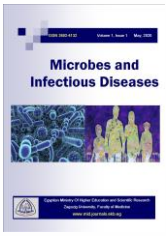
Microbes and Infectious Diseases 2022; 3(4): 1006-1015



Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Antibiotic sensitivity and plasmid assessment of ampicillin-resistant *Vibrio cholerae* from seafoods in coastal areas of Ondo State

Anthony Kayode Onifade, Sawa Joshua Adeyanju *

Department of Microbiology, School of Life Sciences, The Federal University of Technology, Akure, Nigeria.

ARTICLE INFO

Article history:
Received 31 December 2021
Received in revised form 14 January 2022
Accepted 16 January 2022

Keywords:
Seafoods
Vibrio cholerae
Beta-lactamase
Plasmid
Antibiotics

ABSTRACT

Background: *Vibrio cholerae* is an epitome of non-invasive organisms, which only affects the small intestine via the liberation of enterotoxin. The plasmid profile and beta lactamase production in ampicillin-resistant *Vibrio* species isolated from seafoods collected from coastal area of Ondo State, Nigeria were investigated. **Methods:** One hundred and six (106) samples of seafoods were collected in February, 2020 and January, 2021. The isolation and identification of *Vibrio* species, antibiotic sensitivity pattern, plasmid analysis and beta-lactamase detection activity were conducted via pour plate, Kirby-Bauer disc diffusion, alkaline lysis, neutralization, electrophoresis methods, use of 10 % sodium dodecyl sulphate (SDS) and double disk synergistic test (DDST) respectively. **Results:** Pathogenic bacteria such as *Bacillus subtilis*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Staphylococcus aureus*, and *Vibrio cholerae* were isolated from 10 crabs, 54 fishes, and 42 shrimps. Findings also revealed that 66% of *Vibrio cholerae* that showed multiple antibiotic resistance tested positive to beta lactamase production test. The result of plasmid analysis indicated that they were more susceptible to antibiotics after plasmid curing of the microorganisms. **Conclusion:** The resistance of *Vibrio cholerae* in this investigation was found to be plasmid-mediated consequent to its loss after curing. The detection of multidrug resistance, ESBLs-producing *Vibrio* organisms, suggests risks to public health. The risk of seafood-borne infections can be reduced by decrease in pollution levels, ensuring appropriate cooking temperatures, and prevention of cross-contamination during harvesting, handling and processing.

Introduction

The presence of pathogenic bacteria in the marine environment raises concerns of human on food safety due to their potential in causing disease outbreaks depending on the environmental conditions [1]. The genus *Vibrio* comprises about 100 species which are autochthonous inhabitants of aquatic environments and play vital roles in sustaining the aquatic milieu [2]. *Vibrio species* are Gram-negative bacteria, some are halophilic, from the family Vibrionaceae. They are widely distributed in fresh, estuarine, marine and coastal surroundings based on their species and can be isolated from sea foods [3], where they predominantly persist in culturable or non-culturable state, and are considered to exhibit both fermentative and respiratory metabolisms [4].

DOI: 10.21608/MID.2022.113971.1224
* *Corresponding author:* Sawa Joshua Adeyanju
E-mail address: adeyanjusawa@gmail.com

Several investigations have also shown the prevalence of *Vibrio* species in surface water throughout the world, and their prevalence in the environment is influenced by season, location, and the analytical methods employed [4]. They are usually found in a free swimming state, with its motility conferred by a single polar flagellum affixed to inert and animate surfaces including zooplankton, fish, shell-fish or any suspended matter under water [5,6].

Vibrio species cause diarrhoea including cholera infection. *Vibrio cholerae* serogroup O1 is responsible for the majority of cholera cases globally [7]. In rare cases, *Vibrio parahaemolyticus* causes wound and ear infections or septicemia that may be life-threatening to individuals with pre-existing medical conditions [8]. The pathogenic *Vibrio* species of health relevance which are generally transmitted through water and seafood include *Vibrio cholerae*, *Vibrio fluvial*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio tubiashi* [9]. *Vibrio cholerae* is an example of a non-invasive organism, which only affects the small intestine via the release of enterotoxin and is the etiological agent of cholera, whereas *Vibrio parahaemolyticus* and *Vibrio vulnificus* are considered as intrusive microorganisms largely affecting the colon [2]. The aim of this study is to identify and detect extended spectrum beta-lactamases in ampicillin resistant *Vibrio* species from Seafoods in Coastal Areas of Ondo State and determine the plasmid profile of the isolates.

Material and Methods

Description of the study area

Erin-Amah is a town in Aheri kingdom of Ilaje in Ondo State. The coastal areas are largely found in Ilaje Local Government Area. Ilaje is situated between longitude 6º 20` and 6º00 North and latitude 4º45` and 5º45` East. It shares boundary with Ese- Odo Local Government of Ondo State in the North, the Atlantic Ocean in the South, Ogun State in the West and Delta State in the East. The local government has about 80 KM long shoreline, thereby giving Ondo State one of the largest coastlines in Nigeria [10].

