Microbiology Research International
Vol. 12(4), pp. 124-137, October 2024
DOI: 10.30918/MRI.124.24.028
ISSN: 2354-2128
Full Length Research Paper

Prevalence and antibiogram of *Vibrio* species from blood cockles, oysters and periwinkles sold in Port Harcourt

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Accepted 18 October, 2024

ABSTRACT

Vibrio species are autochthonous aquatic pathogens commonly associated with foodborne infections which are difficult to treat, since they are reportedly multi-drug resistant. The aim of the study is to determine the prevalence and antibiogram of *Vibrio* species from blood cockles (*Senilia senilis*), oysters (*Crassostrea gasar*), and periwinkles (*Tympanotonus fuscatus*) sold in Port Harcourt, Rivers State, Nigeria, using cultural techniques. A total of 972 fresh and parboiled samples of blood cockles, oysters and periwinkles were purchased from Creek Road, Mile 1, and Mile 3 Market, in Port Harcourt Local Government Area, Rivers State, Nigeria. *Vibrio* species were isolated using Thiosulphate-Citrate-Bile-Sucrose (TCBS) agar. Antibiotic susceptibility of isolates was ascertained using Kirby-Bauer disc diffusion method. Fresh Oyster samples from the Creek Road Market, recorded the highest Vibrio count 6.4±0.4 x 10^3 CFU/g while parboiled Periwinkle samples from Mile 1 Market recorded the lowest count of 5.3±0.7 x 10^3 CFU/g in the wet season. Fresh periwinkle samples from Mile 3 Market had the highest count of 7.2±0.1 x 10^4 CFU/g and parboiled periwinkle samples from Creek Road Market had the lowest count of 6.9±0.03 x 10^4 CFU/g in dry season. Over 90% of samples were found above FDA safe limits for fresh and boiled seafood's (100 and 10 CFU/g). Prevalence of *Vibrio* species in seafood samples ranged from 29% to 100% and 43% to 71% in the wet and dry seasons respectively. A high resistance to Augmentin 55% and 58% was observed in the wet and dry season respectively. Ofloxacin > ciprofloxacin > perfloxaxin were found to be the most effective drugs. Two isolates *V. vulnificus* (blood cockle) and *V. cholerae* (periwinkle) had the highest multiple antibiotic resistance index of 100% and 67% in the wet and dry season respectively. Twenty Five (71.42%) and Seven (20%) isolates had multidrug resistance (MDR) values of ≥0.4 in the wet and dry season respectively, this is above the arbitrary value of 0.2, indicating high-risk contamination sites where antibiotics are frequently used. The presence of antibiotic resistant *Vibrios* from seafood samples poses a serious concern to public health. Proper seafood handling procedures and antibiotic surveillance strategies/restrictive policies must be established and strictly adhered to.

Keywords: Prevalence, Creek Road, Mile 1, Mile 3, multiple antibiotic resistance index, multiple drug resistance.

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INTRODUCTION

Vibrio species are known to be autochthonous in the aquatic environment, and are among the leading causes of water and foodborne disease outbreaks around the world (Sampson et al., 2022; Osunla and Okoh, 2017). The genus *Vibrio* which consists of 142 species, belongs to the family *Vibrionaceae*. They are gram negative,

oxidase positive (apart from *V. metschnikovii*), “non-spore forming rods that are motile”. More than twenty *Vibrio* spp. have been identified as animal pathogens, and twelve as human pathogens. These human pathogens include: *Vibrio cholerae, V. parahaemolyticus, V. vulnificus, V. fluvialis, V. alginolyticus, V. damsela, V. furnissii, V. hollisae, V. mimicus, V. cincinnatiensis, V. metschnikovii and V. carchariae* (Williams et al., 2010; Kokashvili et al., 2015; Bonnin-Jusserand et al., 2017). *Vibrio* species are classified as halophilic and non-halophilic based on their requirement for sodium chloride. They can be isolated from brackish, marine and estuarine environment where they aid in chitin mineralization on copepod exoskeleton as a source of their nutrient, in doing this they also provide nutrients for the entire aquatic habitat. *Vibrio* species form biofilms on chitin which sustains their growth, survival and environmental persistence (Akoachere and Mbuntcha, 2014; Baker-Austin et al., 2018; Islam et al., 2020). Pathogenic *Vibrios* that cause diseases in humans are classified as gastrointestinal and extra-intestinal pathogens based on the disease conditions they manifest (Osunla and Okoh, 2017).

Non cholera infections can either be a self-limiting gastroenteritis or in severe cases a life-threatening septicemia and necrotizing fasciitis (Maje et al., 2020; Gxalo et al., 2021). Traveling to the Vibrio spp. endemic regions may put an individual at risk of infection. *Vibrio* infections are still a public health threat as they are linked in most cases, to consumption of contaminated water and seafood. The Cholera disease also known as a disease of poverty is contracted by consuming contaminated water/food. The disease is caused by *Vibrio cholerae* O1 (classical or ELT or) and O139. The transmission of diseases among humans is commonly facilitated by poor sanitary conditions, poor hygiene and inadequate water supply. Therefore, the Cholera disease is common in communities lacking proper sewage and water treatment system (Sampson et al., 2022; Adagbada et al., 2012, Sedas et al., 2007). Studies of acute diarrhoea illness in Kolkata, India shows that the incidence of gastroenteritis caused by *Vibrio cholerae* has the highest prevalence followed by *Vibrio parahaemolyticus*. Approximately 10 of the cases of gastroenteritis in patients admitted in Kolkata hospital were caused by *V. parahaemolyticus* and about 70 of gastroenteritis cases were associated with seafood in Japan. *Vibrio parahaemolyticus* can cause food/waterborne gastroenteritis in humans especially after consumption of water, vegetables, and raw or under cooked, contaminated seafood (Rodgers et al., 2014; Prabhakaran et al., 2020). *Vibrio vulnificus* found in sea water and sea food presents with symptoms that include skin blisters, wound infection, and septicemia. Most cases occur in patients with liver diseases. *Vibrio parahaemolyticus and V. vulnificus* have been identified as the most common type of deadly food borne and wound *Vibrio* infections from *Vibrio* species (Froelich and Noble, 2016). Amongst food borne pathogens *V. vulnificus* accounts for the highest case fatality rate (50%). Wound infections can be contracted during swimming, fishing and sea food handling, resulting to a condition known as necrotizing fasciitis or flesh eating diseases at the site of the infection. Three biotypes (biotype 1, 2 and 3) of *V. vulnificus* have been identified. Biotype 1, which is the most common group, was isolated mostly from shellfish in coastal estuarine areas, and is responsible for numerous clinical cases (Efimov et al., 2013).

