IJMAAS International Journal of Microbiology and Applied Sciences
ISSN 1115-4004 Volume 3 issue 2 May, 2024 Research Article

Prevalence of *Campylobacter* and *Vibrio* species Associated with Selected Seafoods; Oyster (*Crassostrea gasar*), Shrimps (*Caridea*) and Prawn (*Penaeus monodon*)

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ABSTRACT

Seafood is a vital component of the human diet globally, contributing essential nutrients and proteins. However, it can also harbour various pathogens including *Campylobacter* and *Vibrio* species, which pose significant threat to public health through improper handling, processing, storage and distribution to consumers. This study was aimed at assessing the prevalence of *Campylobacter* and *Vibrio* species in selected seafood sold in Port Harcourt, Nigeria. A total of 360 seafood samples; Oyster (*Crassostrea gasar*), Shrimps (*Caridea*) and Prawn (*Penaeus monodon*) were purchased from vendors across three major markets. Samples were analysed using standard microbiological techniques to isolate and identify *Campylobacter* and *Vibrio* species. The results revealed a prevalence rate of 70.55% *Campylobacter* and 29.5% of *Vibrio* species in seafood samples examined. Genomic identification revealed the presence of various strains, including *Vibrio alginolyticus Ariake*-S2 (11.36%), *V. parahaemolyticus* (6.82%), *V. fluvialis* (4.55%), *V. mimicus* (15.91%), *V. cholerae* (11.36%), *V. vulnificus* E4010 (9.09%), *V. cholerae* NSTH36 (9.09%), *V. parahaemolyticus* VP35/2 (2.27%) and *Campylobacter jejuni* G-149-05-1 (4.55%), *C. lari* sub sp (9.09%), *C. coli* (2.27%), *C. jejuni* sub sp (9.09%) and *C. lari* (4.55%). This study therefore emphasizes the need for stringent hygienic practices during handling, processing, storage and distribution of seafood to prevent the risk of foodborne illnesses associated with *Campylobacter* and *Vibrio* species. The fact that transfers of resistant bacteria between aquatic environments and humans through handling and consumption of seafood pose a serious hazard to public health, continuous surveillance and adherence to food safety regulations to safeguard consumer health must be sustained.

Keywords: *Campylobacter* sp., *Vibrio* sp., prawn, oyster, shrimps, food safety, public health.

Introduction

Seafood plays a crucial role in the global food industry and it is a major source of nutrition for millions of people worldwide. Significant public health issues are raised by the ingestion of contaminated seafood, which has been linked to a number of bacterial diseases (Iwamoto *et al*., 2010). Fish, shellfish and crustaceans are all examples of seafood that can be contaminated by a variety of bacterial species due to handling, processing, storage and distribution to consumers (Daniels *et al*., 2000). *Vibrio* species, such as *Vibrio parahaemolyticus, Vibrio vulnificus, and Vibrio cholerae*, are among the most often reported bacterial species linked to seafood contamination (Efiuvwevwere and Amadi, 2015)These bacteria may live and grow in a variety of seafood and are naturally found in marine habitats. Other important bacterial species from aquatic environments include *Vibrio* spp. *Shigella* spp., *Listeria monocytogenes*, *Escherichia coli*, *Yersinia* spp. and *Campylobacter* spp.

Campylobacter is a genus of Gram-negative, spiral-shaped bacteria that causes foodborne illnesses worldwide (Facciolà *et al.*, 2017). Among the various species of *Campylobacter*, *Campylobacter jejuni* and *Campylobacter coli* are the most commonly associated with human infections (Sheppard and Maiden, 2015). Similarly, *Vibrio* species, including *Vibrio parahaemolyticus* and *Vibrio cholerae*, are prominent pathogens associated with seafood-related illnesses. They are known to be prevalent in a variety of food sources, including poultry, dairy products and seafood. Their presence in seafood poses a significant risk to public health. *Campylobacter* and *Vibrio* species contaminated seafood can lead to gastroenteritis, characterized by symptoms such as abdominal pain, diarrhea, and fever (Galanis 2007).

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Given the increasing demand for seafood and the potential health risks associated with *Campylobacter* contamination, understanding the molecular epidemiology and pathogenic potential of *Campylobacter* in seafood becomes imperative (Kaakoush *et al.*, 2015). Considering the risk factors associated with *Campylobacter* and *Vibrio* contamination in seafood, it is essential to employ effective control and prevention strategies towards contaminated water sources, cross-contamination during processing and improper storage conditions (Facciolà *et al.*, 2017). Therefore the aim of this study is to investigate the prevalence of *Campylobacter* sp., and *Vibrio* sp., in seafood Oyster (*Crassostrea gasar*), Shrimps (*Caridea*) and Prawn (*Penaeus monodon*) sold in some markets in Port Harcourt metropolis, in Rivers State, Nigeria.

Materials and Methods

Study Area

The study was carried out in three different markets; the Creek Road market, Mile One market, and Rumuokoro market all located within Port Harcourt metropolis. These markets were selected because of the high population density, catchment areas for consumers and its easy accessibility to the purchase of seafood. Seafood samples were purchased randomly from Ten (10) different vendors from each of the three (3) markets. Samples were purchased and aseptically collected using appropriate aseptic methods. Samples were collected in the months of January, May, September and December in the year 2022.

