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Antimicrobial Susceptibility, Microbial Loads and Isolation of *Plesiomonas shigelloides* from African Sharptooth Catfish (*Clarias gariepinus*) Juveniles and Experimental Pond Water

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Abstract The aim of this study was to investigate the antimicrobial susceptibility, microbial loads and isolation of *Plesiomonas shigelloides* isolated from African catfish, *Clarias gariepinus* juveniles and experimental pond water. Microbial loads of pond water and fish tissues (gill, liver, and intestine) were evaluated using standard methods. Isolation and antibiotic susceptibility of the bacterial species were carried out using standard microbiological techniques. Antibiotic susceptibility of the isolates was assessed using a panel of 12 antibiotics by disc diffusion method and standard guidelines. The microbial loads in water from the experimental ponds ranged from 5.60 to 7.00 $\log_{10}$ CFU/mL, while those in gill, liver, and intestine samples ranged from 6.40 to 7.00 $\log_{10}$ CFU/g. The microbial loads were higher than the permissible limits for wastewater and fish tissues. The microscopic cell morphology analysis of presumptive *P. shigelloides* revealed 40 isolates of round-ended, straight rod shape, which were motile, positive to oxidase, catalase, mannitol, and citrate biochemical test, negative to urease, methyl red, and glucose biochemical test. Antibiotic susceptibility results showed that the presumptive *P. shigelloides* were 100% resistant to cefuroxime and cefotaxime, 87.5% to meropenem, and 77.5% to ceftazidime. However, the isolates were 0% resistant to gentamicin and amikacin of aminoglycoside derivatives, suggesting that these might be only two out of the 12 panels of antibiotics used that presumptive *P. shigelloides* might have responded to. The findings highlight the need for routine microbial monitoring, improved pond hygiene, and responsible antimicrobial use in catfish aquaculture. The observed *in vitro* susceptibility to gentamicin and amikacin may provide useful baseline information for future risk assessment and antimicrobial stewardship.

Keywords Antibiotics; Biochemical test; *Clarias gariepinus*; Microbial loads; *Plesiomonas shigelloides*

1 Introduction

Fisheries and aquaculture products worldwide are important sources of high-quality aquatic animal proteins and good sources of income, foreign exchange, and employment. About 950 million people worldwide rely on fisheries and aquaculture directly or indirectly for their livelihoods. Globally, the consumption of fish and fishery products as a protein source has increased considerably over the years, constituting about 20% of total protein (FAO, 2020). The aquaculture industry grows at a fast rate when compared to all other animal food-producing sectors worldwide, with the world average annual growth rate of 8.8% /yr since 1970, compared with only 1.2% for capture fisheries and 2.8% for land-farmed animal production systems (FAO, 2020). However, as aquaculture production increases, aquaculture waste also increases, and this aquaculture wastewater harbours diverse pollutants, including pathogenic organisms, which are detrimental to public health when released into the environment, and this makes fish farmers treat aquaculture effluent with pesticides and antibiotics.

The use of antibiotics can result in drug-resistant strains of diseases, causing bacteria that can harm aquatic animal populations and consumers of aquaculture fish. There are also increasing concerns about foodborne hazards, such as chemicals and microbial contaminants, that might be present in fish. These concerns can also result in decreasing demand for farmed fish (Smallwood and Blaylock, 1991). In an approach to satisfy the growing demand for fish, food safety is a major factor to be considered since these animals can be routes for the transmission of various pathogens such as *Salmonella* spp., *Vibrio* spp., *Aeromonas* spp., *Campylobacter* spp., *Shigella* spp., *Yersinia* spp., *Clostridium* spp., *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Plesiomonas shigelloides* which have been responsible for numerous cases and outbreaks of food-borne diseases in humans worldwide (Cortes-Sanchez et al., 2021).

Plesiomonas shigelloides is an oxidase-positive, facultatively anaerobic, Gram-negative, motile, rod-shaped bacterium commonly found in aquatic environments (Jagger, 2010; Janda et al., 2016). Aquaculture-raised fish for commercial purposes appear to be strongly associated with the presence of P. shigelloides (Janda et al., 2016). Plesiomonas was a common pathogen in the gills, muscles and intestines of fish as well as in rearing waters and pond sediment (Pakingking et al., 2015). Many works of literature reported that *Plesiomonas shigelloides* caused diarrhoea/gastroenteritis in humans via water or fish contaminated with this pathogen (Gonzalez - Rey et al., 2000). Plesiomonads are mesophiles with growth temperatures ranging between 8 ℃~45 °C with (a) pH ranging between 4.5 and 9.0 (Janda et al., 2016). Several factors that encourage the growth of *Plesiomonas shigelloides* include(s) overcrowding, oxygen levels, temperature, and climatic conditions, as well as food sources (Jun et al., 2011). However, little or no information is documented on *Plesiomonas shigelloides* as a causal agent of enteritis in pond water and African catfish (*C. gariepinus*). Hence, this study aimed at evaluating the microbial loads, isolation and antimicrobial susceptibility of *Plesiomonas shigelloides* from experimental pond water and *C. gariepinus*.

