

MULTIPLE ANTIBIOTIC RESISTANT *VIBRIO* PATHOTYPES WITH THE INCIDENCE OF *V. cholerae* AND *V. parahaemolyticus* IN FISH AND FISH STORAGE WATER IN OKITIPUPA AND IGBOKODA AREAS, NIGERIA

Abstract

Vibrio is a genus of bacteria belonging to the family *Vibrionaceae* and is of epidemiological importance. This organism is commonly found in aquatic environments and is associated with water and food-related infectious disease outbreaks of public health concern globally. About 85% of presumptive isolates recovered from fish and fish storage water collected from major markets in Okitipupa and Igbokoda areas of Ondo State, Nigeria were confirmed as *Vibrio* species via polymerase chain reaction (PCR) techniques with the *Vibrio*-specific 16S rRNA gene as a target. Primers for *OmpW* and *toxR* genes were used to identify *V. cholerae* and *V. parahemolyticus* respectively. The isolates were subjected to antibiotic susceptibility testing against 12 antibiotics belonging to 8 classes. The prevalence of *Vibrio cholerae* and *V. parahaemolyticus* was 3.9% and 12.5% respectively. *Vibrio* spp. obtained in this study showed resistance to Meropenem (88.3%), Cefotaxime (81.3%), Ceftazidime (79.7%), Cefuroxime (78.1%), Tetracycline (54.7), Vancomycin (38.3%), Ceftriaxone (26.6%), Cotrimoxazole (21.9), Chloramphenicol (18%), Ciprofloxacin (12.5%), Amikacin (10.9%) and Gentamicin (6.2%). *Vibrio* species obtained from both sampled sites showed the highest susceptibility to Gentamicin (93.8%). Multiple antibiotic resistant Index (MARI) observed among the *Vibrio* species ranged from 0.25 and 0.83. This study revealed high incidence of multi-drug resistant *Vibrio* spp in the fish sold in these major markets, which suggests antimicrobial abuse in the study area. We concluded that the consumption of this aquaculture produce without proper processing and the discharge of the storage water into the environment without treatment pose a public and environmental health threat respectively.

Keywords: *Vibrio* pathotypes, catfish (*Clarias* spp.), Storage water, Antibiotic Resistant, Multiple Antibiotic Resistant Index

INTRODUCTION

Vibrio species are pathogenic bacteria of the family *Vibrionaceae*, which is said to be of great public health and epidemiological importance. *Vibrionaceae* is an essential group of indigenous, Gram negative, facultatively anaerobic, motile, curved rod-shaped bacteria that can be found naturally in marine environment (1). These pathogens are known to be hazardous to human, aquatic organisms and marine animals because they could cause serious infectious diseases such as cholera and other gastrointestinal disorders in man and vibriosis in fishes (2). Several species are members of *Vibrio* genus which include *Vibrio cholerae*, *V. parahaemolyticus*, *V. fluvialis* and *V. vulnificus* that had been recognized as potential human pathogens and as major bacteria that causes illness associated with food and other diseases in humans. *Vibrio hollisae*, *V. fluvialis* and *V. fetus* have also been reported as emerging pathogens associated with human and livestock diseases and economic losses for farmers (3).

One of the world's industries with the quickest rate of growth is aquaculture (Aquaculture is one of the industries with quick rate of growth) and in Nigeria, aquaculture fish production had experienced a tremendous increase in recent years (4). A balanced diet should include seafood because it is nutrient-rich and has a number of positive health effects, thereby leading to increase in the demand for these food products (5). Catfish (*Clarias* species) is a vital freshwater fish in the aquaculture sector in Nigeria due to several important features, which include good price command and increase in its consumption both in the villages and cities (5). Fish and their products are implicated in appreciable number of foodborne infectious diseases globally caused by pathogenic bacteria which includes *Vibrio* spp (6). The main causes of bacteria-associated disease brought on by seafood consumption are *Vibrio vulnificus* and *Vibrio parahaemolyticus*, the two species that are most frequently reported, and *Vibrio cholerae* to a lesser extent (7, 8). *Vibrio vulnificus* is said to be responsible for mortality rate above 50% as a result of toxic gastroenteritis, septicemia as well as wound infections majorly by handling and consuming raw and undercooked seafood (3, 9). *Vibrio cholerae* serotypes O1 and O139 strains, which cause cholera, *V. parahaemolyticus*, which causes food poisoning, and *V. vulnificus*, which causes septicemia, have all received interest due to their known toxicity in humans (10). Vomiting, diarrhea, and dehydration are all signs of diarrhea brought on by *V. cholerae* and *V. parahaemolyticus*. However, several *V. cholerae* non-O1 and O139 serotypes, *V.*

parahaemolyticus, *V. fluvialis*, and *V. alginolyticus* have been identified from riverine systems as etiological agents of enteric illnesses and outbreaks (11, 12).