Health benefits derived from seafood’s are numerous as they constitute an important ingredient in our diet. Oysters (*Crassostrea gasar*) locally known as Ngbe are categorized as shellfish seafood and belong to the family *Ostreidae*. They are usually large and flat and can be eaten either cooked or uncooked. Oysters occur naturally in estuaries, coastal mangrove ecosystem and swampland, and are attached to the aerial prop roots of the mangrove trees and other stationary objects like sea vessels. In Rivers State, oysters can be found in communities such as; Okrika, Abuloma, Andoni, Tema, Dabara, Buguma, Eleme Kalabari and Owoka. Oysters contain high amount of protein, a higher amount of selenium, iron, zinc, magnesium and vitamin B nutrients especially vitamin B12. It has been found effective in reducing blood pressure, increasing blood circulation and weight loss. It is also a sex hormone enhancer especially in men. It also helps in cell development, tissue regeneration, muscle strengthening and increases the vitality of hair, skin, and nails (Maurya, 2021). The West Africa blood cockle (*Anadara selinia*) locally known as Ofingo is a bivalve mollusks and one of the 220 species of bivalves and belongs to the family *Arcidae* (Theerachat et al., 2020; Hossen et al., 2014). The West African blood cockle derives its name from the red liquid observed when sighting or smelling a blood cockle. This red liquid resembles the human blood. The red corpuscle of the blood cockle carries haemoglobin which aids its survival in environments with low oxygen levels. Blood cockles are a cheap source of protein and are usually found in the West African estuaries and lagoons (Udo et al., 2022). Periwinkles are gastropods and belong to the Phylum Mollusca. They are usually found in the sea, mostly in the brackish and littoral regions. It is also a cheap source of protein, vitamins and minerals. In recent years *Tympanotosus fuscatus*, has been in high demand in the Niger Delta region where they are most desired and appreciated because of its culinary value. Although they are highly perishable periwinkles are low in cholesterol, fats and carbohydrate content

(Oghenemowho and Ahaotu, 2021; Nrior et al. 2017; Opara et al., 2020). Their ability to adapt to environmental changes makes them available for harvest all year round. Periwinkles are harvested by hand picking either from a boat or rock surfaces (Oluyemi et al., 2019). Research findings by Nrior et al. (2017) stated that the external body and inner shell fluid of periwinkles are highly contaminated by various species of microorganisms and its rich nutritional content enhances proliferation of pathogens thus increasing the risk of foodborne infections like vibriosis. Despite their numerous nutritional benefits, eating contaminated oysters/seafood can become a public health risk and can cause death in patients with liver diseases like alcoholic cirrhosis, cancer or other chronic diseases that weaken host defenses. As filter feeders oysters have the ability to accumulate and concentrate bacteria like *Vibrio cholerae, Vibrio parahaemolyticus, and Vibrio vulnificus* including their pathogenic and toxic metal forms by 100 times the concentration found in the surrounding water (Froelich and Noble, 2016; Amadi, L., 2016). They do this by pumping water through their gills this way they entrap large planktonic food organisms and bacteria within the cilia and mucus of their respiratory epithelium (Jalal et al., 2009). Several studies have reported the occurrence of *Vibrio* species in bivalve mollusks including oysters, scallops, clams and cockles etc. *Vibrio parahaemolyticus* occurs naturally in the marine environment and some strains appear as opportunistic pathogens to humans as *V. parahaemolyticus* has been isolated severally in clams, cockles, oyster, mussels, crabs and shrimps (Tan et al., 2017). An annual research study of the South West Coast of India reported that 57% of oysters harbored *Vibrio* species in them and are therefore potential reservoirs of disease epidemics particularly in Asia, U.S.A and Sub Saharan Africa where consumption of raw or undercooked oysters are common. Sea food therefore is a significant transmission route for these pathogens due to the ubitiquous nature of *Vibrio* species in the aquatic environment. Prevalence of *V. parahaemolyticus and V. cholerae* isolates in fresh bivalve molluscs was reported to be 30.4% to 32.6%. In another study, *V. cholerae* had the highest prevalence rate (33.3%) in the oyster samples. All fresh oyster samples were positive for at least one *Vibrio* specie. Prevalence of *Vibrio* species was recorded as 77.9%, 8.8%, and 32.3% for *V. parahaemolyticus, V. cholerae, and V. vulnificus* respectively (Viana de Sousa et al., 2004; Rosec et al., 2012; Villicaña et al., 2019). Among these Vibrio strains, *V. parahaemolyticus* had the highest prevalence rate of 23.1% and 39.4% in seawater and bivalves samples respectively. Also, *V. parahaemolyticus* obtained from oysters samples had a prevalence rate > 75% during the summer with frequent occurrence rate of 12.5% to 50.0% during the oyster-harvesting season. In another research study, *Vibrio parahaemolyticus and V. vulnificus* prevalence in deshelled oysters were reported as 95.7% and 60.9% in dry season (Mok et al., 2019; Fernandez-Rendon et al., 2018). Cultivation, handling and processing of seafood is known to introduce and increase the microbial count of *Vibrio* species (Osunla and Okoh, 2017).

The occurrence of antibiotic resistant bacteria (ARB) such as *Vibrio* spp., and enterobacteriaceae as well as multiple antibiotic resistant genes of *Vibrio* species has been reported severally in some aquacultural animals, especially seafood (Preena et al., 2020; Schar et al., 2021). *V. parahaemolyticus* isolates have been reported resistant to penicillin and ampicillin by several research studies (Tan et al., 2017; Zulkply et al., 2019; Alverez-Contereras et al., 2021). Clinical and environmental strains acquire multiple antibiotic resistant genes via several mechanisms like conjugation, lysogenic transfer, chromosomal DNA mutations, enzymatic inactivation, transformation and selective pressure. This also has been greatly enhanced by the incessant/excessive use and abuse of antibiotics in the aquaculture/agriculture sector. Studies have shown that antimicrobial resistant determinants for quinolone and tetracycline can be exchanged by bacteria between the aquatic and terrestrial environments (Cabello et al., 2013). This frequent occurrence of recombination and horizontal gene transfer in the aquatic environment contributes to the evolution of *Vibrio* species (Baker-Austin et al., 2018). In a meta-systematic study on the prevalence of AMR in marine bivalve, the genus *Vibrio* was rated second place after Aeromonas. This suggests that shellfishes/seafood can be potential reservoirs of antibiotic resistant *Vibrio* species (Dahanayake et al., 2019; Albini et al., 2022). Gxalo et al. (2021), and Mancini et al. (2023), observed in their study that multiple antibiotic resistance (MAR) index among the *Vibrio* species was found to be between 0.5 to 0.8 and 0 to 0.69 respectively. While Beshiru et al. (2023), stated that a higher percentage (94.0%) of the isolates obtained in their studies were resistant to more than one antimicrobial drug used.

MATERIALS AND METHODS

Description of study area

The study area was the Port Harcourt Local Government Area of Rivers State, Nigeria. Study locations were decisively selected to be the 3 major retail seafood markets in Port Harcourt City (Creek Road Market, Mile 1 Market and Mile 3 Market). Sampling area was located within longitude 4°50’ N and Latitude 6°55’ E (Figure 1).