2 Materials and Methods

2.1 Study area

This study was conducted at the Department of Fisheries and Aquaculture Technology's Teaching and Research Farm, Olusegun Agagu University of Science and Technology, Okitipupa. Okitipupa is located in the Ondo State of Nigeria, and it is reported to have a geographical coordinate of 6º 27' 25" N,4º 46' 00" E.

2.2 Media preparation and sterilization of materials

All media (Inositol brilliant green agar, nutrient agar, Mueller Hinton agar, nutrient broth, blood agar and alkaline peptone water) were prepared according to the manufacturer's instruction; the media were weighed out accurately and dissolved in an appropriate volume of water. The prepared mixture was homogenized and sterilized by autoclaving at 121 °C for 15 min. All these media were allowed to cool after sterilization to about 45 °C before pouring into Petri dishes. Alkaline peptone water was used as an enrichment medium.

2.3 Sample collection and design

Experimental ponds (6) were randomly selected and used for this study. The experimental ponds were replicated twice and the pond water (aquaculture water) was collected from each experimental pond at 0, 2, 4, 6 and 8 weeks while fish tissues (gill, liver and intestine) were collected from each experimental pond at 0, 4 and 8 weeks. Three fish were sampled from each experimental pond every 4 weeks. The experimental design was completely randomized block design.

2.4 Water quality analysis

A water sample (50 mL) was taken at 25 cm below the water surface by using a Van Dom water sampler (Denmark) from each experimental pond. The water quality parameters such as pH, temperature and total dissolved solids were taken at 0, 2, 4, 6 and 8 weeks as described by Olaifa and Bello (2011).

2.5 Isolation of bacteria/ bacterial counts

One gram (1 g) of gills, intestine and liver sample of C. gariepinus were separately macerated and put into a sterile capped test tube containing 9 mL of sterilized alkaline peptone water and 1 ml of experimental pond water was dispensed into 9 mL of sterilized peptone water (Bello et al., 2012; Bello, 2014). The pond water was also enriched in alkaline peptone water for the isolation of the presumptive *Plesiomonas shigelloides*. Serial dilution was carried out and 0.1 mL each from 10^{-4} and 10^{-5} dilution factor was dispensed into Petri dishes that were appropriately labelled and the molten sterilized medium was poured aseptically into a Petri dish. The plates were swirled gently for even distribution of inoculums and allowed to set/gel and then incubated at 37 ℃ for 24 h. The organism grew into visible different colonies after 24 h. Total viable counts were determined and the results were expressed in $\log_{10}$ CFU/mL for pond water and $\log_{10}$ CFU/g for fish tissues. Also, 3~5 colonies of presumptive *P. shigelloides* were picked, purified and stocked on nutrient agar slant for further study.

2.6 Identification of isolates

Identification of the isolates was based on the procedures described by Mohammed et al. (2026). After observing cultural growth indices, the positive culture was subjected to Gram staining to study staining properties and cellular morphology under a 100X objective of a light microscope. Mixed colonies and Gram-negative bacteria were subcultured on both broth and nutrient agar (Oxoid, UK) and further incubated aerobically for 24 h. Pure culture of single colony type from (both broth and) nutrient agar were transferred onto nutrient slant for a biochemical test including catalase, oxidase, urease test, motility test, indole reaction test and fermentative/oxidative tests, hemolysis on blood agar and Gram staining techniques as described by Quinn et al. (2002) and Medical Research Council (MRC, 2017).

2.7 Gram staining technique

Young growing cultures of 18~24 h of the test isolates were used to prepare smears on clean grease-free microscopic slides. This was done by first cleaning the glass slides with cotton wool soaked with ethanol. A distinct colony of the isolates was picked with a sterile wire loop and emulsified with distilled water to form a smear and fixed. The smear was then stained with aqueous crystal violet for 1 min and was rinsed off gently with water, 95% Lugol iodine was added. The smear was decolourized with acetone until there was no violet colour on the slide. This was then rinsed off gently with water again, counter-stained with safranin for about 30 seconds, and then rinsed with water. The slide was carefully dried and examined under an oil immersion microscope with a 100x objective (MRC, 2017).