Despite the health benefit associated with the consumption of fish and fish products, it could also serve as transmission vehicle for transferring pathogenic bacteria to human through hands-to-mouth route. *Vibrio vulnificus* is also implicated in septicemia, and wound infections, mostly linked to the consumption and handling of raw and undercooked seafood (9). Contamination of fish and fish products had been attributed to environmental pollution from several sources as well as fish feeds resulting in the contamination of fish itself. This, hereby makes the consumption of the contaminated fish detrimental to human health (13). Additionally, contamination of aquaculture systems has been reported to be due to poor water quality caused by pollution of water bodies due to urbanization (14), large number of fish in the system, contaminated feeds and the use of untreated animal manure (4). Ondo State is said to be the highest producer of fish in South-West Nigeria (5). Majority of these fishes are usually taken to the market alive, indicating possible high cross contamination and the storage wastewater is discharged into the environment without any form of treatment. Some of the contaminants that could be found in this storage wastewater are antibiotic resistant pathogens which include *Vibrio* spp.

Antibiotics, one of the most prevalent types of environmental pollutants from effluent and industrial operations, are harmful to ecological safety and public health, which makes the microbial communities of aquaculture systems to be strongly exposed to antibiotics (15). After extended exposure to this category of common contaminants, bacterial resistance to antibiotics may develop. *Use of antibiotics in aquaculture and poultry in abnormal ways had caused the pathogens that are resistant to antibiotics to develop and spread. Thereby, making treatment of infectious diseases in human caused by these resistant strains challenging as a result increased mortality rate* (16). The exposure of aquaculture systems to antimicrobial substances may increase the incidence of antibiotic resistance (17). Effluents from ponds discharged into the environment without treatment also pose a significant threat to the environment, because it could further contaminate freshwater bodies and farmlands especially during runoff. This exposure will compound cases of antibiotic resistance pathogens in the environment, thereby causing a great public health challenge (4). Unpublished background study revealed a concurrent diarrhea-like infection in the study area which could be caused by pathogenic bacteria such as *Vibrio* spp.

Hence, this study is aimed at determining the prevalence of *Vibrio* pathotypes in fish storage water and fish sold at major markets of the Southern region of Ondo State as well as the antibiotic susceptibility profiles of the isolates with the view of assessing the prevalence of MRD strains.

2. MATERIALS AND METHODS

2.1. Description of Study Sites

This study was conducted in Okitipupa and Igbokoda areas in the Southern zone of Ondo State, Southwest, Nigeria. The selected study areas are the most common sources of fresh fish for the community's needs. One of the six states of Southwest Nigeria is Ondo State. The states of Ekiti and Kogi border the state on its northern and western borders, respectively. Additionally, Ondo State is bordered by the Delta and Edo state in the east and by the Atlantic Ocean in the south (5). The state is said to comprise 18 Local Government Areas (LGA), having about 3.4 million inhabitants (18). Ondo State had been reported to have three (3) distinct zones ecologically; to the south is the mangrove forest, the rain forest lies at the center and to the north is guinea savannah. The coordinate of the Okitipupa and Igbokoda sampled sites are 6° 29' 58" N, 4° 47' 12" E and 6° 21' 19" N, 4° 47' 53" respectively.

2.2. Sample Collection and Isolation of *Vibrio* species

A total of 12 fish storage water effluents were collected aseptically from fish sellers using sterile 1000 mL plastic bottles and 12 fish samples were purchased and kept inside zip-lock bags from Okitipupa and Igbokoda areas of Ondo State. Samples were transported using ice to the Microbiology laboratory at Olusegun Agagu University of Science and Technology for analysis, within six hours of collection. The samples were processed in accordance with the American Public Health Association's guidelines (19). Fish storage water samples, fish skin swab and intestinal samples were serially diluted after being enriched in alkaline peptone water (pH 8.6) and incubated at 37 °C for 24 h. After serial dilution, 0.1 ml of dilutions 3 and 4 of each sample were plated on well labeled dried plates of thiosulphate citrate bile salt sucrose agar using spread plate method and incubated at 37 °C for up to 48 h. Water collected directly from storage water source was also analyzed. About 5 – 10 green and yellow colonies were picked, purified on nutrient agar and further characterized by oxidase test followed by hemolytic test on blood agar. The isolates were stocked on nutrient agar slant for further study.

2.3 Extraction of DNA

Deoxyribonucleic acid (DNA) of the isolates was extracted as described by (20). The cells were lysed using AccuBlock (Digital dry bath, Labnet) at 100 °C for 15 min, allowed to cool, and then centrifuged at 13000 rpm using a MiniSpin micro centrifuge for 15 min utilizing single colonies of presumed *Vibrio spp* cultivated overnight on nutrient agar plates at 37 °C. The PCR used the cell lysates as a template.