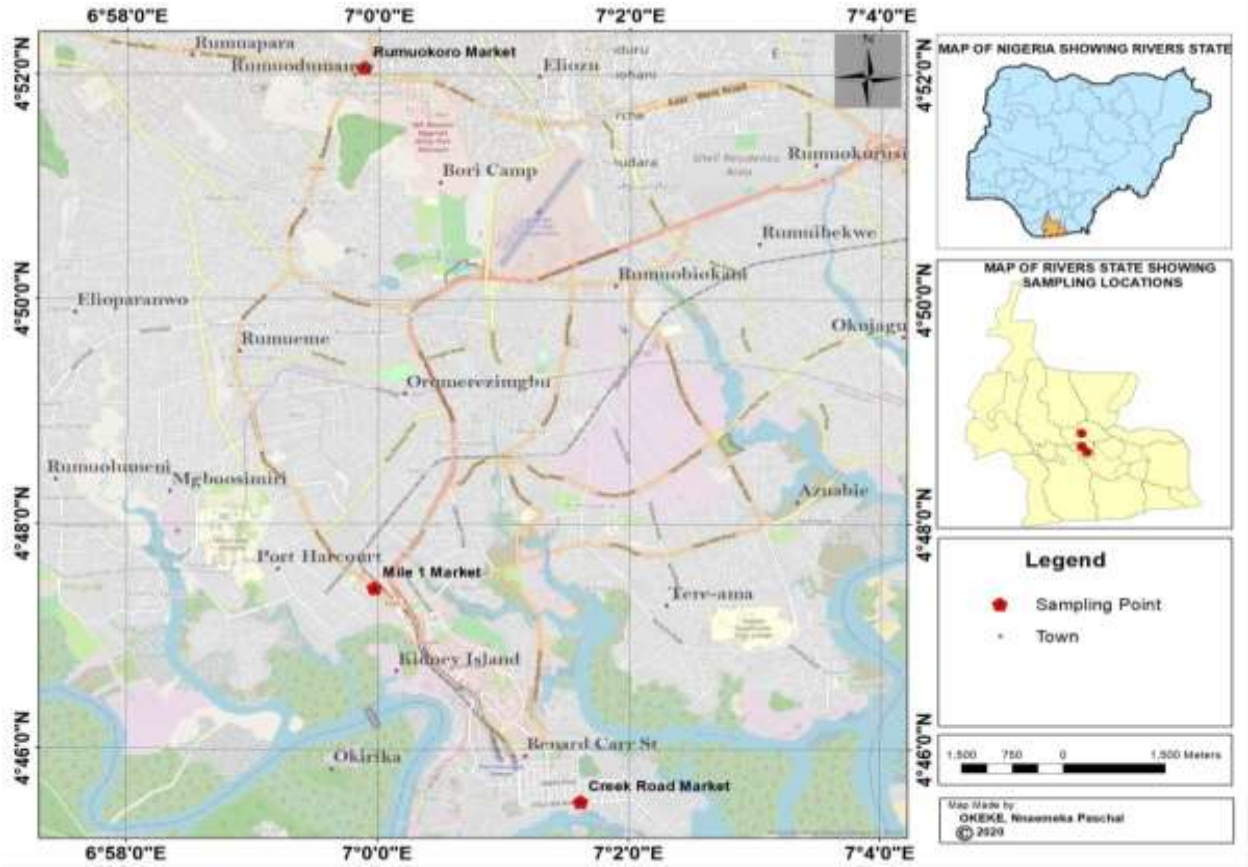

Figure 1: Sampled locations of seafood in Rivers State

Seafood Sample Collection

Seafood samples were purchased from different markets within the Port Harcourt metropolis in sterile bags, labeled and transported in an ice-chest box aseptically to the Department of Microbiology Laboratory Rivers State University for bacteriological analysis.

The seafood samples namely Oyster (*Crassostrea gasar*), Prawn (*Penaeus monodon*) and Shrimps (*Caridea*) in plates 1, 2 and 3 respectively were identified by Dr. Chidinma Amuzie of the Department of Animal and Environmental Biology Rivers State University, Port Harcourt.

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Plate 1: Oyster (*Crassostrea gasar*)

Plate 2: Prawn (*Penaeus monodon*)

Plate 3: Shrimps (*Caridea*)

Preparation of seafood samples

Seafood samples were macerated in physiological saline as homogenate and a 10-fold serial dilution was done for each sample before further inoculation and incubation.

Determination of Total *Vibrio* Count

An aliquot (0.1ml) from 10^{-2} dilution of water samples were inoculated on Thiosulphate citrate bile salt agar plate in triplicate for isolation and enumeration of *Vibrio* species using standard microbiological methods as described by Prescott *et al* (2011). The inocula were spread evenly on the surface of the media using a sterile spreader and incubated at 37^{o}C for 24 hours, after which the colonies that developed were counted and the mean was expressed as colony forming unit per milliliter (Inana *et al* 2019).