2.8 Antibiotic susceptibility

The antibiotic susceptibility profile of presumptive *P. shigelloides* was determined by using the disc diffusion technique as described by Kirby-Bauer with some modified disc diffusion techniques using 12 antibiotic discs (Biotec Lab. the United Kingdom) corresponding to the drugs containing most used in the treatment of human and animal infections caused by bacteria. The antibiotic sensitivity results for presumptive P. shigelloides were interpreted using the recommended guidelines by the Clinical Laboratory Standard Institute (CLSI, 2020). The antibiotics include; Cotrimoxazole (COT) 25 µg, Cefuroxime (CRX) 30 µg, Tetracycline (TET) 10 µg, Gentamicin (GEN) 10 µg, Ceftazidime (CPZ) 30 µg, Chloramphenicol (CHL) 10 µg, Ceftriaxone (CTR) 30 µg, Ciprofloxacin (CPR) 5 µg, Cefotaxime (CTX) 30 µg, Vancomycin (VAN) 30 µg, Amikacin (AMK) 30 µg and Meropenem (MEM) 10 µg. An 18~24 h old culture of all the isolates was prepared, after which the standardized broth culture of the inoculum was used to inoculate solidified pre-sterilized Mueller Hinton Agar plates. The antibiotics disc containing a specific concentration of antibiotics was placed on the Mueller Hinton Agar using sterile forceps and incubated at 32 oC for 24 h. The diameter of zones of inhibition was measured in millimetres and interpreted using CLSI (2020) standard and classified as sensitive, intermediate sensitive and resistant (MRC, 2017).

2.9 Statistical analysis

Bacteriological characteristics and physiochemical analysis resulting from the experiment were subjected to one-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences 2006 version 15.0). Duncan's multiple range test was used to compare differences among individual means.

3 Results

3.1 Water quality parameters of the experimental pond water

The physicochemical properties of the experimental pond water showed a pH range of 7.0~8.5, Temperature 21.3 ℃ ~33.0 °C, and Total suspended solids 25.0~41.0 (mg/L), and there were significant differences ($p < 0.05$) among the treatments in pH, temperature and total dissolved solids (Table 1).

Table 1 Water quality parameters of the experimental pond water

1	0	2	4	6	8
Ph					
Pond 1	7.10±0.01^{a}	8.00±0.06^{b}	7.50±0.01^{ab}	7.20±0.00^{a}	8.50±0.06^{c}
Pond 2	7.40±0.03^{a}	7.20±0.00^{a}	8.00±0.04^{bc}	7.50±0.04^{ab}	7.50±0.04^{ab}
Pond 3	7.10±0.04^{a}	7.50±0.01^{ab}	8.50±0.03^{c}	7.30±0.06^{b}	8.00±0.02^{bc}
Pond 4	7.20±0.02^{a}	7.70±0.03^{ab}	7.70±0.02^{ab}	8.50±0.08^{a}	7.20±0.01^{a}
Pond 5	7.00±0.05^{a}	8.20±0.05^{b}	8.00±0.05^{bc}	7.20±0.03^{ab}	7.60±0.03^{ab}
Pond 6	7.30±0.07^{a}	8.00±0.00^{b}	7.20±0.02^{a}	7.00±0.01^{ab}	7.40±0.04^{ab}
Temperature (°C)					
Pond 1	21.30±0.02^{a}	26.60±0.00^{c}	27.90±0.03^{c}	29.70±0.08^{e}	33.00±0.09^{d}
Pond 2	25.60±0.08^{c}	27.00±0.01^{c}	25.00±0.02^{b}	26.70±0.01^{c}	27.30±0.02^{b}
Pond 3	23.70±0.04^{b}	22.00±0.04^{a}	21.60±0.01^{a}	27.20±0.07^{c}	25.20±0.03^{a}
Pond 4	30.40±0.09^{e}	28.60±0.06^{e}	22.70±0.95^{a}	24.30±0.03^{a}	29.00±0.07^{c}
Pond 5	28.50±0.06^{d}	24.90±0.05^{b}	31.20±0.09^{d}	28.20±0.06^{d}	25.00±0.05^{a}
Pond 6	25.80±0.07^{c}	27.90±0.03^{d}	22.00±0.06^{b}	25.00±0.04^{b}	27.00±0.03^{b}
Total dissolved solid (TDS) (mg/L)					
Pond 1	39.40±0.07^{e}	29.20±0.03^{c}	27.50±0.05^{b}	36.50±0.02^{f}	38.00±0.05^{c}
Pond 2	26.20±0.02^{b}	25.50±0.00^{a}	30.50±0.07^{d}	31.60±0.04^{d}	40.20±0.09^{d}
Pond 3	41.00±0.09^{e}	36.20±0.05^{f}	31.20 ±0.01^{e}	29.60±0.06^{c}	29.80± 0.01^{b}
Pond 4	30.20±0.08^{c}	27.40±0.04^{b}	28.50±0.02^{c}	24.20±0.00^{a}	30.00±0.03^{b}
Pond 5	25.00±0.05^{a}	30.00±0.01^{d}	25.60±0.04^{a}	33.00±0.03^{e}	27.50± 0.02^{a}
Pond 6	33.40±0.03^{d}	31.00±0.2^{e}	30.00 ± 0.06^{d}	26.20±0.01^{b}	28.00± 0.04^{a}