Collection of samples

One hundred and six (106) samples of seafood which comprised ten (10) crabs, forty two (42) shrimps, and fifty four (54) fishes which comprises of the following species: *Cynoglossus brown, Pentanemus quinquarius, Caranx Senigallus, Sardinella Maderensis, Psutolithus elongatus, Caranx rhochuss,* and *Atlantic bumper*; were obtained from landing sites in Erun Amah. Samples were collected in February, 2020 and January, 2021. They were collected from local fishermen in sterile ice packed flask and then transported to the Department of Microbiology Laboratory of Federal University of Technology, Akure.

Preparation of culture media

All culture media were prepared according to the manufacturers specifications. Dehydrated Thiosulfate Citrate Bile Salt Sucrose agar (16.84 g) (Hi-Media, Mumbai, India), Tryptose Soya Agar (8 g) (Hi-Media, Mumbai, India) Mueller-Hinton agar (7.6 g) (Hi-Media, Mumbai, India) were separately dissolved in 200 ml of distilled water in a conical flask. Complete dissolution was achieved on a hot plate at 60 °C for 5 min. Thereafter, the mixture was autoclaved at 121°C for 15 min at 15psi [11].

Detection and identification of *Vibrio species*

Seafood samples were investigated for the presence of *Vibrio* species. The procedure of Oramadike and Ogunbamiwo, [12] was adopted for the isolation. Alkaline peptone water (APW) at pH 8.4 and Alkaline Peptone Salt Broth (APSB) (Hi-Media, Mumbai, India) i.e. (APW supplemented with 3% NaCl) at pH 8.4 were used for the enrichment of the samples. A 25 g portion of each sample (crab, fish and shrimp) was blended separately with sterile blender, 25 g of sediment was mixed with 225 ml of sterile Alkaline peptone water and Alkaline Peptone Salt Broth (APSB) each (i.e. 1:10 dilution).

The alkaline peptone water homogenate was incubated at 37 °C for 6 h. Thereafter, 1 ml of the broth culture showing positive growth (turbidity) was transferred to a sterile Petri dish, then pour plated with sterilized cooled Thiosulphate Citrate Bile Salt Sucrose Agar –TCBS Agar (Hi-Media, Mumbai, India) to obtain discrete colonies for each of the different samples. The plates were incubated for 18 - 24 h at 37 °C, after which the discrete colonies were sub-cultured to obtain pure isolates of *Vibrio* species on dried Tryptose Soya agar (TSA) (Hi-Media, India) supplemented with 3% NaCl and incubated overnight for further identification of the isolates. Typical colonies of *Vibrio* species are either golden yellow (*V cholera, V. alginolyticus, V. fluvialis, V. vulnificus*) or green (*V. parahaemolyticus*), on TCBS.

Characterization and identification of bacterial isolates

All the bacterial isolates were further confirmed using microscopic examination, staining techniques and standard biochemical tests as described by **Tripathi and Sapra,** [13].

Standardization of bacterial inoculum

A loop full of test bacterial isolates was inoculated on nutrient broth and incubated for 24 h. 0.2 ml from the 24 h broth culture of the bacteria was dispensed into

20 ml sterile nutrient broth and incubated for 3 to 5 h to standardize the culture to 0.5 McFarland standards (10^6 CFU/ml) before use. One percent (1%) of Sulphuric acid (H_2SO_4) was prepared by adding 1ml of concentrated Sulphuric acid to 99/ml of distilled water. This solution was mixed properly. Also, 1% solution of barium ($BaCl_2$) chloride was prepared by dissolving 0.5g of dehydrated barium chloride ($BaCl_2H_2O$) in 50/ml of distilled water. About 0.6/ml of barium chloride solution was added to 99.4/ml of Sulphuric acid solution and it was then mixed together to make the McFarland standard. The bacterial suspension turbidity was made sure to be equivalent to the turbidity of McFarland standard. The solution was transferred into capped tube of the same type used for both the control and the test inoculums. The solution was kept at a temperature of 4ºC until it was ready for use [14,15].

Antibiotic sensitivity test

Vibrio isolates were tested for sensitivity to conventional antibiotics (CELTECH, Belgium). Using the disk diffusion method according to guidelines set by the Clinical Laboratory Standards Institute [16]. The test was to determine the susceptibility and resistance patterns of the organisms to the antimicrobial drugs.

Detection of extended spectrum beta-lactamase producing ampicillin resistance *Vibrio* isolates using Double Disc Synergy Test (DDST)

All the ampicillin-resistant *Vibrio* isolates were selected for detection of extended spectrum beta-lactamase using double disc synergy test [17]. A disc of amoxicillin clavulanic acid (30 µg) + ceftazidime (30 µg) + amoxicillin clavulanic acid (30 µg) was placed at appropriate distance of 20mm apart on a Mueller Hinton agar plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards, and then incubated for 18–24 h at 37 °C. An increase in the inhibition zone diameter of > 5mm for a combination disc versus ceftazidime or cefotaxime disc alone was confirmed as ESBLs production.

Plasmid analysis

Extraction of plasmid DNA

Ampicillin-resistant *Vibrio* isolates were revived in Tryptose Soya agar and subsequently, they were respectively grown for 18 – 24 h in Luria-Bertani (LB) broth at 37ºC with aeration using an orbital shaker and plasmid DNA was extracted from lyzed isolates using the Zyppy™ Plasmid Miniprep Kit, (Inqaba Biotech., South Africa) following alkaline lysis method. A 600 µl of *Vibrio* culture grown in Luria-Bertani broth medium were transferred to a 1.5 ml micro centrifuge tube containing the culture for 30s at 14,000 rpm. The supernatant was discarded, 100 µl of 7X lysis Buffer was added and mixed together by inverting the solution in the tube for 4-6 times.