Determination of *Campylobacter* Species

The method described by Penner, (1988) was adopted for the isolation of *Campylobacter* species from the seafoods. Twenty-five (25g) grams of each sample (Prawn, Shrimps and Osyters) were separately homogenized in 225ml sterile thioglycollate broth. Broth samples were incubated at 42°C for 48 hours in a microaerophilic atmosphere). A loopful of enrichment broth was streaked onto Charcoal Cefoperazone Deoxycholate agar (CCDA) plates (Oxoid) and incubated under microaerophilic conditions at 42°C for 48 hours (Persson and Olsen, 2005). The colonies were then subjected to morphological and biochemical examination (Vandamme *et al.,* 2008). The pure cultures were obtained by picking (with sterile inoculating loop) distinct culturally and morphologically different colonies from the various plates (Obire *et al.*, 2003).

Genomic Identification of Bacterial Isolates

Extraction of DNA

DNA extraction is a phenomenon by which DNA is separated from proteins, membranes and other cellular materials contained in the cell (Kelly, 2013). Boiling method was used for the extraction process. A 24 hours old pure culture of the isolates was put in Luria-Bertani (LB) Broth and incubated at 37^{o}C. About 0.5ml of an overnight broth culture of the isolates in Luria Bertani (LB) was put into properly labeled Eppendorf tubes and filling to mark with normal saline and was centrifuged at 14000rpm for 3 min and the supernatant was decanted leaving the DNA at the base. This process was repeated 3 times. The cells were re-suspended in 500ul of normal saline and heated at 95^{o}C for 20 min. The heated bacterial suspension was cooled on ice (About 10minutes) and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5ml micro-centrifuge tube and stored at -20^{o}C for other down-stream reactions (Bell *et al.*, 1998).

DNA Quantification

DNA quantification is a phenomenon carried out to determine the concentration of DNA as well as its purity. The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The Beer Lambert's principle which is used to evaluate the quality and quantity of the genomic DNA is used by the Nanodrop spectrophotometer. The Nanodrop spectrophotometer was connected to a computer with Nanodrop software installed. The software of the equipment was launched by double clicking on the Nanodrop icon. The sample pedestals were properly cleaned. The equipment was initialized using 2µl of sterile distilled water and blanked using 2µl of Normal saline.

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About 2µl of the extracted DNA of the individual isolates of fungi and bacteria was loaded onto the lower pedestal to measure the concentration of the sample, and the upper pedestal was brought down to make contact with the DNA on the lower pedestal. Then, DNA concentration was measured by clicking the "measure" button displayed on the computer screen (Olsen and Morrow, 2012).

16S rRNA Amplification

The 16srRNA Amplification was carried out using an ABI 9700 Applied Biosystems Thermal Cycler and method described by Srinivasan *et al*. (2015). The 16s rRNA region of the rRNA gene of the bacterial isolates were amplified using the forward primer; 27F:5'-AGAGTTTGATCMTGGCTCAG-3' and Reverse primer; 1492R:5'-CGGTTACCTTGTTACGACTT-3'primers at a final volume of 50 micro litres for 35 cycles. The PCR mix includes: (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.5uM and the extracted DNA as template, Buffer 1X and water. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light trans-illuminator for a 1500bp amplicons (Srinivasan *et al*., 2015).

DNA Sequencing

Sequencing of the amplified product was carried out using the Big-Dye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25 ulBigDye® terminator v1.1/v3.1, 2.25ul of 5 x Big Dye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing condition were as follows; 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4minutes (Srinivasan *et al*., 2015).

Phylogenetic Analysis

Similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN prior to the edition of the obtained sequences using the bioinformatics algorithm Trace edit. MAFFT were used to align these sequences. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987).

The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor 1969).

Statistical Analysis of Data

The data obtained was analyzed using analysis of variance (ANOVA) to test for significance and where differences occur Duncan multiple range test was used to separate the means using the Statistical Package for Social Science (SPSS) version 22 (Bewick *et al*., 2004).

Results

The results of the enumeration of *Campylobacter* and *Vibrio* species from oysters, prawns and shrimps in this study are presented in Tables 1, 2, and 3 respectively.

Table 1 shows that *Campylobacter* counts in Oysters from Mile 1 market ranged from 2.0±0.05 $x10^3$ CFU/g in January to 2.8±0.04 $x10^3$ CFU/g in December; *Campylobacter* counts in Oysters from Creek Road market ranged from 2.4±0.04 $x10^3$CFU/g in December to 3.1±0.06 $x10^3$ CFU/g in May; while *Campylobacter* counts in Oysters from Rumuokoro market ranged from 1.8±0.03 $x10^3$ CFU/g in January to 2.3±0.05 $x10^3$ CFU/g in September. Table 1 also shows that *Vibrio* counts in Oysters from Mile 1 market ranged from 3.2±0.06 $x10^3$ CFU/g in May to 5.4±0.09 in September; *Vibrio* counts in Oysters from Creek Road market ranged from 2.5±0.04 $x10^3$ CFU/g in January to 5.4±0.06 in September; while *Vibrio* counts in Oysters from Rumuokoro market ranged from 2.5±0.03 $x10^3$ CFU/g in December to 4.9±0.06 $x10^3$ CFU/g in September. Similar trend were observed in counts for the different markets in others months. Statistical analyses revealed that there were significant differences in the counts between *Campylobacter* and *Vibrio* at P> 0.05 in all the months except for December that had no significant difference.