Means (n =2) in the same column with similar superscripts are not significantly different ($p > 0.05$)

3.2 Bacteria counts of experimental pond water and fish tissues (gill, liver and intestine)

A total of 30 water samples from the experimental fish ponds were analyzed for total bacteria counts at 0, 2, 4, 6 and 8th week and 54 fish tissues (gills, intestine, liver) from *C. gariepinus* juveniles in all the experimental ponds were analyzed for total bacteria counts at 0, 4 and 8th week. Test for the presence of presumptive *P. shigelloides* revealed that the bacterium was present in *C. gariepinus* tissues and experimental pond water. The Plesiomonas count on *C. gariepinus* tissues (gills, liver and intestine) ranges between 6.4 to 7.0 $\log_{10}$ CFU/g while the Plesiomonas count on experimental pond water ranges between 5.6 to 7.0 $\log_{10}$ CFU/mL. There was no significant difference ($p > 0.05$) in the total bacteria observed in the gill, liver and intestine and experimental pond water among the experimental groups except for experimental pond water at the 6th week who recorded significant differences ($p < 0.05$) among the groups (Table 2).

3.3 Isolation of *Plesiomonas shigelloides*

A total of 250 isolates were obtained from both the aquaculture effluent (pond water) and fish tissues (gill, liver and intestine). Morphological identification was analyzed based on the shape, texture and colour of bacteria colonies on inositol brilliant bile green agar. The microscopic cell morphology analysis of the presumptive *P.*

shigelloides however shows only 40 isolates obtained from the selective agar were round-ended, straight rod shapes which are motile (15 isolates from experimental pond water and 25 from fish tissues (Table 3 and Table 4).

3.4 Morphological and biochemical characteristics of isolates of experimental pond water and fish tissues

The 40 isolates further tested positive for oxidase, catalase, mannitol and citrate biochemical tests, they also tested negative for urease, methyl red and glucose biochemical tests (Table 5 and Table 6).

Table 2 Total bacteria counts of experimental pond water and fish tissues (gill, liver and intestine)

Weeks	Pond 1	Pond 2	Pond 3	Pond 4	Pond 5	Pond 6
Water sample						
0	5.70±0.00^{a}	6.00±0.01^{a}	5.60±0.02^{a}	6.10±0.03^{a}	6.00±0.02^{a}	6.00±0.01^{a}
2	7.00±0.02^{a}	7.00±0.03^{a}	7.00±0.01^{a}	7.00±0.06^{a}	6.90±0.09^{a}	7.00±0.03^{a}
4	6.40±0.05^{a}	6.80±0.05^{a}	6.70±0.03^{a}	6.80±0.08^{a}	6.60±0.07^{a}	6.70±0.02^{a}
6	6.20±0.03^{ab}	6.60±0.03^{b}	6.50±0.04^{ab}	6.00±0.03^{ab}	5.80±0.01^{a}	6.50±0.05^{ab}
8	6.50±0.07^{a}	6.70±0.06^{a}	6.30±0.02^{a}	6.60±0.05^{a}	6.30±0.03^{a}	6.60±0.07^{a}
Gill						
0	6.60±0.03^{a}	6.90±0.02^{a}	6.70±0.00^{a}	6.80±0.01^{a}	6.80±0.07^{a}	6.50±0.04^{a}
4	7.00±0.05^{a}	6.80±0.05^{a}	6.90±0.05^{a}	6.80±0.06^{a}	6.60±0.04^{a}	6.60±0.06^{a}
8	6.50±0.02^{a}	6.40±0.00^{a}	6.80±0.03^{a}	6.60±0.02^{a}	6.50±0.01^{a}	6.50±0.02^{a}
Liver						
0	6.90±0.04^{a}	6.70±0.03^{a}	6.80±0.05^{a}	6.80±0.2^{a}	6.70±0.05^{a}	6.60±0.01^{a}
4	6.80±0.02^{a}	6.90±0.07^{a}	7.00±0.08^{a}	6.70±0.2^{a}	6.60±0.03^{a}	6.50±0.04^{a}
8	6.50±0.00^{a}	6.90±0.09^{a}	6.90±0.04^{a}	6.70±0.2^{a}	6.60±0.06^{a}	6.40±0.05^{a}
Intestine						
0	6.50±0.01^{a}	6.90±0.03^{a}	6.80±0.07^{a}	6.60±0.2^{a}	6.50±0.00^{a}	6.50±0.01^{a}
4	6.80±0.02^{a}	6.70±0.01^{a}	7.00±0.09^{a}	6.80±0.2^{a}	6.70±0.04^{a}	6.60±0.02^{a}
8	6.80±0.04^{a}	6.80±0.00^{a}	6.80±0.03^{a}	6.80±0.2^{a}	6.70±0.03^{a}	6.80±0.05^{a}