2.4 Polymerase Chain Reaction

Identification of genus and species of the obtained *Vibrio* isolates was carried out using PCR based techniques with forward and reverse primers for genus *Vibrio*, *V. parahaemolyticus* and *V. cholerae* (Table I). The total reaction mixture was 25 µL which consist of 12.5µL Master Mix (Inqaba Biotech, SA), 1µL of each of oligonucleotide primers (Inqaba Biotech, SA), 6.5µL of nuclease free PCR water and 5 µL of template DNA. *V. parahaemolyticus* DSM 10027 was used as control.

2.5 Antibiotic sensitivity test

In accordance with the protocol of the Clinical and Laboratory Standards Institute (CLSI), antimicrobial susceptibility testing on Mueller-Hinton agar (MHA) was performed using the standard disc diffusion method. Twelve (12) antibiotic discs (Biomark product) belonging to 8 different classes were used in this study and interpreted using CLSI breakpoints (21) (2018). The antibiotics include Tetracycline (30µg), Cotrimoxazole (25µg), Amikacin (30µg), Gentamicin (10µg), Vancomycin (30µg), Cefuroxime (30µg), Ceftazidime (30µg), Ceftriaxone (30µg), Cefotaxime (30µg), Chloramphenicol (30µg), Meropenem (10µg) and Ciprofloxacin (5µg). *Escherichia coli* (ATCC 25922) was used as positive control. Multiple antibiotic resistant phenotypes (MARP) were assessed, after which multiple antibiotics resistant index (MARI) was calculated mathematically using:

$$\text{MARI} = a/b$$

Where, a= number of antibiotics to which isolates were resistant and
b= no of antibiotics to which each isolate was subjected

Likewise, the antibiotic resistance pattern abundance (ARPA) was calculated (22)

$$\text{ARPA} = \text{RT}/\text{TS}$$

RT -the number of resistance types

TS -the total number of strains assayed

Table I: Primers for *Vibrio* Identification

Target species	Sequences (5' 3')	Target gene	PCR Condition	Amplicon size (bp)	Reference
<i>Vibrio</i> genus	F:CGG TGAAATGCGTAGAGAT R: TACTAGCGATTCCGAGTTC	16S rRNA	Initial denaturation took place at 93 °C for 15 min, then there were 35 cycles of 92 °C for 40 s, annealing took place at 45 °C for 1 min, elongation took place at 72 °C for 1.5 min, and final extension took place at 72 °C for 7 min.	663	(23)
<i>V. cholera</i>	F: ACCAAGAAGGTGACTTTATTGTG R: GAACTTATAACCACCCGCG	ompW	Initial denaturation was carried out at 94 °C for 3 min, then there were 35 cycles of 92 °C for 45 s, annealing at 49 °C for 1 min, elongation at 72 °C for 1 min, and final extension at 72 °C for 7 min.	588	(16)
<i>V. parahaemolyticus</i>	F: GTCTTCTGACGCAATCGTTG R: ATACGAGTGGTTGCTGTCATG	toxR	Denaturation at 94 °C for 3 min, 35 cycles of 92 °C for 45 s, annealing at 49 °C for 1 min, elongation at 72 °C for 1 min, and final extension at 72 °C for 7 min	368	(16)

RESULTS

Prevalence and distribution *Vibrio* species

Out of 150 presumptive *Vibrio* spp. isolated, a total of 128 (85.3%) *Vibrio* species were confirmed via 16S rRNA based PCR (Plate 1), with 76 and 52 isolates obtained from Okitipupa and Igbokoda respectively. They occurred in various percentages in water (26.6%), skin (33.6) and the intestine (39.8) of fish (Table II). It was observed that the number of isolates recovered from the fish intestine were higher from both sampled sites. Out of the 128 confirmed *Vibrio* isolates, only five (5) were *V. cholerae* while sixteen (16) were *V. parahaemolyticus* (Plate 2). The rate of isolation of *V. parahaemolyticus* was higher in storage water samples than in the skin and intestine of the fish samples. *Vibrio* species were not recovered from the source of storage water

Table II: Distribution of *Vibrio* pathotypes obtained from samples collected in the two sites

	Okitipupa	Igbokoda	Total (%)	<i>V. cholera</i>	<i>V. parahemolyticus</i>
Water	21	13	34 (26.56)	2	6
Skin	25	18	43 (33.59)	1	5
Intestine	30	21	51 (39.84)	2	5
Total	76(59.37%)	52(40.63%)	128(100%)	5(3.9%)	16(12.5%)