Table 2 shows that *Campylobacter* counts in Prawns from Mile 1 market ranged from 2.0±0.04 $x10^3$ CFU/g in May to 2.6±0.06 $x10^3$ CFU/g in January; *Campylobacter* counts in Oysters from Creek Road market ranged from 1.4±0.0 $x10^3$ CFU/g in December to 3.3±0.05 $x10^3$ CFU/g in May. While *Campylobacter* counts in Oysters from Rumuokoro market ranged from 1.6±0.02 $x10^3$ CFU/g in December to 3.2±0.05 $x10^3$ CFU/g in May.

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Table 1: Total Counts of *Campylobacter* and *Vibrio* in Oysters Sold in the Selected Markets in Port-Harcourt

Month / Market	January, 2022		May, 2022		September, 2022		December, 2022	
	CAMPY C (x 10^3 CFU/g)	TVC (x 10^3 CFU/g)	CAMPY C (x 10^3 CFU/g)	TVC (x 10^3 CFU/g)	CAMPY C (x 10^3 CFU/g)	TVC (x 10^3 CFU/g)	CAMPY C (x 10^3 CFU/g)	TVC (x 10^3 CFU/g)
Mile 1	2.0±0.05 [a]	4.5±0.09 [a]	2.1±0.04[a]	3.2±0.06[a]	2.3±0.05[a]	5.4±0.09[a]	2.8±0.04[a]	3.4±0.04[a]
Creek Road	2.5±0.05 [a]	2.5±0.04 [a]	3.1±0.06[a]	3.2±0.07[a]	2.5±0.05[a]	5.4±0.06[a]	2.4±0.04[a]	2.6±0.04[a]
Rumuokoro	1.8±0.03 [a]	4.6±0.07 [a]	2.3±0.04[a]	4.0±0.08[a]	2.3±0.05[a]	4.9±0.06[a]	2.0±0.03[a]	2.5±0.03[a]
p. value<0.05	**0.51**	**0.71**	**0.25**	**0.64**	**0.94**	**0.85**	**0.30**	**0.22**

*Means with similar superscript down the group (along the column) showed no significant difference (P>0.05)
Key: CAMPY C = *Campylobacter* Count, TVC = Total *Vibrio* Count

Table 2: Total Counts of *Campylobacter* and *Vibrio* in Prawns Sold in the Selected Markets in Port-Harcourt

Month / Market	January, 2022		May, 2022		September, 2022		December, 2022	
	CAMPY C (x 10^3 CFU/g)	TVC (x 10^3 CFU/g)	CAMPY C (x 10^3 CFU/g)	TVC (x 10^3 CFU/g)	CAMPY C (x 10^3 CFU/g)	TVC (x 10^3 CFU/g)	CAMPY C (x 10^3 CFU/g)	TVC (x 10^3 CFU/g)
Mile 1	2.6±0.06[a]	5.6±0.07[a]	2.0±0.04[a]	5.8±0.07[a]	2.2±0.07[a]	3.6±0.06[a]	2.6±0.04[b]	2.3±0.04[b]
Creek Road	1.8±0.03[a]	5.0±0.07[a]	3.3±0.05[a]	6.0±0.06[a]	1.9±0.08[a]	5.0±0.06[a]	1.4±0.03[a]	1.4±0.02[a]
Rumuokoro	2.3±0.04[a]	4.7±0.06[a]	3.2±0.05[a]	7.3±0.04[a]	2.9±0.06[a]	5.5±0.09[a]	1.6±0.02[a]	1.5±0.02[a]
p. value<0.05	**0.45**	**0.62**	**0.14**	**0.17**	**0.56**	**0.18**	**0.02**	**0.05**

*Means with similar superscript down the group (along the column) showed no significant difference (P>0.05)
Key: CAMPY C = *Campylobacter* Count, TVC = Total *Vibrio* Count

Table 3: Total Counts of *Campylobacter* and *Vibrio* in Shrimps Sold in the Selected Markets in Port-Harcourt

Month / Market	January, 2022		May, 2022		September, 2022		December, 2022	
	CAMPY C (x 10^3 CFU/g)	TVC (x 10^3 CFU/g)	CAMPY C (x 10^3 CFU/g)	TVC (x 10^3 CFU/g)	CAMPY C (x 10^3 CFU/g)	TVC (x 10^3 CFU/g)	CAMPY C (x 10^3 CFU/g)	TVC (x 10^3 CFU/g)
Mile 1	1.9±0.07[a]	4.2±0.09[a]	3.5±0.05[b]	4.8±0.12[a]	2.1±0.09[a]	4.5±0.06[a]	2.1±0.03[a]	3.0±0.04[a]
Creek Road	1.7±0.06[a]	3.1±0.08[a]	1.4±0.07[a]	3.2±0.09[a]	1.1±0.04[a]	3.2±0.07[a]	2.3±0.03[a]	2.3±0.03[a]
Rumuokoro	2.5±0.08[a]	5.0±0.12[a]	1.3±0.05[a]	2.1±0.09[a]	1.2±0.06[a]	3.7±0.09[a]	2.3±0.04[a]	2.4±0.05[a]
p. value<0.05	**0.70**	**0.38**	**0.01**	**0.18**	**0.53**	**0.49**	**0.88**	**0.41**