Thereafter, the solution was centrifuged at 14000 rpm for 2-4 minutes. However, 900 µl of the supernatant was transferred into a provided Zymo-Spin™ IIN column, placed into a collection tube and centrifuged at the proceeding measure (14000 rpm) for 15 seconds. The flow-through in the column was discarded and the column was placed back into the same collection tube. Thereafter, 200 µl of Endo-Wash buffer was added to the column and centrifuged for 30 seconds after which 400 µl of Zyppy™ wash buffer was added to the column and centrifuged for 1 minute. The column was transferred into a clean 1.5 ml micro centrifuge tube, 30 µl of Zyppy™ elution buffer was added to the column matrix and left alone for one minute at room temperature. Hence, the constituent was centrifuged to obtain the plasmid DNA. The column was then removed after the centrifugation and the micro-centrifuge tubes containing the plasmid DNA was stored at -20°C until used [18].

Plasmid profiling

Plasmid profiling was done starting with the preparation of agarose gel. 1g of agarose powder was measured into a sterile conical flask and mixed with 100ml of Tris-acetate-ethylene diaamine tetra-acetic acid (TAE). The solution was heated for homogenization for about 1-3minutes. The agarose solution was allowed to cool for 5 minutes and 10µl EZ vision™ DNA dye was added in order to visualize the DNA under ultraviolet (UV) light. The solution was poured in a gel tray and allowed to solidify at room temperature for about 20-30 minutes [18].

Once the gel was ready i.e. solidified, the purified plasmid DNA was loaded and run on the agarose gel by the addition of loading buffer. Now, the gel was placed into the gel box (electrophoresis unit) and filled with 1xTAE until the gel is covered. The molecular weight ladder was loaded carefully to the first lane of the gel, purified plasmid DNA is loaded into the remaining wells of the gel. The current was supplied to the tank at about 90V for 1 hour, the resulting bands were observed under UV trans-illuminator and compared with100bp and 1kb ladder.

Plasmid curing

Plasmid curing was performed by inoculating 100ml of culture grown in Tryptose Soya broth containing 10% of Sodium Dodecyl Sulfate (SDS). After Inoculation, the cultures were incubated at 37 °C for 24 hrs with constant shaking with the aid of water bath. After 24hrs incubations, the extraction of plasmid DNA procedure as explained earlier on, was used to purify the plasmid [14].

Post-curing sensitivity test

Freshly prepared Tryptose Soya broths supplemented with 3% NaCl were again prepared and then inoculated with the plasmid-cured tested cultures and incubated at 37 °C for 24 hours. After incubation, the standardized inocula of these bacteria were swabbed into the Mueller Hinton Agar (MHA) (Hi-Media, Mumbai, India) plates and incubated at 37 °C for 18hrs as a confirmatory antibacterial assay. Then Antibiotic sensitivity was carried out again according to Kirby-Bauer disc diffusion method, as previously described by **Kebede et al.** [19] using same convention antibiotics earlier described. Results were examined and compared with the formal for successful examination.

Statistical analysis

All experiments were carried out in triplicate, and data obtained were subjected to one way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) version 20 and treatment means were separated using Duncan`s New Multiple Range Test (DNMRT) at $p \leq 0.05$ level of significance.

Results

Biochemical characteristics of bacterial isolates

Probable microorganisms were isolated from Shrimp, Crab and Fish samples following the morphological and biochemical characteristics exhibited by the isolates. *Vibrio cholerae* and other pathogenic microorganisms such as *Bacillus subtilis*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Staphylococcus aureus* were identified as shown in **table (1)**.

Antibiotic sensitivity patterns of *vibrio* species isolates from seafood samples

The results of the antibiotics sensitivity pattern of the three *Vibrio cholerae* isolates are presented in **table (2)**. The zones of inhibition ranges from 0.00±0.58 to 17.00±1.58 with ofloxacin being the most effective on the *Vibrio cholerae*; while ampicillin, ceftriaxone sulbactam, nalidixic acid augmentin, cefotaxime, imipenem, and cefexime were least effective with no zone of inhibition.

Occurrence of extended spectrum beta lactamases in isolated *Vibrio* species

Two of the three tested ampicillin resistant *Vibrio cholerae* showed beta-lactamases production with an increase in the inhibition zone diameter of > 5mm towards amoxicillin-clavulanate disc from the two extended spectrum beta-lactamase disc of ceftazidime and cefotaxime.