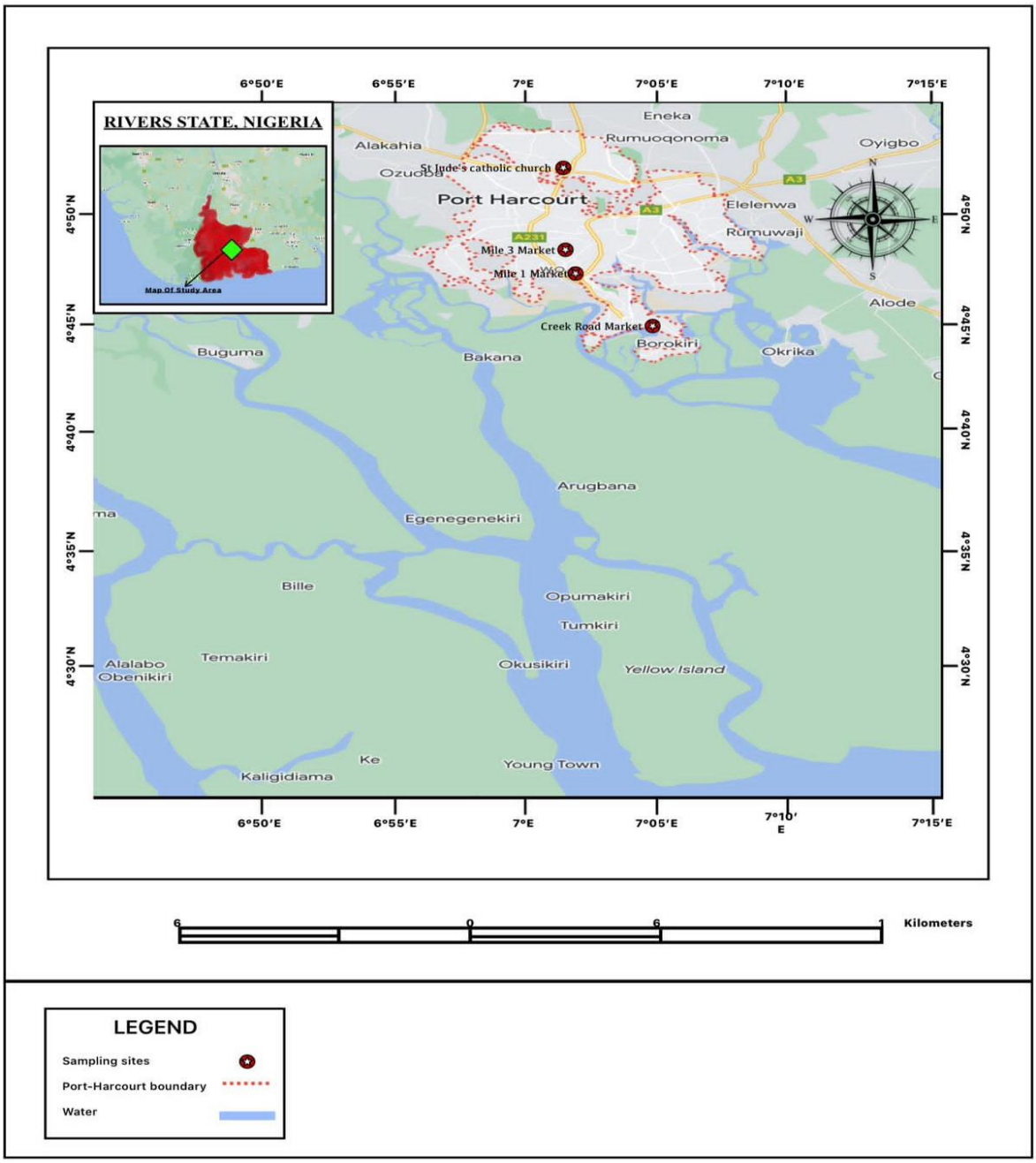

Figure 1. Study area indicating sampled locations.

Sample collection

Samples were collected from three Markets (Creek Road Market, Mile 1 Market and Mile 3 Market) in Port Harcourt. Fresh and parboiled samples of blood Cockles oysters, and periwinkles (shelled) were collected from each sample location at two different intervals (the wet (March-October) and dry (November - February) seasons) (Edokpa 2020), using sterile plastic containers, which were placed in a thermos box and transported to the laboratory for analyses. Samples were analyzed immediately after collection. Samples were analyzed in the different seasons as research studies have shown that the appearance and abundance of *Vibrio* species are influenced by variations in seasons and are linked to cholera disease outbreaks (Huq et al., 2005; Akoachere

and Mbuntcha, 2014). Blood cockles were not available in Mile 3 market during the study period, therefore no cockle sample was collected from this location.
The sample size was determined using the formula:

$$N = \frac{Z^2 P (1-P)}{d^2}$$ (Naing et al., 2006)

N = Desired sample size
Z= Z statistics for level of confidence at 95% is 1.96
P= Expected prevalence
D= precision (0.05 i.e. 5%).
P= 18% (0.18) (Nsofor et al., 2014).

Blood cockles (144), oysters (360) and periwinkles (468) were purchased and subjected to microbiological, and biochemical analysis. A total of 972 fresh (72,180, and 234) and parboiled (72,180, and 234) blood cockles, oyster and periwinkles respectively were analysed. All boiled samples were parboiled at point of sale. They are usually processed by pouring hot water on samples to facilitate the shucking process while the periwinkle samples were parboiled for 2-3 minutes so the fleshy meat can be extracted.

Bacteriological analysis

Seafood samples were scrubbed under tap water to remove debris, and shucked. Samples were allowed to dry, and were disinfected with 70% ethanol, The flesh and intervalve liquid of oysters and blood cockles were aseptically removed from the shell with a sterilized sharp knife, while the periwinkle samples were extracted with a sterile needle. Each sample was blended in a sterile blender for 1 minute. Twenty five grams (25g) of each sample was homogenized by agitation for 60 seconds in a sterile conical flask containing 225ml of alkaline peptone saline water (APSW) modified with 2% Sodium Chloride (NaCl). One milliliter (1ml) of each sample was collected and dispensed into 9ml of the sterilized diluent (alkaline peptone water). A tenfold serial dilution was prepared before dispensing an aliquot (0.1ml) from serial dilution tubes directly onto Thiosulfate-Citrate-Bile-Sucrose (TCBS) agar (Titan Biotech, India) using the spread plate technique. Agar plates were incubated at 37 degrees centigrade for 24 hours and observed for growth. Isolation and enumeration of *Vibrio* species was done according to the method adopted by Viana de Sousa et al. 2004, Azwai et al. (2016) and Laboratory Protocol, (2017) with slight modification. Yellowish and greenish colonies 2 - 3mm on agar plates were recorded as *Vibrio* species. Pure cultures of suspected *Vibrio* spp. were subcultured onto nutrient agar for further analysis.

Identification of *Vibrio* species

Vibrio species were isolated based on their colonial/morphological and cultural characteristics such as the size, margin, shape, colour, elevation, texture and opacity. Several Biochemical test were used for presumptive identification of isolates these include: Gram stain, oxidase, and string test, methyl-red, Voges-Proskauer, indole production, citrate utilization, salt tolerance, and sugar fermentation tests (Cheesbrough, 2006; Brenner, 2005).

Antibiotic susceptibility testing of *Vibrio* species.