*Means with similar superscript down the group (along the column) showed no significant difference (P>0.05)
Key: CAMPY C = *Campylobacter* Count, TVC = Total *Vibrio* Count

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Table 2 also shows that *Vibrio* counts in Prawns from Mile 1 market ranged from 2.3±0.04 x10^3 CFU/g in December to5.8±0.07 x10^3 CFU/g in May; *Vibrio* counts in Oysters from Creek Road market ranged from 1.4±0.02 x10^3 CFU/g in December to 6.0±0.06 x10^3 CFU/g in May; while *Vibrio* counts in Oysters from Rumuokoro market ranged from 1.5±0.02 x10^3 CFU/g in December to 7.3±0.04 x10^3 CFU/g in May. Similar trend were observed for creek road and Rumuokoro markets respectively.

There were significant differences in the count between *Campylobacter* and *Vibrio* species in January, May and September at P>0.05 but shows no significant differences in the month of December. Also, results of T - test revealed that there was no significant difference in *Campylobacter* and *Vibrio* species counts across the three sampled markets.

Table 3 shows that *Campylobacter* counts in Shrimps from Mile 1 market ranged from 1.9±0.07 x10^3 CFU/g in January to 3.5±0.05 x10^3 CFU/g in May; *Campylobacter* counts in Oysters from Creek Road market ranged from 1.1±0.04 x10^3 CFU/g in September to 2.3±0.03 x10^3 CFU/g in December; while *Campylobacter* counts in Oysters from Rumuokoro market ranged from 1.2±0.06 x10^3 CFU/g in September to 2.5±0.08 x10^3 CFU/g in January.

Table 3 also shows that *Vibrio* counts in Shrimps from Mile 1 market ranged from 3.0±0.04 x10^3 CFU/g in December to 4.8±0.12 x10^3 CFU/g in May; *Vibrio* counts in Oysters from Creek Road market ranged from 2.3±0.03 x10^3 CFU/g in December to 3.2±0.09 x10^3 CFU/g in May; while *Vibrio* counts in Oysters from Rumuokoro market ranged from 2.1±0.09 x10^3 CFU/g in May to 5.0±0.12 x10^3 CFU/g in January. Similar counts of higher *Vibrio* counts were observed in all the months of sample collection and there were significant differences in all the months except for May and December.

Figure 2 presents the Phylogenetic tree of the evolutionary distances between the *Campylobacter* and *Vibrio* species isolated from the selected seafood and percentage relatedness with their close relatives in the gene bank.