Plasmid profile and curing of ampicillin resistant *Vibrio* species

As shown in **plate (1A)**, isolated plasmids from the *Vibrio cholerae* isolates indicated the presence of plasmids of high molecular weight in all samples. Plasmids of molecular weight ranging from about 3.8Kbp to 9Kbp were isolated from *Vibrio cholerae* sample 1 and 2 while plasmids of molecular weight of about 9Kbp were isolated from samples 3. Additionally, samples 1 indicated the presence of another plasmid of molecular weight at about 700bp while sample 2 indicated the presence of two additional plasmids of molecular weight of about 580bp and 700bp similar to that of sample 1. However, plasmid curing was successful in the lower molecular weight plasmids possessed by sample 1 and 2 represented by 4 and 5 on **plate (1B)**, however, the high 9kbp plasmid were not cured. this was illustrated by the absence of plasmid lower than 9Kbp in samples 1 and 2 cured. Plasmid curing for samples 3 represented by 6 on **plate (1B)** was successful as no plasmid was found after curing.

Post-curing antibiotics sensitivity pattern of ampicillin resistant *Vibrio* isolates

The results of the post-curing antibiotics sensitivity pattern of the three *Vibrio cholerae* isolates are presented in **table (4)**. The zones of inhibition ranges from 0.00±0.00 to 24.00±2.08 with ofloxacin being the most effective on the *Vibrio cholerae*; followed by entamycin 23.33±3.33, levofloxacin, ciprofloxacin, ceftriaxone-sulbactam and cefexime 21.00±2.08, cefuroxime and augmentin were 16.67.±8.82 and 14.00±7.02 respectively, while ampicillin, nalidixic acid, cefotaxime, and imipenem, were least effective with no zone of inhibition.

Table 1. Biochemical characteristics of bacteria isolated from seafood samples

Gram's Rxn	Catalase Test	Motility	Indole Test	Triple Sugar Iron Test				Citrate	Urease	Methyl Red		Probable Organisms
				Slant	Butt	H_2S	Gas			Methyl	V.P	
+ rod	+	+	-	-	-	-	-	+	-	-	+	*Bacillus subtilis*
-rod	+	+	-	+	+	-	+	+	-	-	+	*Enterobacter cloacae*
+ cocci	-	-	-	+	-	-	-	-	-	-	+	*Enterococcus faecalis*
- rod	+	+	+	+	+	-	+	-	-	+	-	*Escherichia coli*
-rod	+	+	-	+	-	+	+	+	+	+	-	*Proteus vulgaris*
-rod	+	+	-	-	-	-	+	+	-	-	-	*Pseudomonas aeruginosa*
-rod	+	+	-	-	+	+	+	+	-	+	-	*Salmonella enterica*
+ cocci	+	-	-	+	+	-	-	+	+	+	+	*Staphylococcus aureus*
-rod	+	+	-	+	+	-	-	+	+	-	+	*Vibrio cholerae*

Keys: V. P: Voges Proskauer; rxn: reaction; + (Positive); -(Negative), CFU: Colony forming unit.

Table 2. Antibiotic sensitivity pattern of *Vibrio* isolates (mm).

	CPR S = ≥21 I =16-20 R = ≤15	CRO S = ≥21 I =18-20 R = ≤17	LBC S = ≥17 I =14-15 R = ≤13	OFX S = ≥16 I =13-15 R = ≤12	GN S = ≥15 I =13-14 R = ≤12	AMP S = ≥17 I =14-16 R = ≤13	NA Not applicable	CXM S = ≥18 I =15-17 R = ≤14	AUG S = ≥15 I =13-14 R = ≤12	CTX S = ≥26 I =23-25 R = ≤ 24	IMP S = ≥23 I =20-22 R = ≤19	ZEM Not applicable
VC (1)	10.67 ± 1.33^{a}	0.00 ± 0.00^{a}	5.00 ± 5.00^{a}	16.00 ± 0.58^{a}	8.00 ± 23.16^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	6.67 ± 6.67^{b}	0.00 ± 0.00^{a}
VC (2)	12.67 ± 0.33^{ab}	0.00 ± 0.00^{a}	6.00 ± 5.10^{ab}	17.00 ± 1.58^{ab}	9.00 ± 33.16^{ab}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	7.67 ± 6.77^{b} ^{c}	0.00 ± 0.00^{a}
VC (3)	11.67 ± 1.33^{a}	0.00 ± 0.00^{a}	5.00 ± 5.00^{a}	16.00 ± 0.58^{a}	8.00 ± 23.16^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	6.67 ± 6.67^{b}	0.00 ± 0.00^{a}

Values represent mean ±standard error. Superscripts carrying the same alphabet in the same column are not significantly different (p>0.05).
Keys: S-Sensitive; I-Intermediate; R-Resistance; CPR-Ciprofloxacin; CRO-Ceftriaxone-Subactam; LBC-Levofloxacin; OFX- Ofloxacin; GN-Gentamycin; AMP-Ampicillin; NA-Nalidixic Acid; CXM-Cefuroxime; AUG-Augmentin; CTX-Cefotaxime; IMP-Imipenem; ZEM-Cefexime.

Table 3. Deduced antibiotics sensitivity pattern of multiple antibiotic resistant *Vibrio cholerae.*

	CPR	CRO	LBC	OFX	GN	AMP	NA	CXM	AUG	CTX	IMP	ZEM
VC 1	R	R	R	S	R	R	R	R	R	R	R	R
VC 2	R	R	R	S	R	R	R	R	R	R	R	R
VC 3	R	R	R	S	R	R	R	R	R	R	R	R

Keys: VC- *Vibrio cholerae*; S-Sensitive; I-Intermediate; R-Resistance; CPR-Ciprofloxacin; CRO-Ceftriaxone-Subactam; LBC-Levofloxacin; OFX-Ofloxacin; GN-Gentamycin; AMP-Ampicillin; NA-Nalidixic Acid; CXM-Cefuroxime; AUG-Augmentin; CTX-Cefotaxime; IMP Imipenem; ZEM-Cefexime.

