	6	CRO; CXM; ACX; CTX; IMP; ZEM; NA; GN; AUG; NF; C; E; W	0.8
	2	CRO; CXM; ACX; CTX; IMP; OFX; ZEM; GN; AUG; NF; LBC; C; E; W	0.9

NOTE: CRO: Ceftriaxone Sulbactam, CXM: Cefuroxime, ACX: Ampiclox, CTX: Cefotaxime, IMP: Imipenem/Cilastin, OFX: Ofloxacin, ZEM: Cefexime, NA: Nalidixic acid, GN: Gentamycin, AUG: Amoxicillin-Clavulanate, NF: Nitrofurantoin, LBC: Levofloxacin, CIP: Ciprofloxacin, C: Chloramphenicol, E: Erythromycin, W: Trimethoprim.

100% resistant to Cefotaxime (CTX), Ampiclox (ACX), Cefuroxime (CXM), Imipenem/Cilastin (IMP), Ceftriaxone Sulbactam (CRO), Cefexime (ZEM) and Gentamycin (GN), 93.3% resistant to Amoxicillin Clavulanate (AUG), Nitofurantoin, Erythromycin (E) and Trimethoprim (W), 73.3% resistant to Nalidixic Acid (NA), 80% resistant to Chloramphenicol (C), and 53.3% resistant to Ofloxacin (OFX). The *Vibrio parahaemolyticus* isolates were 86.7% and 73.3% sensitive to Levofloxacin (LBC) and Ciprofloxacin (CIP), respectively (Figure 6). The multiple antibiotic resistance index of the isolated *Vibrio parahaemolyticus* ranges between 0.6 and 0.9 (Table 2).

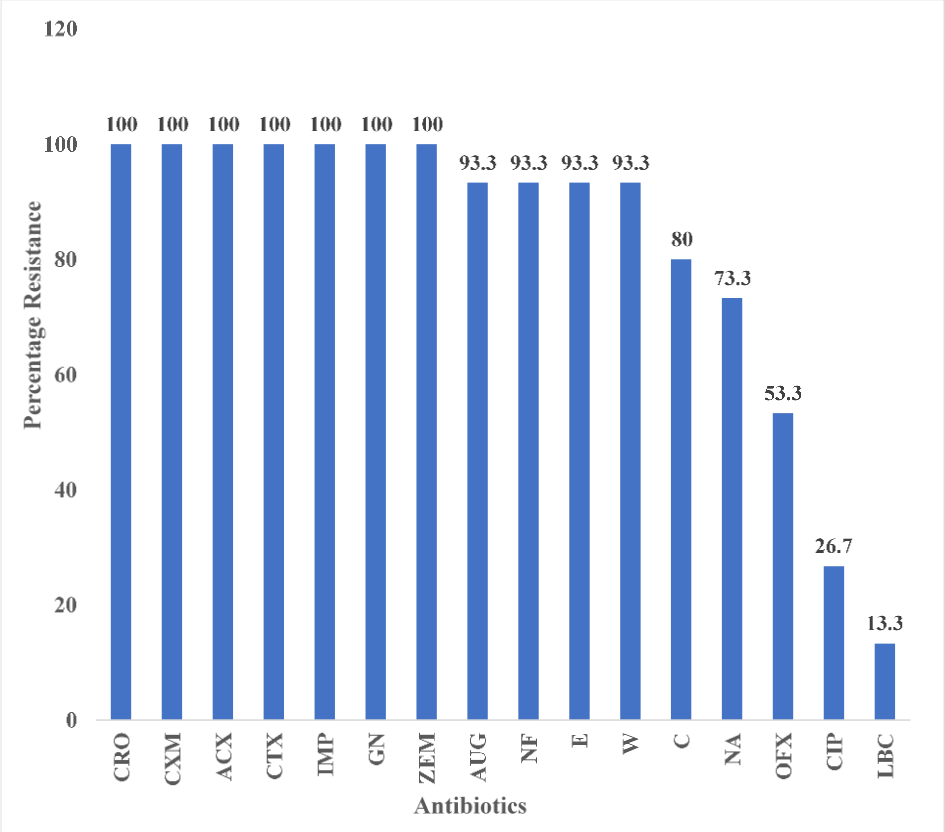


Figure 6. Antibiotic Resistance Pattern *Vibrio parahaemolyticus* isolated from Wild-caught fish obtained from Asejire and Eleyele Dams in Ibadan, Oyo State.