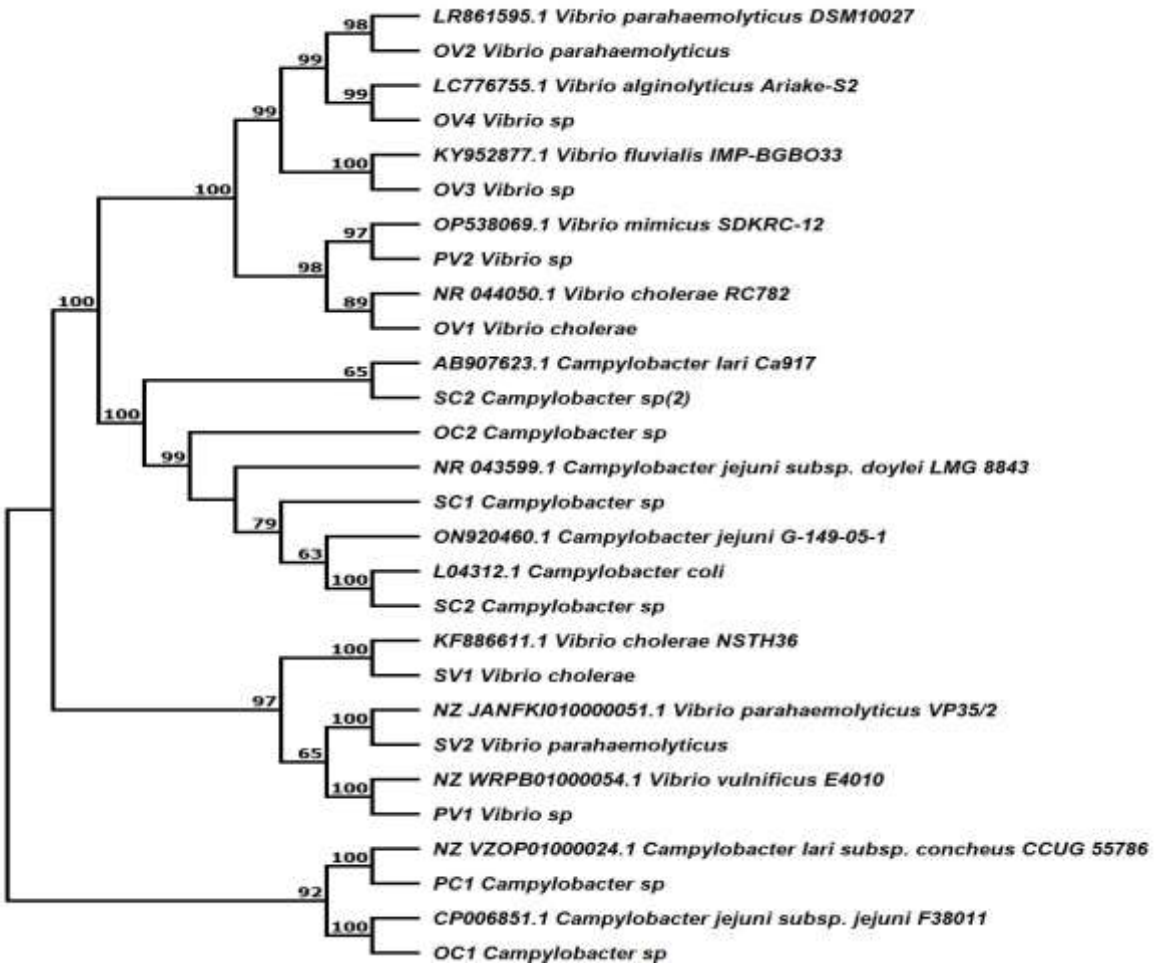

Figure 2: Phylogenetic Tree Showing Evolutionary Distance Between Bacterial Isolates

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Figure 3 presents the frequency of distribution (%) of *Vibrio* and *Campylobacter* species isolated from the selected seafood sold in Mile 1 market. Values for *Vibrio* species shows that *V. mimicus* 15.91% had the highest, *V. alginolyticus Ariake*-S2 recorded 11.36%, and *V. cholerae* 11.36%, while *V. parahaemolyticus* VP35/2 recorded the least value of 2.27%. Values for *Campylobacter* species shows that *Campylobacter lari sub sp* and *Campylobacter jejuni sub sp* recorded the highest value of 9.09% each while *C. coli* recorded the lowest percentage of 2.27%. The *Vibrio* species were more prominent than the *Campylobacter* species during the study.

Figure 4 presents the frequency of distribution (%) of *Vibrio* and *Campylobacter* species isolated from the Selected Seafood sold in Creek Road.

The values recorded for the isolates were as follows; *V. alginolyticus Ariake*-S2 (10.39%), *V. parahaemolyticus* 7.79%, *V. fluvialis* 6.49%, *V. mimicus* 10.39%, *V. cholerae* 12.99%, *V. vulnificus* E4010 9.09%, *V. cholerae* NSTH36 10.39%, *V. parahaemolyticus* VP35/2 2.60%, *C. jejuni* G-149-05-1 6.49%, *C. lari* sub sp 7.79%, *C. coli* 5.20%, *C. jejuni* sub sp 7.79% and *C. lari* 2.60%.

Figure 5 shows the Percentage frequency distribution of *Vibrio* and *Campylobacter* species from Rumuokoro market in Port Harcourt, results shows that *V. parahaemolyticus* (14.04%), had the highest percentage followed by *V. cholerae* NSTH36 (12.28%) and *V. alginolyticus Ariake*-S2 (10.53%), *and C. lari* sub sp (3.51%), recorded the lowest percentage.

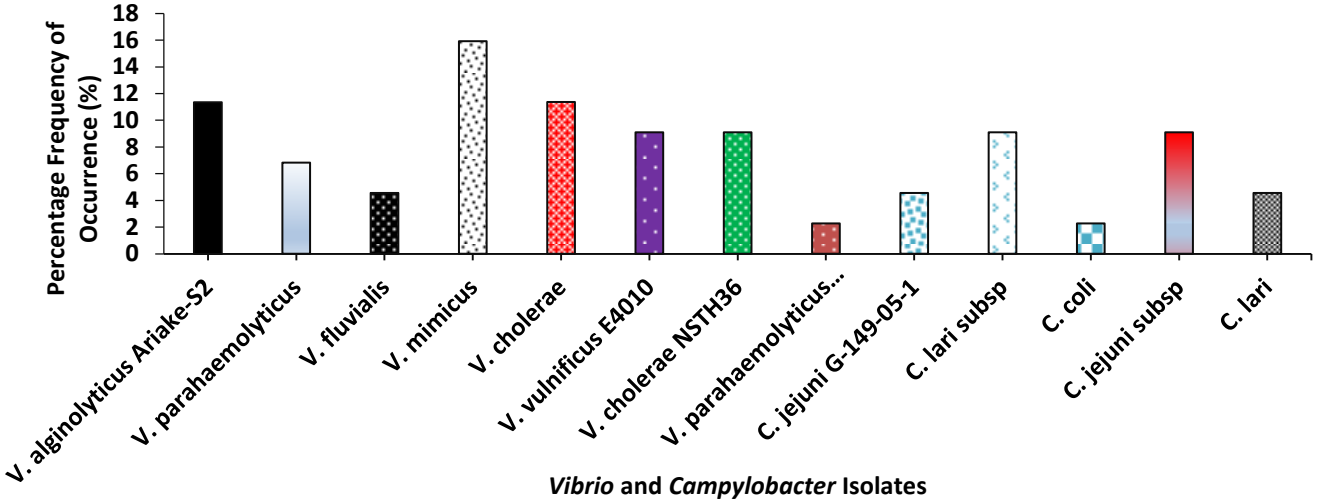

Figure 3: Frequency (%) of *Vibrio* and *Campylobacter* Species Isolated from Seafood in Mile 1 Market