Table 4. Post curing antibiotics sensitivity pattern of ampicillin resistant *Vibrio cholerae*

	CPR S = ≥21 I =16-20 R = ≤15	CRO S = ≥21 I =18-20 R = ≤17	LBC S = ≥17 I =14-15 R = ≤13	OFX S = ≥16 I =13-15 R = ≤12	GN S = ≥15 I =13-14 R = ≤12	AMP S = ≥17 I =14-16 R = ≤13	NA Not applicable	CXM S = ≥18 I =15-17 R = ≤14	AUG S = ≥15 I =13-14 R = ≤12	CTX S = ≥26 I =23-25 R = ≤24	IMP S = ≥23 I =20-22 R = ≤19	ZEM Not applicable
V.C (1)	20.00±0.00^{b}	21.00±0.00^{b}	20.00±0.00^{b}	24.00±2.08^{b}	23.33±3.33^{b}	3.00±3.00^{b}	0.00±0.00^{a}	16.67±8.82^{b}	14.00±7.02^{b}	0.00±0.00^{a}	0.00±0.00^{a}	20.00±0.00^{b}
V.C (2)	20.00±0.00^{b}	20.00±0.00^{b}	21.00±0.00^{b}	24.00±2.08^{b}	23.33±3.33^{b}	3.00±3.00^{b}	0.00±0.00^{a}	16.67±8.82^{b}	14.00±7.02^{b}	0.00±0.00^{a}	0.00±0.00^{a}	21.00±0.11^{bc}
V.C (3)	21.00±0.00^{b}	20.00±0.00^{b}	20.00±0.00^{b}	24.00±2.08^{b}	23.33±3.33^{b}	3.00±3.00^{b}	0.00±0.00^{a}	16.67±8.82^{b}	14.00±7.02^{b}	0.00±0.00^{a}	0.00±0.00^{a}	20.00±0.00^{b}

Values represent mean ±standard error. Superscripts carrying the same alphabet in the same column are not significantly different (p>0.05).
Key: S-Sensitive; I-Intermediate; R-Resistance; CPR-Ciprofloxacin; CRO-Ceftriaxone-Subactam; LBC-Levofloxacin; OFX-Ofloxacin; GN-Gentamycin; AMP-Ampicillin; NA-Nalidixic Acid; CXM-Cefuroxime; AUG-Augmentin; CTX-Cefotaxime; IMP-Imipenem; ZEM-Cefexime .

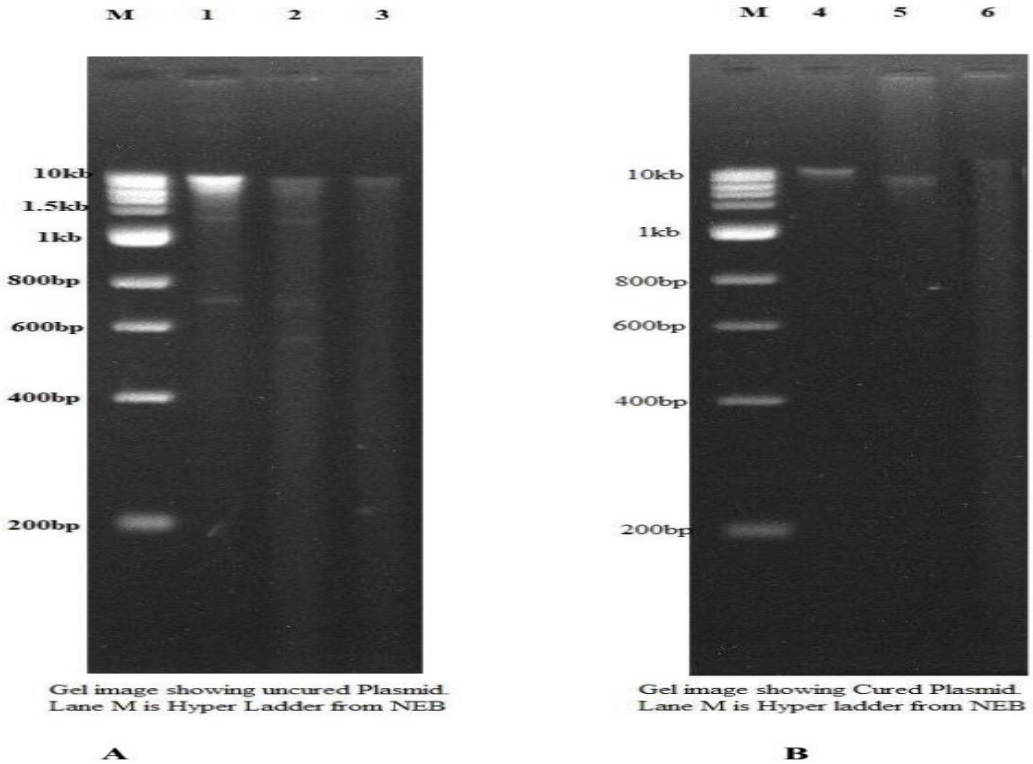
Table 5. Deduced antibiotics sensitivity pattern of multiple antibiotic resistant bacteria after plasmid curing.