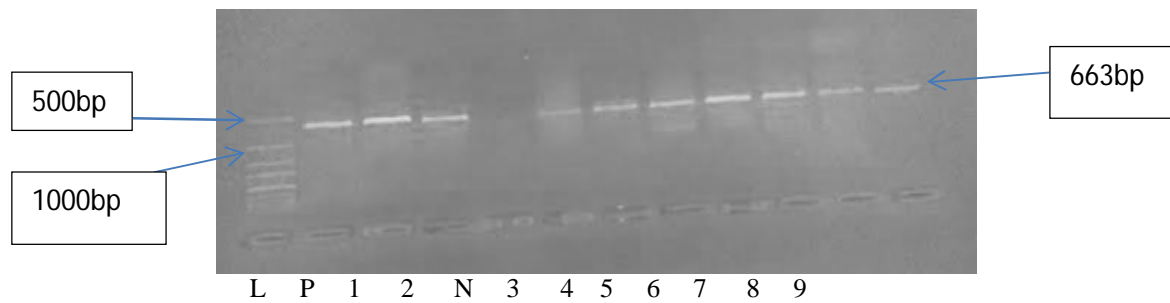


Plate 1: Gel electrophoresis of genus *Vibrio*. Lane L- Ladder (1kb), lane P-Positive control, lane N-Negative control, lane 1-9—isolates.



Plate 2: Gel electrophoresis of genus *Vibrio*. Lane 1- Ladder 100bp, lane 2- positive control, lane 3-10- isolates

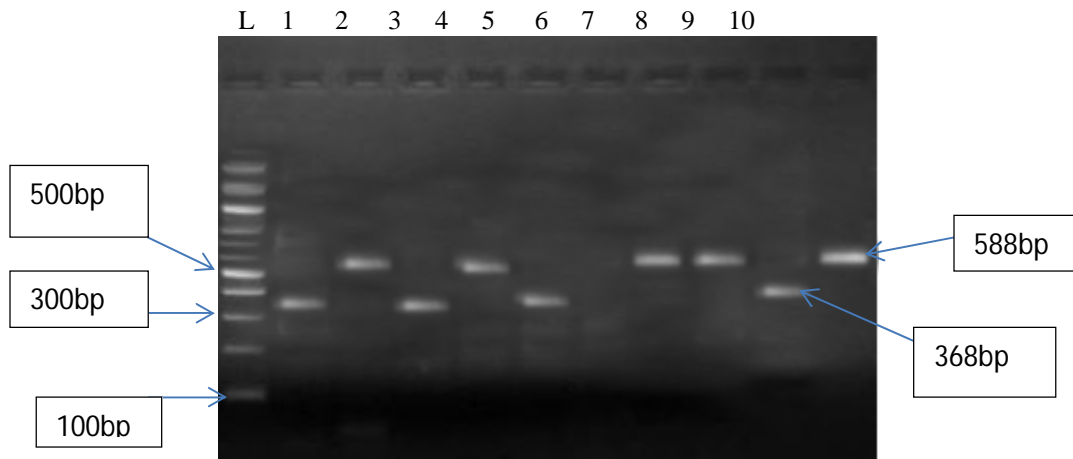


Plate 3: Gel electrophoresis for *V. cholerae* (588bp) and *V. parahemolyticus* (368bp). Lane L- 100bp ladder, lane 1-10- isolates

All the confirmed *Vibrio spp.* were subjected to antibiotic susceptibility test and the study revealed that all the isolates were resistant to two or more antibiotics. The percentage of antibiotic resistant *Vibrio spp.* obtained from water, skin and intestine of fish from the two sampled sites are shown in Figure 1 and 2. The isolates obtained from Okitipupa showed resistance to the tested antibiotics in the following trend: Meropenem (92.1%), Cefotaxime (86.8%), Ceftazidime (84.2%), Cefuroxime (78.9%), Tetracycline (59.2), Vancomycin (38.2%), Ceftriaxone

(28.9%), Cotrimoxazole (25.0), Chloramphenicol, Ciprofloxacin (14.5%), Amikacin (9.2%) and Gentamicin (7.9%). Also, isolates obtained from Igbokoda follows the same trend. *Vibrio* spp. obtained from Igbokoda showed highest susceptibility to Ciproflaxacin (90.4%) and lowest susceptibility to Cefuroxime (23.1%) (Table III).