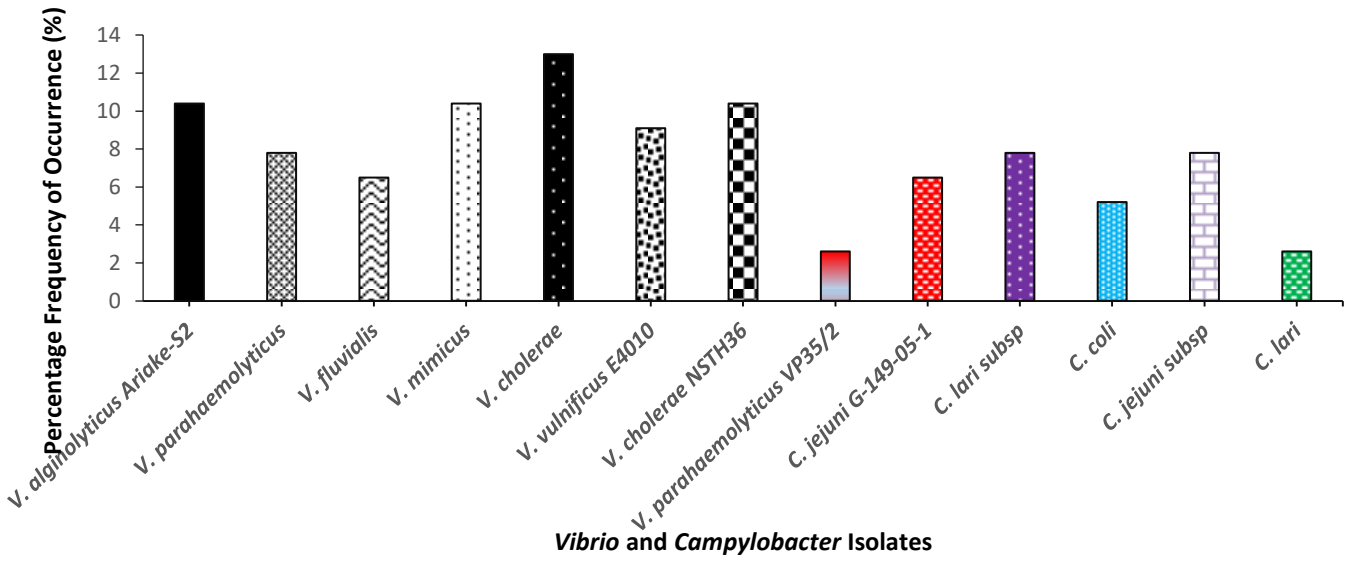

Figure 4: Frequency (%) of *Vibrio* and *Campylobacter* Species Isolated from Seafood in Creek Road market

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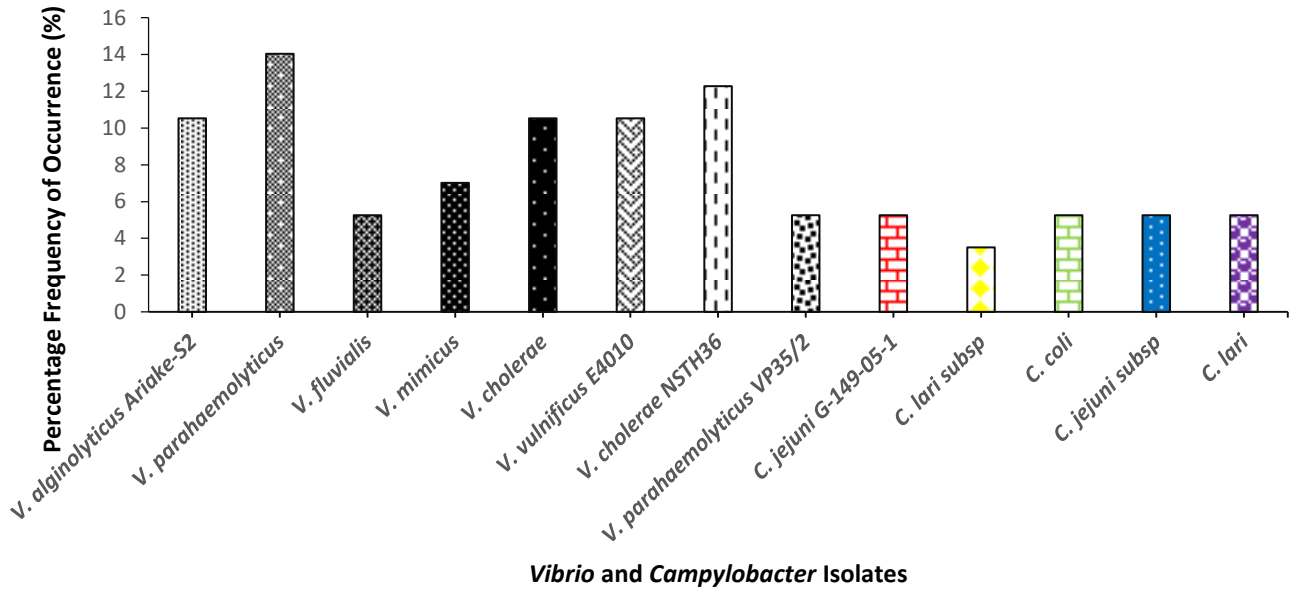