The Kirby Bauer disc diffusion technique was used for antibiotic susceptibility testing. Each sample was streaked onto nutrient agar and incubated at 35ºC for 24 hours to obtain isolate of interest. Each colony was transferred to a tube containing sterilized normal saline. The suspension was compared to 0.5 McFarland solution to adjust the turbidity. A sterile swab stick was dipped into the test tube containing a suspension of the isolate of interest (after adjusting the turbidity) and used to swab Mueller Hinton agar (Titan Biotech, India) plates. Using sterile forceps, discs impregnated with antibiotics (MaxiCare) were placed evenly on the surface of the inoculated plates. Agar plates were inverted and incubated at 35ºC for 16 hours, after applying the discs. Zones of clearance on agar plates were measured, and recorded as susceptible, intermediate and resistant (CLSI, 2018). In cases where CLSI break points were not available for *Vibrio species* interpretative criteria was based on different studies on *Vibrio* species *(*Bier et al., 2015*)*.

Determination of multiple antibiotic resistance (MAR) index

Multiple Antibiotic Resistance (MAR) is the resistance of bacteria to three or more antibiotics. The MAR Index of isolates was determined using the formula:

MAR Index= a/b

Where

a = number of antibiotics to which the test isolates shows resistance.
b = the total number of antibiotics the isolate was subjected to (Krumperman, 1985). The most resistant isolate/isolates with the highest MAR index were screened for the presence of antibiotic resistance genes.

Data analysis

Data obtained from the study were analyzed using Microsoft excel. One way Anova (Analysis of Variance) statistical tool was used to test for significance using the statistical software package SAS-JMP version 12. Expressive prevalence statistics were presented in frequency tables, mean, percentages and standard deviations with their corresponding 95% Confidence intervals. The prevalence data was expressed via appropriate cross-tabulations. p values ≤ 0.05 was considered significantly different. Results were recorded and summarized.

RESULTS

Total *Vibrio* counts for the three seafood samples ranged from 5.3±0.7 to 6.4±0.4 x 10^3 CFU/g and 6.9±0.03 to 7.2±0.1x 10^4 CFU/g in the wet and dry seasons respectively (Table 1a and 1b). Total *Vibrio* count (TVC) for fresh blood cockles, oysters and periwinkles in Creek Road market were 6.4±0.03, 6.4±0.4; and 6.3±0.1 x 10^3 CFU/g and 7.2±0.1; 7.0±0.2; 7.1±0.04 x 10^4 CFU/g in the wet and dry season respectively (Table 1a and 1b). Total *Vibrio* count (TVC) for parboiled blood cockles, oysters and periwinkles in Creek Road market were 5.9±0.1; 5.9±0.4; and 6.1±0.1 x 10^3 CFU/g and 7.0±0.1; 6.9±0.3; and 6.9±0.03 x 10^4 CFU/g in the wet and dry season respectively (Table 1a and 1b). Total *Vibrio* count (TVC) for fresh *blood cockles, oysters and periwinkles* in Mile 1 market were 6.2±0.1; 5.9±0.3; and 5.9±0.09 x 10^3 CFU/g and 7.1±0.1; 7.1±0.1; and 7.1±0.2 x 10^4 CFU/g in the wet and dry season respectively (Table 1a and 1b). Total *Vibrio* count (TVC) for parboiled blood cockles, oysters and periwinkles in Mile 1 market were 6.1±0.1; 5.8±0.4; and 5.3±0.7 x 10^3 CFU/g and 6.9±0.1; 6.9±0.3; and 6.9±0.2 x 10^4 in the wet and dry season respectively (Table 1a and 1b). Total *Vibrio* count (TVC) for fresh oysters and periwinkles in Mile 3 market were 6.3±0.03; and 6.3±0.1 x 10^3 CFU/g and 7.0±0.3; and 7.2±0.1 x 10^4 CFU/g in the wet and dry season respectively (Table 1a and 1b). Total *Vibrio* count (TVC) for parboiled blood cockles, oysters and periwinkles in Mile 3 market were 6.1±0.1; and 6.1±0.4 x 10^3 CFU/g and 6.9±0.3; and 6.9±0.1 x 10^4 CFU/g in the wet and dry season respectively (Table 1a and 1b). With respect to the sample type, the fresh blood cockles from the Creek Road market, recorded the highest count of 6.4±0.03 x 10^3 CFU/g while parboiled periwinkle samples from Mile 1 market recorded the lowest count of 5.3±0.7 x 10^3 CFU/g in the wet season (Table 1a and 1b). Also, the fresh periwinkle samples from the Mile 3 market had the highest count of 7.2±0.1 x 10^4 CFU/g while the parboiled periwinkle samples from Creek Road market had the lowest count of 6.9±0.03 x 10^4 CFU/g in the dry season (Table 1a and 1b). During the survey period, the detection rate of *Vibrio* species in seafood samples was highest in the dry season. With respect to the different locations studied, there was a significant difference ($P<0.05$) between the Blood Cockle samples obtained from Creek Road and those of the Mile 1 and Mile 3 samples (Table 1a and 1b). Overall, there was a significant difference ($p\leq0.05$) between the fresh and parboiled cockles analyzed in this study but there was no significant difference ($p\geq0.05$) between the fresh and parboiled oysters and periwinkle samples (Table 1a and 1b). Fresh and parboiled cockle samples were not available at the Mile 3 Market therefore, blood cockle samples were not collected from Mile 3 Market during our study period.

Prevalence pattern for *Vibrio* species isolated from seafood samples are presented in Table 2. The prevalence of *Vibrio* species in different seafood samples ranged from 29% to 100% and 43% to 71% in the wet and dry seasons respectively (Table 2). *Vibrio alginolyticus* and *V. vulnificus* had the highest prevalence of 100% and 71% in the wet and dry seasons respectively while *V. vulnificus* and other *Vibrio* species had the lowest prevalence rate of 29% and 43% in the wet and dry seasons respectively (Table 2). Overall, there is no significant difference ($p> 0.05$) in the occurrence of *Vibrio* species in seafood samples in the wet and dry seasons (Table 2).

Isolates were screened for their susceptibility to antibiotics. The assessment of antibiotic susceptibility of the *Vibrio* species isolated from seafood (Table 3a-4c) revealed that isolates were most resistant to Augmentin and most sensitive to Ofloxacin. Results obtained from this study shows that isolates exhibited resistance to Amoxicillin> Chloramphenicol> Gentamycin> Augmentin in the wet season and Augmentin>Chloramphenicol> Gentamycin >Amoxicillin in the dry season (Table 3a-4c). Overall resistance of tested *Vibrio* isolates varied in the wet season as follows: Ofloxacin—7%, Ciprofloxacin —10%, Pefloxacin—13%, Streptomycin—45%, and Gentamicin—58%, Amoxicillin—74%, Chloramphenicol—74%, Augmentin-55% (Table 3a-3c) and in the dry season as follows: Ofloxacin—3%, Pefloxacin—0%, Streptomycin—9%, Ciprofloxacin—3%, Amoxicillin—21%, Gentamicin—21%, Chloramphenicol—42%,and Augmentin—58% (Table 4a-4c). Ofloxacin, ciprofloxacin and pefloxacin were found to be the most effective drugs but ciprofloxacin gave the highest clearance among these three drugs (Table 3a-4c). Data on antibiotic susceptibility test of isolates obtained from blood cockles in the wet season showed that *Vibrio cholerae* were most resistant to the antibiotics tested followed by *V. angulliarum*

Table 1a. *Vibrio* count for fresh and parboiled *Senilia senilis* (blood cockles), *Crassostrea gasar* (oyster), and *Tympanotonus fuscatus* (periwinkles) sampled from Creek Road, Mile 1 and Mile 3 markets in the wet season.