Means (n =2) in the same row with similar superscripts are not significantly different ($p > 0.05$)

Table 3 Colony characteristics of isolates from experimental pond water

Weeks	Isolate code	Colony shape	Elevation	Edge	Surface	Pigmentation	Opacity
4	Dctr	Short rod	Raised	Regular	Smooth	Pink	Opaque
	Dctr	Short rod	Raised	Regular	Dull	Pink	Opaque
	DTii	Short rod	Raised	Regular	Smooth	Pink	Opaque
	DTii	Short rod	Raised	Entire	Smooth	Pink	Opaque
	DTiii	Short rod	Raised	Regular	Smooth	Pink	Opaque
	DTiv	Short rod	Raised	Entire	Smooth	Pink	Opaque
	DTv	Short rod	Raised	Regular	Smooth	Pink	Opaque
	DTvi	Short rod	Raised	Regular	Dull	Pink	Opaque
	DTvi	Short rod	Raised	Irregular	Rough	Pink	Opaque
8	Ectr	Short rod	Raised	Regular	Smooth	Pink	Opaque
	ETii	Short rod	Raised	Regular	Smooth	Pink	Opaque
	ETiii	Short rod	Raised	Regular	Smooth	Pink	Opaque
	ETiv	Short rod	Raised	Regular	Smooth	Pink	Opaque
	ETv	Short rod	Raised	Entire	Smooth	Pink	Opaque
	ETvi	Short rod	Raised	Regular	Dull	Pink	Opaque

Keys: D, water sample at week 4; E, water sample at week 8; Ctr, experimental pond 1; Tii, experimental pond 2; Tiii, experimental pond 3; Tiv, experimental pond 4; Tv, experimental pond 5; Tvi, experimental pond 6; SR, Short rod

Table 4 Colony characteristics of isolates from gill, liver and intestine

Weeks	Isolate code	Colony shape	Elevation	Edge	Surface	Pigmentation	Opacity
	FGctr	Short rod	Raised	Regular	Dull	Pink	Opaque
	FGTii	Short rod	Raised	Entire	Smooth	Pink	Opaque
0	FITv	Short rod	Raised	Regular	Smooth	Pink	Opaque
	Fiiv	Short rod	Raised	Regular	Smooth	Pink	Opaque
	FLTiii	Short rod	Raised	Irregular	Rough	Pink	Opaque
	GGTii	Short rod	Raised	Entire	Smooth	Pink	Opaque
	GGTiii	Short rod	Raised	Regular	Smooth	Pink	Opaque
	GGTv	Short rod	Raised	Regular	Smooth	Pink	Opaque
	GGTiv	Short rod	Raised	Entire	Smooth	Pink	Opaque
4	GIctr	Short rod	Raised	Regular	Smooth	Pink	Opaque
	GITiv	Short rod	Raised	Regular	Dull	Pink	Opaque
	GITv	Short rod	Raised	Regular	Dull	Pink	Opaque
	GLctr	Short rod	Raised	Regular	Smooth	Pink	Opaque
	GLTii	Short rod	Raised	Irregular	Rough	Pink	Opaque
	GLTv	Short rod	Raised	Regular	Smooth	Pink	Opaque
	HGTii	Short rod	Raised	Regular	Smooth	Pink	Opaque
	HGTv	Short rod	Raised	Entire	Smooth	Pink	Opaque
	HGTvi	Short rod	Raised	Irregular	Rough	Pink	Opaque
	HITiii	Short rod	Raised	Regular	Smooth	Pink	Opaque
8	HITiv	Short rod	Raised	Regular	Smooth	Pink	Opaque
	HLctr	Short rod	Raised	Entire	Smooth	Pink	Opaque
	HLctr	Short rod	Raised	Regular	Dull	Pink	Opaque
	HLTii	Short rod	Raised	Regular	Smooth	Pink	Opaque
	HLTiii	Short rod	Raised	Regular	Dull	Pink	Opaque
	HLTvi	Short rod	Raised	Regular	Smooth	Pink	Opaque