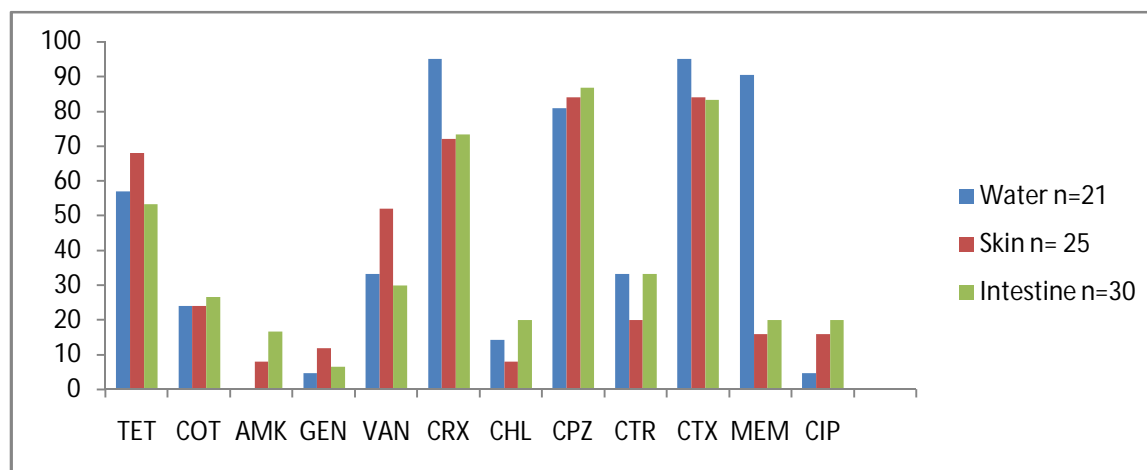


Figure 1: Percentage resistance of *Vibrio* species obtained from Okitipupa

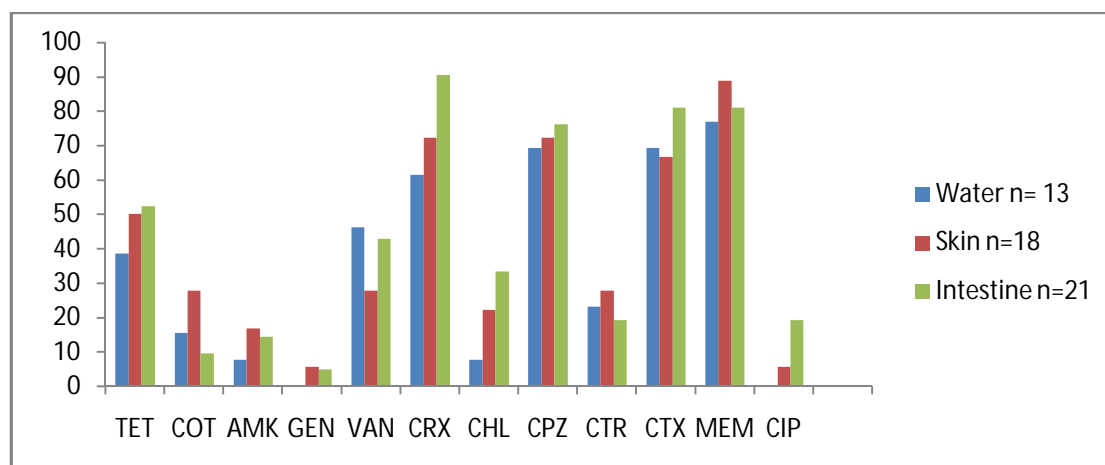


Figure 2: Percentage resistance of *Vibrio* species obtained from Igbokoda

Keys: TET-Tetracycline, COT-Cotrimoxazole, AMK-Amikacin, GEN-Gentamicin, VAN- Vancomycin; CRX- Cefuroxime, Ciprofloxacin; CHL-Chloramphenicol, CPZ- Ceftazidime; CTR-Ceftriaxone, CTX- Cefotaxime, MEM-Meropenem.

Table III: Antibiogram Profile of *Vibrio* species from the two Sampling Locations

Antimicrobial Family	Antimicrobial Agent (Disc)	Code	Potency (µg)	No of Antimicrobial Resistant Isolates		
				OPA	IGB	Total (%) N=128
Tetracycline	Tetracycline	TET	30	45	25	70(54.69)
Sulfonamides	Cotrimoxazole	COT	25	19	9	28(21.88)
Aminoglycosides	Amikacin	AMK	30	7	7	14(10.94)
	Gentamicin	GEN	10	6	2	8(6.25)
Glycopeptides	Vancomycin	VAN	30	29	20	49(38.28)

Cephalosporins	Cefuroxime	CRX	30	60	40	100(78.13)
	Ceftazidime	CPZ	30	64	38	102(79.69)
	Ceftriaxone	CTR	30	22	12	34(26.56)
	Cefotaxime	CTX	30	66	38	104(81.25)
Phenicol	Chloramphenicol	CHL	30	11	12	23(17.97)
Carbapenem	Meropenem	MEM	10	70	43	113(88.28)
Fluoroquinolones	Ciprofloxacin	CIP	5	11	5	16(12.5)

Keys: OPA- Okitipupa, IGB- Igbokoda, TET-Tetracycline, COT-Cotrimixazole, AMK-Amikacin, GEN-Gentamicin, VAN- Vancomycin; CRX- Cefuroxime, Ciprofloxacin; CHL-Chloramphenicol, CPZ- Ceftazidime; CTR-Ceftriaxone, CTX- Cefotaxime, MEM-Meropenem.