	Blood cockle (CFU/g) x 10^3		Oyster (CFU/g) x 10^3		Periwinkle (CFU/g) x 10^3	
	Fresh	Parboiled	Fresh	Parboiled	Fresh	Parboiled
Location	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Creek Road	6.4±0.03	5.9±0.1	6.4±0.4	5.9±0.4	6.3±0.1	6.1±0.1
Mile 1	6.2±0.1	6.1±0.1	5.9±0.3	5.8±0.4	5.9±0.1	5.3±0.7
Mile 3	0	0	6.3±0.03	6.1±0.1	6.3±0.1	6.1±0.4

There was a significant difference ($p \leq 0.05$) between the blood cockles (p- 0.0037) obtained from different locations studied in the wet and dry season.

Table 1b. *Vibrio* count for fresh and parboiled *Senilia senilis* (blood cockles), *Crassostrea gasar* (oyster), and *Tympanotonus fuscatus* (periwinkles) sampled from Creek Road, Mile 1 and Mile 3 market in the dry season.

	Blood cockle (CFU/g) x 10^4		Oyster (CFU/g) x 10^4		Periwinkle (CFU/g) x 10^4	
	Fresh	Parboiled	Fresh	Parboiled	Fresh	Parboiled
Location	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Creek Road	7.2±0.1	7.01±0.1	7.0±0.2	6.9±0.3	7.1±0.04	6.9±0.03
Mile 1	7.1±0.1	6.9±0.1	7.1±0.1	6.9±0.3	7.1±0.2	6.9±0.2
Mile 3	0	0	7.0±0.3	6.9±0.3	7.2±0.1	6.9±0.1

A significant difference existed ($p \leq 0.05$) between the fresh and parboiled blood cockles (p- 0.036631) obtained in the wet and dry season.

Table 2. Prevalence of *Vibrio* species in seafood samples in the wet and dry seasons.

Isolates	Wet season (%)	Dry season (%)	Mean±Stdv	P-value
V. cholerae	0.23(35)	0.43(65)	0.33±0.14	0.0887
V. paraheamolyticus	0.06(40)	0.09(60)	0.07±0.02	
V. vulnificus	0.06(29)	0.14(71)	0.10±0.06	
V. auguilliarum	0.2(100)	0(0)	0.10±0.14	
V. alginolyticus	0.11(100)	0(0)	0.06±0.08	
V. xuii	0.09(38)	0.14(62)	0.11±0.04	
Other *Vibrio* species	0.23(57)	0.17(43)	0.20±0.04	

There was no significant difference ($p \geq 0.05$) between the seafood samples obtained in the wet and dry seasons.

whereas in the dry season, *Vibrio cholerae* from blood cockles was most resistant to the antibiotics used (Table 3a-4c). Also, in the wet season *V. angulliarum* isolates obtained from the oyster samples were the most resistant to the antibiotics used, however, in the dry season, *Vibrio cholerae* isolates from oyster samples were the most resistant to the antibiotics used (Table 3a-4c). Furthermore, *Vibrio cholerae* isolates obtained from the periwinkle samples were the most resistant to antibiotics used in this study in the wet and dry season (Table 3a-4c).

The *Vibrio* isolates tested in this study, demonstrated multiple antibiotic resistant (MAR) indices ranging from 0.25 to 1.0 and 0.25 to 0.63 in the wet and dry seasons respectively (Table 5a-6c). Two isolates *V. vulnificus* (blood cockle) and *V. chole*rae (periwinkles) had the highest MAR index of 100% and 63% in the wet and dry seasons respectively as shown in Table 5a-6c. Overall, 61.42% of isolates exhibited resistance to more than one antibiotic used in this study hence, presented to be multidrug resistant (MDR). A total of 32 isolates (45.71%) had MAR indiex of more than ≥0.4. This is above the arbitrary value of 0.2, indicating high-risk contamination sites where antibiotics are frequently used (Table 5a-6c). The blood cockle and the periwinkle samples had the highest number of isolates with the highest MAR index in the wet season (Table 5a-5c), while in the dry season the periwinkle samples had the highest number of isolates with the highest MAR index followed by the oyster samples (Table 6a-6c).

Table 3a. Antibiotic susceptibility test of isolates obtained from blood cockles in the wet season.

	Zone diameter (mm) Antibiotics											
***Vibrio* spp.**	**CH 30 µg**	**CPX 5 µg**	**AM 30 µg**	**AU 30 µg**	**GN 10 µg**	**PEF 5 µg**	**OFX 5 µg**	**S 10 µg**	**MAR Index**	**R (%)**	**S (%)**	**I (%)**
V. aesturianus	(R)	(S)	(S)	(R)	(S)	(S)	(S)	(I)	0.25	25	63	12
V. cholerae	(R)	(I)	(R)	(R)	(R)	(R)	(I)	(R)	0.88	88	0	12
V. fluvialis	(R)	(S)	(S)	(R)	(S)	(I)	(I)	(I}	0.25	25	37	38
V. cholerae	(S)	(S)	(S)	(R)	(S)	(S)	(S)	(S)	0.12	12	88	0
V. cholerae	(R)	(R)	(R)	(R)	(I)	(R)	(R)	(S)	0.75	75	13	12
V.cholerae	(S)	(R)	(R)	(R)	(R)	(R)	(S)	(R)	0.75	75	25	0
V. alginolyticus	(R)	(S)	(R)	(R)	(S)	(S)	(S)	(R)	0.5	50	50	0
V. cholerae	(R)	(S)	(R)	(R)	(R)	(S)	(S)	(S)	0.5	50	50	0
V. hepatarius	(R)	(S)	(R)	(R)	(R)	(I)	(S)	(R)	0.63	63	25	12
V. vulnificus	(R)	(R)	(R)	(R)	(R)	(R)	(R)	(R)	1	100	0	0
V. anguilliarium	(R)	(S)	(R)	(R)	(R)	(S)	(S)	(I)	0.5	50	38	12
V. paraheamolytics	(I)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	0	0	88	12
V.anguillarum	(R)	(S)	(R)	(R)	(S)	(S)	(S)	(R)	0.5	50	50	0

Key: R = Resistant, I = Intermediate, S = Susceptible, CH = Chloramphencol, CPX = Ciprofloxacin, AM = Amoxicillin, AU = Augmentin, GN = Gentamycin, PEF = Pefloxacin, OFX = Ofloxacin, S = Streptomycin.