FG, fish gills at 0 weeks; FI, fish intestine at 0 weeks; FL, fish liver at 0 weeks; GG, fish gill at 4 weeks; GI, fish intestine at 4 weeks; GL, fish liver at 4 weeks; HG, fish gill at 8 weeks; HI, fish intestine at 8 weeks; HL, fish liver at 8 weeks; Ctr, experimental pond 1; Tii, experimental pond 2; Tiii, experimental pond 3; Tiv, experimental pond 4; Tv, experimental pond 5; Tvi, experimental pond 6

3.5 Antibiotic sensitivity test

The antibiotic sensitivity test for presumptive *Plesiomonas shigelloides* was interpreted using the recommended guidelines by the Clinical Laboratory Standard Institute (CLSI, 2020) and is shown in table 5. Presumptive *Plesiomonas shigelloides* that were observed showed 100% resistance to cefotaxime, and cefuroxime, which belongs to the antibiotic class of cephems, followed by meropenem 87.5%, which belong to the antibiotic class of Carbapenems, Ceftazidime 77.5%, which belong to the antibiotic class of cephems, Vancomycin 70% which belong to the antibiotic class of glycopeptides tetracycline 40% which belong to the antibiotic class of tetracycline, ceftriaxone 37.5% which belong to the antibiotic class of cephems, chloramphenicol 20% which belong to the antibiotic class of phenicols, ciprofloxacin 20% which belong to a class of fluoroquinolones, cotrimoxazole 17.5% which belong to a class of sulfonamides, gentamicin and amikacin 0% which belong to a class of aminoglycosides (Table 7).

3.6 Multiple antibiotic resistance phenotypes of *Plesiomonas shigelloides*

All presumptive *P. shigelloides* obtained from this study exhibited resistance to at least one antibiotic. Meanwhile, most of the isolates (85%) showed resistance to three (3) or more classes of antibiotics. Resistant to four (4) classes of antibiotics had the highest frequency of occurrence. Out of the seventeen isolates resisting the effect of four (4) classes of antibiotics, resistance to Tetracycline, Cephalosporins, Carbapenem and Glycopeptides (76.5%) was seen to be the highest compared to other phenotypes (Table 8).

Table 5 Morphological and biochemical characteristics of isolates of gill, liver and intestine

Weeks	Isolate code	Cell shape	Gram reaction	Catalase	TSI reaction	Urease	Motility test	H_2S test	Citrate reaction	Methyl red	Hemolysis	Glucose	Mannitol	Oxidase reaction	Possible organisms
0	FGctr	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	FGTii	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	FITv	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	FITiv	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	FLTiii	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
4	GGTii	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	GGTiii	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	GGTiv	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	GGTv	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	GIctr	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
	GITiv	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	GITv	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
	GLctr	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
	GLTii	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	GLTv	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
8	HGTii	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
	HGTv	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
	HGTvi	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
	HITiii	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	HLTii	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	HITiv	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	HLctr	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	HLctr	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
	HLTii	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	HLTvi	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides

FG, fish gills at 0 weeks; FI, fish intestine at 0 weeks; FL; fish liver at 0 weeks; GG, fish gill at 4 weeks; GI, fish intestine at 4 weeks, GL, fish liver at 4 weeks, HG, fish gill at 8 weeks, HI, fish intestine at 8 weeks; HL, fish liver at 8 weeks; Ctr, experimental pond 1; Tii, experimental pond 2; Tiii, experimental pond 3; Tiv, experimental pond 4; Tv, experimental pond 5; Tvi, experimental pond 6; SR, Short rod; +, Positive; β, Beta; -, Negative

Table 6 Morphological and biochemical characteristics of isolates of experimental pond water