	CPR	CRO	LBC	OFX	GN	AMP	NA	CXM	AUG	CTX	IMP	ZEM
V.C 1	I	S	S	S	S	R	R	I	I	R	R	S
V.C 2	I	I	S	S	S	R	R	I	I	R	R	S
V.C 3	S	I	S	S	S	R	R	I	I	R	R	S

Keys: VC- *Vibrio cholerae*; S-Sensitive; I-Intermediate; R-Resistance; CPR-Ciprofloxacin; CRO-Ceftriaxone-Subactam; LBC-Levofloxacin; OFX-Ofloxacin; GN-Gentamycin; AMP-Ampicillin; NA-Nalidixic Acid; CXM-Cefuroxime; AUG-Augmentin; CTX-Cefotaxime; IMP-Imipenem; ZEM-Cefexime

Plate 1. Electrophoretic image of plasmid DNA of the *Vibrio cholerae* isolates.

(A: Electrophoretic image before plasmid curing and B: Electrophoretic image after plasmid curing).



Discussion

Vibrio cholerae implicated in this study is a significant diarrhoeic pathogen which when ingested causes cholera and gastroenteritis. *Vibrio cholerae* isolated from crabs corroborates with the investigations of **Sivasubramanian et al.** [20], and **Soundarapandian and Sowmiya** [21]. Also, **Ashiru et al.** [22] report supported the findings of this research as they isolated *Vibrio* species including the *V. cholerae* from the intestine of crabs. **Okonkwo et al.** [23] did not isolate any *Vibrio* species from crab samples in their findings; a result which supported absolutely with the result gotten from this findings. *Vibrio cholerae* reported by **Jahan et al.** [24] supported the findings from this study as two *V. cholerae* were identified from fish samples. No *Vibrio* species was isolated from shrimp samples in their findings, an outcome which was parallel to **Okonkwo et al.** [23].

Two of the three *Vibrio* tested positive for the production of extended spectrum beta-lactamases which were identified as *Vibrio cholerae* were positive and this correlated with the findings of **Canellas et al.** [25] who reported that four strains of tested *Vibrio cholerae* were producers of extended-spectrum beta-lactamases and their antimicrobial susceptibility testing confirmed resistance to aminoglycosides, beta-lactams (including carbapenems and third-generation cephalosporins), fluoroquinolones, sulfonamides, and tetracyclines. The report also correlates with the findings of **Ceccarelli et al.** [26] whose finding was positive for detection of *Vibrio* beta-lactamases producers among screened isolates.

The electrophoresis band pattern of plasmid profiling for uncured and cured three (3) Ampicillin resistant *Vibrio* isolates indicated that 100% of the isolates harbored plasmid with molecular weight as high as 9 Kbp. During curing process, *Vibrio* isolates 1 and 2 lost plasmids of molecular weights 580 bp and 700 bp but retained 9 Kbp size, and remained resistant to ampicillin, nalidixic acid, cefotaxime and imipenem. However, *Vibrio* isolate 3 was successfully cured but also remained resistant to ampicillin, nalidixic acid, cefotaxime and imipenem. The 3 isolates were moderately resistant to Cefuroxime, and Augmentin. Isolate 2 was moderately resistant to ciprofloxacin and ceftriaxone, while isolate 1 was moderately resistant ciprofloxacin, and Isolate 3 to ceftriaxone. the results suggested that the resistance to antibiotics in isolated *Vibrio* species might be due to chromosomal and plasmid-borne as the isolates shows sensitivity to other antibiotics. The results correlated with the reports from **Amalina et al.** [27] and **Garben et al.** [7]. *Vibrio cholerae* isolates in this study showed high susceptibility to Ofloxacin, and high levels of resistance to Ampicillin, and other antibiotics similar to findings of **Das et al.** [28] and **Garbern et al.** [7].

Antimicrobial drug resistance in *Vibrio* species can develop through mutation or through acquisition of resistance genes transferred between bacteria [6]. Thus, plasmid is one of the mediators that play an important role in spreading of resistance genes since it consists most of the genetic determinants of antibiotic resistance. Acquisition of plasmids by the microorganisms in collected in Ondo State Seaside is possible as the site is about 35min to the urban centre of densely industrialized Lekki Zone in Lagos, Nigeria. The phenomenon which has been supported by several studies on the effect of released effluents, industrial and household waste and feacal contaminants into the water body. This study also shows correlation between plasmid and antibiotic resistance among *Vibrio* species which has been reported by **Amalina et al.** [27]. The post-curing antibiotic susceptibility profiles obtained in current study indicate clearly that ofloxacin, levofloxacin, gentamycin, cefexime, and ciprofloxacin are effective against tested *Vibrio cholerae*. Emergence of high resistance *Vibrio* strains against Ampicillin and many other WHO recommended antibiotics was related with the extensive used of the antibiotics and could influence the disease management in aquaculture system (Food and Agriculture Organization) **[FAO],** [29]. Thus, ampicillin is ineffective for treatment of *Vibrio* infection.