Table 3b. Antibiotic susceptibility test of isolates obtained from oyster samples in the wet season.

	Zone diameter (mm) Antibiotics											
***Vibrio* spp.**	**CH 30 µg**	**CPX 5 µg**	**AM 30 µg**	**AU 30 µg**	**GN 10 µg**	**PEF 5 µg**	**OFX 5 µg**	**S 10 µg**	**MAR Index**	**R (%)**	**S (%)**	**I (%)**
V. anguillarum	(R)	(S)	(R)	(R)	(R)	(I)	(S)	(S)	0.5	50	38	12
V. natrigenes	(R)	(S)	(R)	(R)	(S)	(S)	(S)	(I)	0.38	38	50	12
V. anguilliarium	(R)	(S)	(R)	(R)	(R)	(I)	(S)	(R)	0.63	63	25	12
V. vulnificus	(R)	(S)	(R)	(R)	(R)	(I)	(S)	(I)	0.5	50	25	25
V. diazotrophicus	(R)	(S)	(R)	(R)	(R)	(I)	(S)	(R)	0.63	63	25	12
V. alginolyticus	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	0	0	100	0

Key: R = Resistant, I = Intermediate, S = Susceptible, CH = Chloramphencol, CPX = Ciprofloxacin, AM = Amoxicillin, AU = Augmentin, GN = Gentamycin, PEF = Pefloxacin, OFX = Ofloxacin, S = Streptomycin.

Table 3c. Antibiotic susceptibility test of isolates obtained from periwinkle samples in the wet season.

	Zone diameter (mm) Antibiotics											
***Vibrio* spp.**	**CH 30 µg**	**CPX 5 µg**	**AM 30 µg**	**AU 30 µg**	**CN 10 µg**	**PEF 5 µg**	**OFX 5 µg**	**S 10 µg**	**MAR Index**	**R (%)**	**S (%)**	**I (%)**
V. anguillarum	(I)	(S)	(R)	(R)	(R)	(S)	(S)	(R)	0.5	50	38	12
V. cholerae	(R)	(S)	(R)	(R)	(R)	(S)	(S)	(S)	0.5	50	50	0
V. xuii	(R)	(S)	(I)	(R)	(S)	(S)	(S)	(S)	0.25	25	63	12
V. xuii	(R)	(I)	(R)	(R)	(R)	(S)	(S)	(R)	0.63	63	25	12
V. xuii	(I)	(S)	(I)	(R)	(S)	(S)	(S)	(I)	0.12	12	50	38
V. natrigenes	(R)	(S)	(R)	(R)	(S)	(S)	(S)	(S)	0.37	37	63	0
V. aesturianus	(R)	(S)	(R)	(R)	(R)	(I)	(S)	(R)	0.63	63	25	12
V. cicinatiensis	(R)	(S)	(R)	(R)	(R)	(S)	(S)	(R)	0.63	63	37	0
V. anguillarum	(R)	(I)	(R)	(R)	(R)	(S)	(S)	(S)	0.5	50	38	12
V. anguillarum	(I)	(S)	(R)	(R)	(R)	(S)	(S)	(R)	0.5	50	38	12
V. alginolyticus	(R)	(S)	(R)	(R)	(R)	(S)	(S)	(R)	0.63	63	37	0
V. paraheamoyticus	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	0	0	100	0

Key: R = Resistant, I = Intermediate, S = Susceptible, CH = Chloramphencol, CPX = Ciprofloxacin, AM = Amoxicillin, AU = Augmentin, GN = Gentamycin, PEF = Pefloxacin, OFX = Ofloxacin, S = Streptomycin.

Table 4a. Antibiotic susceptibility test of isolates obtained from blood cockles in dry season.

	Zone diameter (mm) Antibiotics											
***Vibrio* spp.**	**CH 30 µg**	**CPX 5 µg**	**AM 30 µg**	**AU 30 µg**	**CN 10 µg**	**PEF 5 µg**	**OFX 5 µg**	**S 10µg**	**MAR Index**	**R (%)**	**S (%)**	**I (%)**
V. cholerae	(R)	(S)	(R)	(R)	(R)	(S)	(S)	(R)	0.5	63	37	0
V. xuii	(S)	(S)	(R)	(I)	(S)	(S)	(S)	(I)	0.12	12	63	25
V. cholerae	(R)	(S)	(R)	(R)	(S)	(S)	(S)	(S)	0.37	37	63	0
V .cholerae	(S)	(S)	(S)	(R)	(S)	(S)	(S)	(S)	0.12	12	88	0
V. vulnificus	(I)	(S)	(I)	(I)	(S)	(S)	(S)	(S)	0	0	63	37
V. xuii	(I)	(S)	(S)	(I)	(S)	(S)	(S)	(I)	0	0	63	37
V .cholerae	(R)	(S)	(S)	(R)	(I)	(S)	(S)	(I)	0.25	25	50	25

Key: R = Resistant, I = Intermediate, S = Susceptible, CH = Chloramphencol, CPX = Ciprofloxacin, AM = Amoxicillin, AU = Augmentin, GN = Gentamycin, PEF = Pefloxacin, OFX = Ofloxacin, S = Streptomycin.

Table 4b. Antibiotic susceptibility test of isolates obtained from oyster samples in dry season.

	Zone diameter (mm) Antibiotics											
***Vibrio* spp.**	**CH 30 µg**	**CPX 5 µg**	**AM 30 µg**	**AU 30 µg**	**GN 10 µg**	**PEF 5 µg**	**OFX 5 µg**	**S 10 µg**	**MAR Index**	**R (%)**	**S (%)**	**I (%)**
V. xuii	(R)	(S)	(R)	(R)	(S)	(S)	(S)	(I)	0.38	38	50	12
V. porteresia	(R)	(S)	(S)	(R)	(R)	(S)	(S)	(S)	0.37	37	63	0
V. vulnificus	(R)	(S)	(I)	(R)	(S)	(S)	(R)	(S)	0.38	38	50	12
V. cholerae	(I)	(S)	(S)	(R)	(R)	(S)	(S)	(I)	0.25	25	50	25
V. fluvialis	(S)	(S)	(I)	(I)	(S)	(S)	(S)	(S)	0	0	75	25
V. cholerae	(I)	(R)	(S)	(R)	(R)	(S)	(S)	(S)	0.38	38	50	12
V. cholerae	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	0	0	100	0
V. vulnificus	(I)	(S)	(S)	(R)	(S)	(S)	(S)	(S)	0.13	13	75	12
V. cholerae	(R)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	0.12	12	88	0
V. vulnificus	(R)	(S)	(S)	(R)	(S)	(S)	(S)	(S)	0.25	25	75	0

Key: R = Resistant, I = Intermediate, S = Susceptible, CH = Chloramphencol, CPX = Ciprofloxacin, AM = Amoxicillin, AU = Augmentin, GN = Gentamycin, PEF = Pefloxacin, OFX = Ofloxacin, S = Streptomycin.

Table 4c. Antibiotic susceptibility test of isolates obtained from periwinkle samples in dry season.