Weeks	Isolate code	Cell shape	Gram reaction	Catalase	TSI reaction	Urease	Motility test	H_2S test	Citrate reaction	Methyl red	Hemolnysis	Glucose	Mannitol	Oxidase reaction	Possible organisms
4	Dctr	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	Dctr	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	DTii	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
	DTii	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	DTiii	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	Dtiv	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	DTv	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	Dtvi	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	Dtvi	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
8	Ectr	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	Etii	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	Etiii	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	Etiv	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	Etv	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	ETvi	SR	-	+	-	-	-	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides

D-, water sample at week 4; E, water sample at week 8; Ctr, experimental pond 1, Tii, experimental pond 2; Tiii, experimental pond 3; Tiv, experimental pond 4; Tv, experimental pond 5; Tvi, experimental pond 6; SR, Short rod; +, Positive; β, Beta; -. Negative

Table 7 Antimicrobial susceptibility profile for *Plesiomonas* isolate

Antibiotic class	Antibiotic tested			Susceptibility pattern					
		Disc code	P (μg)	Sensitive		Intermediate		Resistant	
				N	%	N	%	N	%
Fluoroquinolones	Ciprofloxacin	CIP	5	24	60	8	20	8	20
Tetracyclines	Tetracycline	TET	10	23	57.5	0	0	17	42.5
Sulfonamides	Cotrimoxazole	COT	25	28	70	5	12.5	7	17.5
Aminoglycosides	Gentamicin	GEN	10	40	100	0	0	0	0
	Amikacin	AMK	30	40	100	0	0	0	0
Cephems	Ceftazidime	CPZ	30	4	10	5	12.5	31	77.5
	Cefotaxime	CTX	30	0	0	0	0	40	100
	Cefuroxime	CRX	30	0	0	0	0	40	100
	Ceftriaxone	CTR	30	14	35	11	27.5	15	37.5
Phenicols	Chloramphenicol	CHR	10	26	65	5	12.5	9	22.5
Glycopeptides	Vancomycin	VAN	30	8	20	4	10	28	70
Carbapenems	Meropenem	MEM	10	2	5	3	7.5	35	87.5

Keys: N – number of isolates

Table 8 Multiple antibiotic-resistant phenotypes of *Plesiomonas shigelloides*

No of antibiotics	Resistance Pattern	Frequency
2	Ceph- carb	4
3	Ceph- Carb- phe	1
	Tet- Ceph- Carb	1
	Ceph- Gly- Carb	8
4	Sul- Ceph- Phe- Carb	1
	Flu- Phe- Gly- Carb	2
	Ceph- Gly- Carb- Phe	1
	Tet- Ceph- Carb- Gly	13
5	Tet- Ceph- Sul - Gly- Carb	4
	Tet- Ceph- Phe- Gly- Carb	2
6	Flu- Tet- Ceph- Phe- Gly- Carb	1

KEY: Ceph, Cephems; Gly, Glycopeptides; Carb, Carbapenems; Tet, Tetracyclines; Sul, Sulfonamides; Phe, Phenicols; Ami, Aminoglycosides; Flu, Fluoroquinolones

5 Discussion

The result of the study revealed that water quality parameters such as pH, temperature and total dissolved solids measured every two weeks show variation in the values obtained among these parameters and there was a significant difference ($p< 0.05$) among the experimental ponds. The water quality values observed in this study are comparable to those reported by Olaifa and Bello (2011) who reported a temperature of 25℃~28℃ and pH of 6-8.5 for *C. gariepinus* on feed supplemented with walnut leaves and onion bulb-based diet. This finding supports the report of Olusola and Olorunfemi (2017) who observed a temperature range of 28 ℃~30 ℃ and pH of 5.70 - 6.19 for *C. gariepinus* fed guava *(Psidium guajava)* leaves and drumstick (*Moringa oleifera)* leaves extracts supplemented diet. This finding also aligns with the report of Omotayo et al. (2006). The value of total dissolved solid obtained was within the acceptable limit (2000 mg/L) by Food and Agriculture Organization, FAO (2013) for culture water.

Most microbes are transients in aquatic animals and may change rapidly with the intrusion of microbes coming from water and food. The growing demand for fish, and food safety is an essential element to consider since these animals can be vehicles for the transmission of various pathogens (Cortes-Sanchez et al., 2021). The result of this study revealed that the value of bacteria counts in experimental pond water was higher in 2 weeks in all the experimental ponds and the values decreased in 4, 6, and 8 weeks. Pond 2 recorded higher values of bacteria counts at 8 weeks when compared to other experimental ponds and there was no significant difference ($p > 0.05$) among the experimental ponds except 6 weeks that recorded a significant difference ($p < 0.05$) among the experimental ponds. The value of bacteria counts in the gill, liver and intestine was higher in pond 3 at 4 and 8 weeks when compared to other experimental ponds and there was no significant difference ($p > 0.05$) among the experimental ponds. The value of microbial loads obtained in the gill, liver and intestine were higher than the world health organization (WHO) acceptable limits 3.0 $\log_{10}$ CFU/g for fish and 6.2~6.5 $\log_{10}$ CFU/mL for wastewater.