Vibrio cholerae in the seafoods from coastal area of Ondo State which might be released into this environment from human activities such as release of effluents which results in the modification of the genetic makeup of the pathogens and in-turn acquaintance of resistance genes and plasmids which aid antimicrobial resistance; and faecal contamination of the water body by tourist and those who visit the beach for recreation activities.

Conclusion

Pathogenic organisms such as *Vibrio cholerae* are recognized as pathogenic

microorganisms of seafood-borne illnesses among which are diarrhoeic agents. Marine environment harbor varieties of seafood and pathogenic microorganisms which tolerates the salty environment. They cause diseases in aquatic life and also in the consumers of these sea foods. Seawater an environment where *Vibrio* species are autochthonous which leads to unsafe seafood. This findings indicate the presence of antimicrobial-resistant and potentially pathogenic However, foodborne pathogen are consumed via consumption of uncooked or insufficiently heat-cooked seafood or the toxins produced by these pathogens. The detection of multidrug resistance, ESBLs-producing *Vibrio* organisms, suggests risks to public health. The risk of seafood-borne infections can be reduced by decrease in pollution levels, ensuring appropriate cooking temperatures, and prevention of cross-contamination during harvesting, handling and processing. By doing so, exposure to aquatic environments and consumption of seafood can be limited to minimize the risks of infection. Findings from the study has shown that sea foods from coastal area of Ondo State consequent to the presence of *Vibrio cholerae*; antibiotic sensitivity patterns of the *Vibrio* species from the sea foods and plasmid as the basis of the antibiotic resistance shown by the *Vibrio cholerae*.

Acknowledgement

All authors appreciate the output of laboratory technicians at the Department of Microbiology, Federal University of Technology Akure (FUTA) for the success of the study.

Authors' contributions

Author A. K. Onifade designed the study concept. Author S. J. Adeyanju developed the methodology, acquired, analyzed and interpreted the data. Author S. J. Adeyanju the manuscript. A. K. Onifade corrected and fine-tuned the manuscript. Both authors read and approved the final manuscript.

Funding: No funding received.

Conflict of Interests: Authors declare no conflict of interests

References

1-**Ceccarelli D, Hasan NA, Huq A, Colwell RR.** Distribution and Dynamics of Epidemic and Pandemic *Vibrio parahaemolyticus* Virulence Factors. Frontier in Cellular and Infectious Microbiology 2013; 3(97):1-9.

2-**Osunla CA, Okoh AI.** Vibrio Pathogens: A Public Health Concern in Rural Water Resources in Sub-Saharan Africa. International Journal of Environmental Research and Public Health 2017; 14: 1188-1215.

3-**Letchumanan V, Mutalib NA, Wong SH, Chan K, Lee L.** Determination of Antibiotic Resistance Patterns of *Vibrio parahaemolyticus* from Shrimp and Shellfish in Selangor, Malaysia. Progress in Microbes and Molecular Biology 2019; 2(1): a0000019.

4-**Alam M, Chowdhury WB, Bhuiyan NA, Islam A, Hasan NA, Nair GB, et al**. Serogroup, Virulence, and Genetic Traits of *Vibrio parahaemolyticus* in the Estuarine Ecosystem of Bangladesh. Applied and Environmental Microbiology 2009; 75: 6268–6274.

5-**Gode-Potratz CJ, Kustusch R.J, Breheny PJ, Weiss DS, McCarter LL.** Surface Sensing in *Vibrio parahaemolyticus* Triggers a Programme of Gene Expression that Promotes Colonization and Virulence. Molecular Microbiology 2011; 79: 240–263.

6-**Elbashir S, Parveen S, Schwarz J, Rippen , Jahncke M, dePaola A.** Seafood Pathogens and Information on Antimicrobial Resistance: A Review. Food Microbiology 2017; (70) 85-93.

7-**Garbern SC, Chub T, Yangc P, Gaineyd M, Nasrine S, Kanekarc S.** Clinical and Socio-Environmental Determinants of Multidrug-Resistant *Vibrio Cholerae* 01 in

Older Children and Adults in Bangladesh. International Journal of Infectious Diseases 2021; 105: 436-441.

8-**Zhang L, Orth K.** Virulence Determinants for *Vibrio parahaemolyticus* Infection. Currents Opinion in Microbiology 2013; 16: 70–77.

9-**Hassan ZH, Zwartkruis-Nahuis JTM, de Boer, E.** Occurrence of *Vibrio parahaemolyticus* in Retailed Seafood in the Netherlands. International Food and Research. Journal 2012; 19: 39–43.

10-**Thomas OS.** Housing Characteristics of Coastal Dwellers in Ondo State, Nigeria. Analele Universitatii din Oradea, Seria Geografie 2017; 272109-749, 255-263.

11-**Mailoa MN, Tapotubun AM, Matrutty TEA.** Analysis Total Plate Count (TPC) On Fresh Steak Tuna Applications Edible Coating *Caulerpa* sp During Stored at Chilling Temperature. IOP Conference Series of Earth and Environmental Sciences 2017; 89:012014.

12-**Oramadike C, Ogunbamiwo ST.** Prevalence and Antimicrobial Susceptibility of *Vibrio parahaemolyticus* Isolated from Seafoods in Lagos Lagoon Nigeria. Cogent Food & Agriculture 2015;1: 1041349.