Table IV displays the pattern of the *Vibrio spp* multiple antibiotic resistance phenotypes. In the present investigation, all of the isolates showed resistance to more than two different types of antibiotics. Antibiotic resistant phenotypes expressed by *Vibrio spp* in this study ranged from resistance to three (3) classes of antibiotics to seven (7) classes of antibiotics. Resistance to five (5) distinct antibiotic classes was found to have the highest frequency of occurrence in both sampled sites. *Vibrio spp* isolated from Okitipupa possess highest resistance to six (6) classes of antibiotics, while three of the isolates obtained from Igbokoda showed resistant to seven (7) classes of antibiotics. *Vibrio cholerae* obtained in this study exhibited resistant to three (3) to five (5) classes of antibiotics while *V. parahaemolyticus* resisted the action of three (3) to five (5) classes of antibiotics. The MAR_{index} of the *Vibrio* species from the two sampled sites were discovered to be greater than 0.2 threshold value. The calculated MAR_{index} ranged from 0.25 and 0.83 which is higher than the critical limit of 0.2.

Table IV: Multiple antibiotic resistance phenotype (MARP) patterns in different *Vibrio* species

No of Antimicrobials	Resistant Pattern	Frequency	MAR index	ARPA
OKITIPUPA				
3	Gly-Ceph-Car	4	0.42	0.18
	Ceph-Car-Tet	4	0.83	
	Sul-Gly-Ceph	6	0.25	
4	Ceph-Car-Gly-Sul	3	0.50	
	Tet-Ceph-Car-Phe	3	0.42	
	Ami-Tet-Ceph-Car	3	0.58	
	Tet-Sul-Phe-Ceph	2	0.50	
5	Tet-Sul-Gly-Ceph-Car	5	0.58	
	Sul-Ceph-Flu-Car-Ami	5	0.58	
	Tet-Sul-Ceph-Phe-Car	7	0.67	
6	Tet-Sul-Gly-Ceph-Phe-Car	6	0.50	
	Tet-Sul-Gly-Ceph-Car-Flu	7	0.50	
	Tet-Ami-Ceph-Car-Phe-Gly	5	0.67	
	Sul-Ami-Gly-Ceph-Car-Flu	3	0.50	
IGBOKODA				
3	Gly-Ceph-Car	5	0.42	0.25
	Tet-Phe-Ceph	4	0.50	
	Tet-Ceph-Gly	4	0.33	
4	Tet-Gly-Ceph-Car	3	0.50	
	Tet-Ceph-Car-Phe	3	0.42	
	Ami-Tet-Ceph-Car	2	0.58	
5	Tet-Sul-Car-Gly-Ceph	5	0.58	
	Sul-Ceph-Flu-Car-Ami	3	0.50	
	Tet-Sul-Ceph-Phe-Car	6	0.58	
6	Tet-Sul-Gly-Ceph-Phe-Car	3	0.50	
	Sul-Ami-Gly-Ceph-Car-Flu	3	0.75	

7	Tet-Sul-Gly-Ceph-Phe-Car-Flu	2	0.67
	Ceph-Gly-Ami-Car-Phe-Tet-Flu	1	0.83
<i>Vibrio cholera</i>			
3	Gly-Ceph-Car	1	0.42
4	Ami-Tet-Ceph-Car	1	0.58
5	Tet-Sul-Gly-Ceph-Car	1	0.58
	Tet-Sul-Ceph-Phe-Car	2	0.50
<i>Vibrio parahaemolyticus</i>			
3	Gly-Ceph-Car	2	0.42
	Ceph-Car-Tet	1	0.83
	Sul-Gly-Ceph	1	0.25
	Tet-Phe-Ceph	1	0.50
4	Ami-Tet-Ceph-Car	1	0.58
	Tet-Ceph-Car-Phe	1	0.42
5	Tet-Sul-Gly-Ceph-Car	3	0.58
	Sul-Ceph-Flu-Car-Ami	1	0.50
6	Tet-Sul-Gly-Ceph-Phe-Car	3	0.50
	Tet-Ami-Ceph-Car-Phe-Gly	1	0.67
	Sul-Ami-Gly-Ceph-Car-Flu	1	0.75

Keys: Ami-Aminoglycoside, Ceph-Cephalosporins; Car-Carbapenem, Flu-Fluoroquinolones, Gly-Glycopeptide, Phe-Phenicol, Sul-Sulfonamides; Tet-Tetracycline

DISCUSSION

Generation of wastewater is very common globally and treatment of this wastewater before discharge into the environment is so minimal (20). Aquaculture is prone to contamination due to poor quality of water used as well as the addition of fish feeds, which enriches the water with organic matter, making it a rich medium for microbial growth (3). Pollution of aquatic environment is an important factor that influences the microbial load and strains of organisms found in the water bodies and their animals (14). Pollutants such as pathogenic bacteria from aquaculture environment can be transferred to the market via aquaculture fish, which could contaminate the environment and be transmitted to humans via hand to mouth route (24).