	Zone diametre (mm) Antibiotics											
***Vibrio* spp.**	**CH 30 µg**	**CPX 5 µg**	**AM 30 µg**	**AU 30 µg**	**GN 10 µg**	**PEF 5 µg**	**OFX 5 µg**	**S 10 µg**	**MAR Index**	**R (%)**	**S (%)**	**I (%)**
V. cholerae	(R)	(S)	(S)	(R)	(S)	(S)	(S)	(S)	0.25	25	75	0
V. cholerae	(R)	(S)	(S)	(R)	(R)	(S)	(S)	(S)	0.37	37	63	0
V. xuii	(I)	(S)	(S)	(I)	(S)	(S)	(S)	(S)	0	0	75	25
V. paraheamolyticus	(I)	(S)	(S)	(R)	(S)	(S)	(S)	(I)	0.12	12	63	25
V. cholerae	(R)	(S)	(I)	(R)	(S)	(S)	(S)	(S)	0.25	25	63	12
V. porteresia	(S)	(S)	(I)	(I)	(R)	(S)	(S)	(S)	0.12	12	63	25
V. cholerae	(R)	(S)	(R)	(R)	(R)	(S)	(S)	(R)	0.63	63	37	0
V. parahaemolyticus	(S)	(S)	(R)	(S)	(S)	(S)	(S)	(S)	0.12	12	88	0
V. xuii	(S)	(S)	(S)	(I)	(S)	(S)	(S)	(S)	0	0	88	12
V. paraheamolyticus	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	0	0	100	0
V. cholerae	(R)	(S)	(S)	(R)	(S)	(S)	(S)	(I)	0.25	25	63	12
V. cholerae	(I)	(S)	(S)	(R)	(S)	(S)	(S)	(S)	0.13	13	75	12
V. furnishi	(I)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	0	0	88	12
V. furnishi	(R)	(S)	(R)	(R)	(S)	(S)	(S)	(R)	0.5	50	50	0
V .cholerae	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	0	0	100	0
V. vulnificus	(I)	(S)	(S)	(I)	(S)	(S)	(S)	(S)	0	0	75	25

Key: R = Resistant, I = Intermediate, S = Susceptible, CH = Chloramphencol, CPX = Ciprofloxacin, AM = Amoxicillin, AU = Augmentin, GN = Gentamycin, PEF = Pefloxacin, OFX = Ofloxacin, S = Streptomycin.

Table 5a. MAR index of isolates obtained from blood cockles in the wet season.

	MAR Index								
Isolated bacteria	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**
V. cholerae	0(0.00)	0(0.00)	0(0.00)	1(0.50)	0(0.00)	2(0.75)	1(0.88)	0(0.00)	0(0.00)
V. fluvialis	1(0.25)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
V. aesturianus	1(0.25)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
V. alginolyticus	0(0.00)	0(0.00)	0(0.00)	1(0.50)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
V. hepatarius	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(0.63)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
V. vulnificus	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(1.00)
V. anguilliarium	0(0.00)	0(0.00)	0(0.00)	2(0.50)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Total	2	0	0	4	1	2	1	0	1

Table 5b. MAR index of isolates obtained from oysters in the wet season.

	MAR Index								
Isolated bacteria	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**
V. anguillarum	0(0.00)	0(0.00)	0(0.00)	1(0.50)	1(0.63)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
V. natrigenes	0(0.00)	1(0.38)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
V. vulnificus	0(0.00)	0(0.00)	0(0.00)	1(0.50)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
V. diazotrophicus	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(0.63)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Total	0	1	0	2	2	0	0	0	0

Table 5c. MAR index of isolates obtained from periwinkles in the wet season.

	MAR Index								
Isolated bacteria	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**
V. cholerae	0(0.00)	0(0.00)	0(0.00)	1(0.50)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
V. anguillarum	0(0.00)	0(0.00)	0(0.00)	3(0.50)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
V. xuii	1(0.25)	0(0.00)	0(0.00)	0(0.00)	1(0.63)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
V. natrigenes	0(0.00)	1(0.37)	0(0.00))	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
V. alginolyticus	0(0.00)	0(0.00)	0(0.00)	1(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
V. aesturianus	0(0.00)	0(0.00)	0(0.00)	1(0.00)	1(0.63)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
V. cicinatiensis	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(0.63)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
V. anguillarum	0(0.00)	0(0.00)	1(0.44)	2(0.56)	1(0.63)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Total	1	1	1	4	4	0	0	0	0

Table 6a. MAR index of isolates obtained from blood cockles in the dry season.

	MAR Index								
Isolated bacteria	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**
V. cholerae	1(0.25)	1(0.37)	0(0.00)	0(0.00)	1(0.63)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Total	1	1	0	0	1	0	0	0	0

Table 6b. MAR index of isolates obtained from oysters in the dry season.

	MAR Index								
Isolated bacteria	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**
V. cholerae	1(0.25)	1(0.38)	0(0.00)	0(0.00)	0(0.0)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
V. porteresia	0(0.00)	1(0.37)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
V. xuii	0(0.00)	1(0.38)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
V. vulnificus	1(0.25)	1(0.38)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Total	2	4	0	0	0	0	0	0	0

Table 6c. MAR index of isolates obtained from periwinkles in the dry season.

	MAR Index								
Isolated bacteria	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**
V. cholerae	3(0.25)	1(0.37)	0(0.00)	0(0.00)	1(0.63)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
V. furnishi	0(0.00)	0(0.00)	0(0.00)	1(0.50)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Total	3	1	0	1	1	0	0	0	0

DISCUSSION

The seafood and water samples evaluated in this study were heavily contaminated with *Vibrio* species. Over 90% of the counts for *Vibrio* species were above the FDA (FDA, 2022) minimum recommended limit (100/10 CFU/g**)** in fresh and boiled bivalve mollusk/gastropods. *Vibrio* colony count were higher in the dry season than in the wet season, this is similar to the findings reported by Venggadasamy et al. (2021), who stated that higher densities of *Vibrio* species were found in 14/16 groups of shellfish. Odu et al. (2011), observed similar values for *Vibrio* counts in oyster, and periwinkle samples analysed in their study. Also, research reports by Abioye et al. (2021), in a study conducted in the Agadir Bay (Morocco), on antimicrobial resistance characterization of *Vibrio* and *Salmonella* strains from mussels, sediment, and water samples isolated at a high frequency. They reported that *Vibrio* species density also varied across seasons with relatively high density recorded in the summer. Mancini et al. (2023), in their research on occurrence and densities of *V. parahaemolyticus* in molluscs and their positive correlation to water temperatures, reported that outbreaks occur mainly during the warmer months in temperate zones and can be linked to outbreaks caused by *Vibrio cholerae nonO1-nonO139, V. parahaemolyticus, and V. vulnificus* in several European countries. This research reports may explain why a higher occurrence of *Vibrio* species was detected in the warmer months specifically in the dry season.