13-**Tripathi N, Sapra A (2021).** Gram Staining. StatPearls. Treasure Island (FL): StatPearls PMID: 32965827. 20121. Available at: https://pubmed.ncbi.nlm.nih.gov/32965827/ #affiliation-2.

14-**Onifade AK, Alaofin S, Owoyemi OO.** Isolation and Characterization of Plasmid-Bearing Multiple Antibiotic Resistance Bacteria from Different Aquatic Sources in Akure, Nigeria. Microbiology Research Journal International 2019; 1-12.

15-**Bayode MT, Olalemi AO, Oladejo BO.** Multiple antibiotic resistant (MAR) index and detection of qnrS and qnrB genes in bacterial consortium of urine samples from clinical settings. European Journal of Biological Research 2021; 11(1): 45-56.

16-**Clinical and Laboratory Standards Institute (CLSI).** Performance Standards for Antimicrobial Disk Susceptibility Tests. CLSI Standard M02, M07, and M11. 28th edition, Wayne, PA: USA. 2018. 1-296.

17-**Falodun OI, Morakinyo YM, Fagade OE.** Determination of Water Quality and Detection of Extended Spectrum Beta-Lactamase Producing Gram-Negative Bacteria in Selected Rivers Located in Ibadan, Nigeria. Jordan Journal of Biological Sciences 2018; 11(1): 107 – 112.

18-**Onifade AK, Palmer OG.** Plasmid Profile Analysis and Curing of Multidrug-Resistant Bacteria Isolated from Hospital Environmental Surfaces in Akure Metropolis, Ondo State, Nigeria, American Journal of Information Science and Technology 2018; 2(1), 18-23.

19-**Kebede A, Kemal J, Alemayehu H, Habte Mariam S.** Isolation, Identification, and Antibiotic Susceptibility Testing of *Salmonella* from Slaughtered Bovines and Ovines in Addis Ababa Abattoir Enterprise, Ethiopia: A Cross-Sectional Study. International Journal of Bacteriology 2016: 1–8.

20-**Sivasubramanian K, Ravichandran S, Rajan, DK.** Isolation of Gut Associated Bacteria from Mangrove Crabs Collected

from Different Mangrove Regions of Tamil Nadu, South East Coast of India. African Journal of Microbiology Research 2017; 11(14), 586-595.

21-**Soundarapandian P, Sowmiya S.** Microbial Contamination of Commercially Important Crabs, *Portunus pelagicus* (Linnaeus) and *P. sanguinolentus* (Herbst). Open Access Scientific Reports 2012; 2:593.

22-**Ashiru AW, Uaboi-Egbeni PO, Odunlade AK, Ashade OO, Oyegoke TM, Idika CN.** Isolation of *Vibrio* Species from the Gut of Swimming Crabs (Callinectus sp.) and Their Antibiotic Susceptibility. Pakistan Journal of Nutrition 2012; 11 (6): 536-540.

23-**Okonko IO, Ogunnusi TA, Ogunjobi AA, Adedeji AO, Adejoye OD, Babalola ET, et al.** Microbial Studies on Frozen Shrimps Processed in Ibadan and Lagos, Nigeria. Scientific Research and Essay 2008; 3(11): 537-546.

24-**Jahan S, Jewel MAS, Haque MA, Hasan J, Mita MM, Aktar A, et al.** Current Status of Bacteria Contamination in Some Fish Species of Bakkhali River Estuary, Bangladesh. Archives of Agriculture and Environmental Science 2019; 4 (1): 96-100

25-**Canellas ALB, Lopes IR, Mello MP, Paranhos, R, de Oliveira BFR, Laport MS.** *Vibrio* Species in an Urban Tropical Estuary: Antimicrobial Susceptibility, Interaction with Environmental Parameters, and Possible Public Health Outcomes. Microorganisms 2021; 9: 1007: 1-18.

26-**Ceccarelli D, Alam M, Huq A, Colwell RR.** Reduced Susceptibility to Extended-Spectrum β-Lactams in *Vibrio cholerae* Isolated in Bangladesh. Frontier in Public Health 2016. doi.org/10.3389/fpubh.2016.00231 www.frontiersin.org/articles/10. 3389/ fpubh.2016.00231/full (Accessed: 29th December, 2019).

27-**Amalina NZ, Santha S, Zulperi D, Amal MN A, Yusof MT, Zamri-Saad M, Ina-Salwany MY.** Prevalence, Antimicrobial Susceptibility and Plasmid Profiling of *Vibrio* Spp. Isolated from Cultured Groupers in Peninsular Malaysia. BMC Microbiology 2019; 19:251; 1-15.

28-**Das SK, Klontz EH, Azmi IJ, Ud-Din AIMS, Christi MJ, Afrad MH.** Characteristics of Multidrug Resistant *Shigella* and *Vibrio cholerae* O1 Infections in Patients Treated at an Urban and Rural Hospitals in Bangladesh. International Scholarly Research Notices 2013*:* 12-18.

29-**Food and Agriculture Organization of the United Nations FAO.** Antimicrobial Resistance (AMR) in Aquaculture. Rome. 2017. Available from :http://www.fao.org/cofi/aq/90408/en/.