Vibrio spp. are aquatic microorganisms that cause disease in both humans and animals ranging from cholera to vibriosis. A total of 150 presumptive *Vibrio* species were obtained based on selective isolation and oxidase test as well as hemolytic test, so as to ascertain the pathogenic potential of the isolates by lysing the red blood cell. Meanwhile, 85% (128) of the presumptive isolates were *Vibrio spp.* by PCR. The highest number of *Vibrios* was obtained from Okitipupa (76) while, the intestine of the fish harbored the largest number of *Vibrio spp.* This could be due to feed quality or the *Vibrio spp.*, are indigenous to the aquatic environments of the fish (25); Incidence of *Vibrio* species in high number agree with the findings of Adesiyani *et al.* (16), who also obtained high quantity of presumptive *Vibrio spp.* from water resources in Southwest Nigeria as well as other places (16). Haenen *et al.* (13) also recovered *Vibrio spp.* from sick eel. High prevalence of *Vibrio spp.* confirms the reason for the frequency of cholera-like epidemic that usually occur in the study area. All these findings confirm that *Vibrio spp.*, are inhabitants of aquatic environment. In this study, the percentage occurrence of *Vibrio* in storage water was lower (26.7%) compared to the skin (33.6%) and the intestine (39.8%) of fish. The absence of *Vibrio spp.* in the water source suggest that *Vibrio spp.* recovered from the samples are possibly from the aquaculture pond which confirms the findings of Baumeister *et al.* (8), who recorded high incidence of *Vibrio spp.* in sea catfish. The incidence of higher percentage in the intestine of the fish showed the ability of the bacterium to survive in adverse environmental condition as previously reported by other scientists (26, 16). The presence of *Vibrio spp.*, *V. cholerae*, and *V. parahaemolyticus* in fish storage water and fish in the current investigation implies that if these fish are consumed in

a minimally processed state, foodborne infectious illness may ensue (3). This could also lead to cross contamination of other food products in these markets, thereby resulting in diarrhea associated infection in humans.

Vibrio cholerae and *V. parahaemolyticus*'s occurrence in fish storage water as well as fish and its absence in storage water source indicate that the pond is contaminated with *Vibrio* spp. It also suggests that there is a healthy carrier of *Vibrio* spp. releasing it inside aquatic surroundings (1). It had been stated that the most frequent pathogenic species were *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, and *V. alginolyticus*, but the most prevalent pathogens in humans were *V. cholerae* and *V. parahaemolyticus*. Infections in human was reported to be initiated via exposure to contaminants in water-bodies or by consuming raw or minimally processed seafoods that are contaminated, thereby resulting in a variety of signs and symptoms in humans. *Vibrio parahaemolyticus* was reported to be first known as the agent responsible for foodborne associated illness in Osaka, Japan in 1951, which was linked to the consumption of seafoods in some countries in Asia (3). Likewise, the incidence of *V. parahaemolyticus* in this research confirms the findings of Baumeister *et al.* (8), who said that this organism is associated with sea catfish. Due to the significant consumption of seafood in the sample locations, the prevalence of *Vibrio* spp. could also cause serious infectious diseases and also constitute an economic danger to aquaculture (27) farmers. Furthermore, food-borne pathogenic bacteria like *V. parahaemolyticus* and *V. vulnificus* are linked with raw seafood resulting in syndromes such as gastroenteritis, septicemia and wound infections (28). *V. parahaemolyticus* is a common species that had been obtained from seafood like shrimp (29), tilapia (3) and catfish (8). Therefore, high incidence of *Vibrio* spp. in aquaculture product assessed in this study is detrimental to human health especially through hand to mouth route of transmission and hence a threat to the public health.

Antibiotic resistant bacteria are a commonly known global challenge due to their ubiquitous nature. One of the means through which their spread increases includes horizontal gene transfer as well as transfer from aquatic environments to man (16). This is because aquatic environments are prone to contamination from diverse sources. In this study, *Vibrio* spp. obtained from Igbokoda market were more susceptible to Gentamicin (97.2%) and Ciprofloxacin (90.4%) while those obtained from Okitipupa market were more susceptible to Gentamicin (92.1%) and Amikacin (90.8%). High rates of susceptibility to Gentamicin (93.7%), Amikacin (89.1%) and Ciprofloxacin (87.5%) in this study confirm the recommendation of US Center for Diseases prevention and control (CDC) for antibiotics belonging to Aminoglycoside class to be used for the treatment of *Vibrio* associated infection in children (30).