DISCUSSION

The detection of *V. cholerae* and *V. parahaemolyticus* in wild-caught fish raises significant public health concerns, particularly when proper hygiene principles are not followed throughout the fish food production chain. This aligns with the findings of Morris and Acheson (2003) and Dutta *et al.* (2021), who reported that *Vibrio* infections extend beyond aquatic environments, thereby posing health risks to humans through raw or undercooked seafood consumption. *Vibrio* spp. have always been a public health problem due to their widespread and persistent presence in the environment and fisheries, thus increasing the difficulty in reducing their spread (Morris and Acheson, 2003). The obtained water quality parameters in this study, such as high temperature (28–30°C), moderate salinity, alkaline pH, enhanced turbidity (TSS), and organic pollution indices (NH_3, NO_2^-, BOD), may likely be part of the environmental factors that allowed the proliferation of *V. cholerae* and *V. parahaemolyticus* in the two studied dams and fishes. These conditions are in line with global observations showing that warmer, nutrient-enriched waters with moderate salinity and particulate matter increase the prevalence of *Vibrios* in aquaculture and wild-caught fish habitats (Raghunath, 2019; Brumfield *et al*., 2021).

In this study, *V. cholerae* exhibited a higher prevalence than *V. parahaemolyticus*, which is consistent with the reports of Senderovich *et al*. (2010) and Ali *et al*. (*2023*), where 87% and 66% prevalence were recorded for *V. cholerae* and 60% and 35% for *V. parahaemolyticus,* respectively, which reinforced that non-pathogenic and pathogenic *V. cholerae* serogroups are frequently present in fish from contaminated water bodies. Similarly, Ma *et al*. (2023) reported a higher prevalence of *V. cholerae* over *V. parahaemolyticus,* showing that *V. cholerae* is a significant contaminant across fish species and environments. In the same vein, Ibangha *et al*. (2023) also reported a higher prevalence of *V. cholerae* (59.5%) when compared with *V. parahaemolyticus* (10.4%) in water sources, which underscored a greater likelihood of detecting *V. cholerae* in fish and water environments. In contrast, Agboola *et al*.

(2022) reported a higher prevalence of *V. parahaemolyticus* over *V. cholerae,* which demonstrated the variability of relative prevalence depending on environment and sampling context.

The present study demonstrated varying prevalence in the tissues of wild-caught fish from Asejire and Eleyele dams, which suggested widespread contamination of fish tissues by *Vibrio* species, an indication that edible tissues, particularly muscle, may likely serve as important reservoirs for transmission to humans. The presence of exceptional high prevalence of *Vibrio cholerae* in the muscle tissues was in contrast with several previous reports where intestinal organs (intestine, kidney, and liver have been regarded predilection sites for *Vibrio* organisms) usually harboured higher bacterial loads than edible muscle tissues (Senderovich *et al*., 2010; Halpern and Izhak, 2017).

The antibiotic susceptibility results in this study are consistent with recent reports of multidrug-resistant *V. cholerae* in fish and seafood environments. According to Sultana *et al*. (2025), isolated *V. cholerae* from Tilapia exhibited resistance to several clinically relevant antibiotics, while *V. parahaemolyticus* exhibited varying resistance patterns, demonstrating variable resistance patterns reflective of local antibiotic exposure pressures. Similarly, Abioye *et al*. (2023) reported *V. cholerae* isolates from South African seafood with high resistance to β-lactam antibiotics and a range of MAR indices above 0.2, underscoring the widespread nature of AMR in aquatic Vibrio spp. Additionally, studies in Nigeria by Agboola *et al*. (2022) highlighted significant resistance of *Vibrio* species to cefotaxime, meropenem, and other antibiotics, with MAR indices between 0.2 and 0.83, indicating considerable antimicrobial pressure in fish environments, aligning closely with our findings. On a global scale, systematic analyses have shown that environmental *V. cholerae* isolates frequently possess resistance to multiple antibiotic classes, reinforcing the public health significance of AMR in aquatic reservoirs (Yuan *et al*., 2022).

Conclusion

The presence and high prevalence of *V. cholerae* and *V. parahaemol*yticus isolates in sampled tissues of wild-caught fish in the two main dams in Ibadan, Oyo State, is of major public health concern. Of importance is the isolation of the two *Vibrios* in the edible part of the wild-caught fish. Also, their MDR status with high MAR index above recommended 0.2 suggested the extent of the environmental pollution which may be as a result of several factors, untreated effluent discharges into the water body, agricultural runoff, and resistant gene transfer. These findings emphasise the need for prospective active surveillance for *Vibrio* occurrence and antibiotic resistance status of the *Vibrio* organisms. This also represents a call for One Health inclusivity in the eradication of *Vibrio* diseases in both fish and humans.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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