Morphological identification was analyzed based on the shape, texture and colour of bacteria colonies on inositol brilliant bile agar. Forty (40) isolates tested positive for oxidase, catalase, mannitol and citrate biochemical tests, they also tested negative for urease, methyl red and glucose biochemical tests. This study supports the report of Wang et al. (2020) who observed a similar trend in the morphology and biochemical test of *P. shigelloides* during isolation and characterization from wastewater and tissues of *Ictalurus punctatus*. The identification of presumptive *P. shigelloides* in the pond water and fish tissues (gill, liver and intestine) is aligned with the observation of Krovacek et al. (2000) that fish and shellfish are the natural habitats of *P. shigelloides*. Jon et al. (2013) also isolated *P. shigelloides* from farm-cultured eels (*Anguilla japonica*) and their environmental waters in Korean eel farms. This study also agrees with Adesiyan et al. (2019) who stated that *P. shigelloides* is one of the indigenous bacteria of an aquatic environment.

This study was evaluated to look at the pattern of resistance or susceptibility to some of the commonly used 12 antibiotics in Nigeria and the forty presumptive *P. shigelloides* isolates were obtained from the pond water and gill, liver and intestine of *C. gariepinus*. This study revealed that cefuroxime and cefotaxime, a derivative of cephems is the least effective because all the 40 presumptive *P. shigelloides* were (100%) resistant to this class of antibiotics. The resistance of the isolates is also high in meropenem (87.5%) and ceftazidime (77.5%), which are the derivatives of carbapenems and cephems, respectively. Vancomycin (70%) a derivative of glycopeptides, tetracycline (42.5%), a derivative of tetracycline, ceftriaxone (37.5%), a derivative of cephems, chloramphenicol (22.5%) a derivative of phenols, ciprofloxacin (20%), a derivative of fluoroquinolones and cotrimoxazole (17.5%), a derivative of sulfonamides. However, presumptive *P. shigelloides* exhibited 100 % susceptibility to gentamicin and amikacin, a derivative of aminoglycosides. Gentamicin and amikacin proved to be excellent options for the treatment of infection associated with this organism. This study supports the report of Wang et al. (2020) who reported a high resistance value of *Plesiomonas shigelloides* in *Ictalurus punctatus* against cefotaxime, ciprofloxacin, ceftazidime and chloramphenicol.

The continuous use of antibiotics in veterinary medicine have resulted in a prompt selective potency in the emergence of drug resistance among several Gram-negative bacteria. However, the presence of antibiotic resistance mediated by extrachromosomal elements or R-plasmid is common among the members of Enterobacteriaceae (Some et al., 2021). This study revealed a high occurrence/ frequency of antibiotic resistance in experimental pond water and tissue of *C. gariepinus*. It was found from this study that presumptive *P. shigelloides* were resistant to multiple antibiotics which suggests consumption of such fishes could be detrimental to human health and wastewater from the pond could serve as a means of transmitting antibiotic-resistant bacteria such as *P. shigelloides* which are of public health importance into the environment. Multiple antibiotic-resistant phenotypes with tetracycline, cephems, carbapenems and glycosides (13) were observed to have the highest frequency of occurrence and this aligned with the report of Cooke (1976) and Reinthaler et al. (2003) who stated that natural water sample, effluents and aquatic organisms were more resistant to multiple antibiotics. Multiple antibiotics resistant phenotypes exhibited by the larger percentage of presumptive *P. shigelloides* obtained from this study in an indication of abuse of antibiotics in aquaculture settings.

6 Conclusion

This study corroborates other studies that showed *P. shigelloides* is indigenous to the aquatic environment. Meanwhile, gentamicin and amikacin, a derivative of aminoglycosides, are shown to be more promising antibiotics that can be employed in aquaculture. The study provides baseline information for microbial risk assessment, antimicrobial resistance monitoring, and pond hygiene management in catfish aquaculture. It is therefore recommended that future studies should include molecular identification, resistance gene detection, and broader field sampling.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Study limitations

Lack of molecular confirmation, and absence of resistance gene testing for the study were observed.

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