In this study, the occurrence of *Vibrio parahaemolyticus* occurrence was higher in the dry season, this is similar to the results obtained by Ojesanmi and Ibe (2012), who reported that the population of *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio alginolyticus* were higher in the dry season than in the wet season. Also, Odoemelam et al. (2023), reported V. parahaemolyticus 18.6%, as the most predominant isolate, followed by *V. Vulnificus* 17.7%, *V. Cholerae* 14.6%, *V. alginolyticus* 11.6%, *V. furnissii* 7.3%, V. anguilarum 6.3%, and V. diazotrophicus 3.1% this report is contrary to the reports in this study where only 3 seafood samples were contaminated with *V. parahaemolyticus* and zero prevalence rate was recorded for *V. parahaemolyticus* isolated from oyster samples, Although, 8% and 19% prevalence rate was recorded for the *V. parahaemolyticus* isolated from blood cockles and periwinkle samples in the wet and dry seasons respectively. Overall, *Vibrio* counts were observed to be higher in the dry season than in the wet season. Odu et al. (2011) stated that this may be due to the filter feeding nature of oysters. The lower counts observed for *Vibrio* species in the wet season may therefore be due to the dilution effect of rain water on the river water. Blood cockles from the Creek Road market had the highest count. The very high count in blood cockles from the Creek Road market may be due to the high nutritional composition of blood cockles. The Creek Road market is also a major wet market in Port Harcourt where a variety of seafood samples can be assessed from different Local Government Areas in Rivers State, therefore, samples obtained from this location may vary in the microbial load from other smaller seafood markets like Mile 1 and Mile 3 markets. The parboiled bivalve samples were processed by pouring hot water on samples to facilitate the shucking process before sales to the public while the periwinkle samples were parboiled for 2-3 minutes so the fleshy meat can be extracted. This may be the reason why the counts for the parboiled samples were still a bit high as these samples were not thoroughly boiled before sales. The boiling process is not sufficient to kill these microorganisms as it is used mainly for shucking and extraction of the flesh meat, therefore, seafood's especially parboiled seafood's purchased from these wet markets must be thoroughly cooked to ensure food safety.

Among *Vibrio* species, *Vibrio cholerae*, had the highest occurrence in the wet and dry seasons respectively. *V. cholerae* has been observed to adapt and survive under various conditions for extended periods (Okello, 2019). Akoachere and Mbuntcha (2014) stated that the effect of salinity triggered the occurrence of *Vibrio cholerae* in the dry season this may be the reason why *V. cholerae* occurrence was higher in dry season in this study. Also *V. cholerae* can thrive in saline (brackish) and nonsaline (fresh water) environment unlike most *Vibrio's* that are strictly halophiles. This unique nature of *V. cholerae* may account for its persistence and high prevalence in the study. The differences observed in the prevalence of *Vibrio spp*. in this study and other research studies may also be attributed to the sample size, sample type and different geographical locations which are constantly

influenced by different environmental conditions like temperature, pH and salinity (Emch et al., 2008; Adagbada et al., 2012).

Vibrio species were most susceptible to ofloxacin, ciprofloxacin, pefloxacin, and were most resistant to augmentin, chloramphenicol, gentamycin, and amoxicillin. This is similar to research reports by Udoekong et al. (2021), who observed that V*ibrio* species were susceptible (100%) to the quinolones. They also reported that all *Vibrio* isolates were sensitive to chloramphenicol, amoxicillin/clavulanic, gentamycin, streptomycin and amikacin this is in contrast to the findings in this study especially in the wet season where isolates exhibited high resistance to Amoxicillin—74%, Chloramphenicol—74%, and Augmentin-55%. Also, research reports by Mancini et al. (2023), stated that none of the isolates were resistant to chloramphenicol and gentamycin this is in contrast to reports in this study in the wet season where *Vibrio* isolates were resistant to chloramphenicol (74%) and gentamycin (58%). Inana et al. (2024) observed a high resistance of *Vibrio* isolates to Augmentin in their study this is similar to findings in this study. The penicillin group is usually prescribed as an antibiotic for the primary treatment of food-borne illnesses in Nigeria because it is more affordable than other antibiotics. The emergence of penicillin-resistant bacteria has drastically dwindled its efficacy. Resistance to Augmentin among *Vibrio* species was reported higher in this study and other study reports (Beshiru et al., 2023), this has a major public health implication as it may no longer be used for therapeutic purposes in the future despite its affordability.

The MAR index from this study was found between 0.25 - 1.0. This is similar to a study by Mancini et al. (2023) who recorded MAR index values from 0 to 0.69. In this study, Thirty-two 32(46%) isolates had a MAR index value of ≥0.4 in the wet and dry season. This is similar to a study by Gxalo et al. (2021), who observed that multiple antibiotic resistance patterns among the *Vibrio* species had a range of 0.5 to 0.8. On the contrary research reports by Beshiru et al. (2023), stated that a higher percentage (94.0%) of the isolates were resistant to more than one antibiotic used and Dahanayake et al. (2019), observed in their study that 27 (84%) isolates were multidrug resistant. The MAR index of isolates in this study is far above the arbitrary value of 0.2, indicating high-risk contamination sites. The high multiple antibiotic resistance indexes observed may be due to inappropriate use/abuse of antibiotics in the area where these seafood are harvested from, this culminates in the development and spread of superbugs. This study's findings further support the possible dissemination of *Vibrio* species and its resistant determinants. This information will be useful in designing microbiological risk assessment models to estimate the incidence and prevalence of *Vibrio* species and there antibiotic resistant determinants.

CONCLUSION

Seafood is an important food source globally, with a variety of nutritional and health benefits. Despite these benefits eating contaminated seafood can become a public health risk and can cause death in patients. The occurrence *Vibrio* species in bivalve mollusks and gastropods have been reported severally, However, the total *Vibrio* counts and the prevalence rates of *Vibrio* species varies amongst several studies, this could be attributed to the difference in the reservoir (sample type), geographical location, seasonal variation and other environmental factors like temperature, pH and salinity influencing the host species. A majority of isolates showed resistance to Augmentin especially in the wet season. This finding suggests that in the near future commonly used antibiotics like Augmentin may become ineffective for the treatment of vibriosis. The Creek Road market samples had the most contaminated shellfish. This raises serious concerns about food safety and public health, as these retail seafood products were found to be resistant to multiple antibiotics and therefore, may serve as a constant reservoir for multidrug-resistant bacteria that can be transmitted to humans through the food chain. Proper food handling procedures and antibiotic surveillance/control strategies must be established and strictly adhered to. Understanding AMR dissemination among *Vibrio* species is necessary to reduce AMR in Rivers State. Therefore, it important to obtain data regularly on *Vibrio* species for biosafety/micro risk assessment of the distribution of AMR as part of the AMR monitoring program. Regular surveillance, improved policy development and preparedness should be a standard approach for developing a conceptual framework to decipher factors that might influence the prevalence of *Vibrio* spp. in bivalve mollusks and gastropods in Port Harcourt, Rivers State, this will help prevent occurrence of an outbreak in the future.

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Citation: Moro KO, Amadi LO, Ugboma CJ, Peekate LP, **2024**. Prevalence and antibiogram of *Vibrio* species from blood cockles, oysters and periwinkles sold in Port Harcourt. Microbiol Res Int, 12(4): 124-137.