The highest number of resistant strains was observed against Meropenem (88.3%) followed by the Cephalosporin groups such as Cefuroxime, Cefotaxime (81.3%) and Ceftazidime (79.7%) while only 35 (27.3%) isolates was found to resist the antibacterial effect of Ceftriaxone. More than 50% of the isolates were resistant to Tetracycline which is one of the most commonly used antibiotics for diarrhea related infections. Resistance observed against tetracycline also corroborate the findings of Haenen *et al.* (13) who reported a sudden acquired multiple antibiotics resistance in *Vibrio* species. Other researchers also reported tetracycline resistant in *vibrio* species obtained from diverse sources of water such as wastewater effluent (20), abattoir effluent (31) and surface water (16).

In this study, only few *Vibrio* species were resistant to quinolones (ciprofloxacin) which contradicts the report of Haenen *et al.* (13) who recorded resistant to quinolones which he attributed to acquisition of resistance from the use of lower-dose of flumequine baths for a long period. The low rate of resistance to Chloramphenicol observed in this study (17.97%) conforms to the findings of other researchers. It had been previously reported that the use of Chloramphenicol in fish farming are stopped in some nations as a result of its toxic nature in human beings (32, 16). Therefore, decrease in prevalence of resistance in *Vibrio* spp. to the activity of Chloramphenicol had been a common phenomenon observed in many areas (34, 16).

The present research shows that fish sold in these two major markets harbor *Vibrio* spp., which could be carry-over contaminants from the ponds. High incidence of many *Vibrio* species that are resistant to antibiotics in fish storage water and fish indicates a potential health threat to both human beings and aquatic animals. Besides, continuous distribution of MDR strain of *Vibrio* could hinder treatment of infections caused by these bacteria efficiently in the study areas.

Vibrio spp. obtained in the current research showed MARI that were higher than 0.2, indicating that the aquaculture fish are source of antimicrobial resistant bacteria in Okitipupa and Igbokoda area of Ondo State Nigeria (34). The distribution of these multiple antibiotic resistant *Vibrio* spp. in aquaculture produce poses a serious threat to both human and animal health on a global scale (35, 33, 16). Abundance of *Vibrio* spp. in the aquaculture produce seen in this investigation could be explained by contamination of freshwater environment which could be from effluents, human as well as agricultural runoff because discharge of waste had been shown to be one of the means through which antibiotics enter the water bodies (36, 16). All the *V. cholerae* and *V. parahaemolyticus* isolated also exhibited resistant to three or more classes of antibiotics, which indicates public health concern due to the possibility of these strain causing infectious diseases. The trend of antibiotic resistance seen in this study could be linked to

inappropriate use of antibacterial materials for prevention or treatment of infectious diseases as well as in preservation of agriculture produce (37). Despite the advantages associated with the use of antibiotics, indiscriminate use continued to outweigh these advantages especially in food safety and productivity. Hence, it has been recommended that non-antimicrobial approaches such as the use of probiotics as well as plant based antibacterial agents be used in managing and controlling *Vibrio* strains and other pathogenic bacteria in aquaculture. This is to minimize the threat it poses on human health when aquaculture produce are consumed raw or minimally processed.

Conclusion

The presence of *Vibrio* spp. in the fish storage water and fish samples may be an indication for future outbreak of gastrointestinal infection via consumption of contaminated fish. However, chances of cross contamination of other food products in the market as well as through kitchen materials or via handling could result in infectious diseases. The presence of *V. cholera* and *V. parahaemolyticus* in the samples could indicate contamination of pond or water resources and poor hygiene practices by fish handlers, which is of great public health concern. The high incidence of resistant *Vibrio* species against Glycopeptides, Cephalosporins, tetracycline and Carbapenem implies increased resistance against some antibiotics of choice which could be a threat to the effective treatment of infection associated with *Vibrio* spp. in the study area. This suggests abuse of antibiotics usage around the study areas and aquaculture settings as well as water bodies. Therefore, this study showed that the fishes sold in this area serve as potential reservoirs of multidrug resistant *Vibrio* spp. that may cause outbreaks of diarrhea associated infection in Southern part of Ondo State, Nigeria.

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Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

Agboola Temitope: Planned, designed, analyzed the molecular aspect, supervised the research and prepared the first manuscript. Nmema Eucheria: Executed some molecular aspect of the research and reviewed the manuscript. Samuel Busayo: Executed the presumptive isolation aspect of the research. Odetoyin Babatunde: Designed, analyzed molecular aspect of the research and reviewed the manuscript.

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