Figure 5: Frequency (%) of *Vibrio* and *Campylobacter* Species Isolated from Seafood in Rumuokoro Market

Discussion

Seafood are becoming very important dietary components due to the increased awareness of the health benefits associated with their nutritional values (Ashiru *et al.*, 2012; Oramadike and Ogunbanwo, 2015). However, the bacteriological quality of some seafood continues to be of great concern because, shellfish, oysters and shrimps most especially, have been shown to be reservoirs for some pathogenic *Vibrio* species (Lutz *et al.*, 2015). This study presents a comprehensive examination of the *Campylobacter* and *Vibrio* counts of Oyster (*Crassostrea gasar*), Shrimps (*Caridea*) and Prawn (*Penaeus monodon*) sold in some selected markets in Port Harcourt metropolis, in Rivers State, Nigeria in the year 2022. This study was aimed at providing valuable insights essential for effective risk assessment and mitigation of health risks in seafood consumption.

Safeguarding the integrity of seafood, especially oysters, prawns and shrimps is important due to the latent health risks associated with microbial contamination. Seafood are generally safe for consumption but their exposure to handling practices, processing and distribution may occasionally cause health risks (Yang *et al.*, 2017). Understanding these microbial intricacies is crucial for maintaining public health standards and ensuring the resilience of the predominant food distribution network. Significant difference in *Campylobacter* and *Vibrio* count as shown in this study is a crucial indicator of overall microbial load among seafood.

The frequency of distribution (%) of *Vibrio* species recorded in this study could be as a result of pollution of water bodies due to beehive of anthropogenic activities such as indiscriminate disposal of wastes into the surrounding rivers. These *Vibrio* species naturally, inhabit estuarine, coastal waters and marine sediments throughout the world, since these areas have the appropriate salinity required for their growth (Miyoshi, 2013, Huehn *et al.*, 2014). They have been known to colonize sea foods such as corals, fish , molluscs, shrimps and zooplankton (specifically copepods) (Xu *et al*., 2015), as well as oyster, clams, mussels, periwinkles and prawns (Lutz *et al.*, 2015 , Huehn *et al.*, 2014) Oramadike and Ogunbanwo, 2015), all of which are an embodiment of the marine food chain.

This could be due to rapid growth of human population, and soil erosion into the aquatic ecosystem (Chindah *et al*., 2003; Ideriah *et al.*, 2012). These pollutants accumulate and persist on and in sediments and may be ingested by aquatic organisms including the seafoods (prawns, oysters and shrimps) in this study as they graze on benthos micro-plants. This may result in deleterious health effects on man through sufficient bioaccumulation (Davies *et al.*, 2006). This is because a host of the population use streams, springs and wells (Adagbada *et al.*, 2012) which normally are supplied by surface runoff, containing untreated wastes from homes, workshops, factories and industries. Thus, the microbiological quality of the polluted water sources is usually highly compromised (Kuitcha *et al*., 2010).

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Ashiru *et al*. (2012), observed shrimps were the most contaminated of all the sea foods. This suggests that these water environments may be good reservoirs that generate new strains of infectious *Vibrio* species (Ashiru *et al*. 2012).

This present study was able to identify that all cases of harvested and sold raw oysters at the various selected markets in Port Harcourt and throughout the period of study, confirmed the isolation of *Campylobacter jejuni* and *Campylobacter coli*. Campylobacteriosis is the most common cause of bacterial gastroenteritis, with symptoms ranging from abdominal pain, fever, mild watery diarrhea to bloody stools (Ecdc, 2013). *Campylobacter* spp. identification for contamination cases from this study revealed high prevalence of *C. jejuni* and *C. lari*. *Campylobacter jejuni* is responsible for over 95% of the diagnosed cases of Campylobacteriosis in sea foods. This condition is attributed to improper cooking or recontamination may occur through contaminated hands which indicate lack of sanitary processing practice (Lutz *et al.*, 2013). This finding is in agreement with that of Caron *et al*. (2021), who documented that all sick individuals consumed raw oysters from Harvest Area A in Rhode Island which was the only food exposure in common among all ill individuals.

The results of this present study shows that intake of oysters should be taken into account when determining the cause of *Campylobacter* infections and that contaminated raw oysters can pose a health risk. Furthermore, according to Carton *et al.* (2021), Campylobacter species, specifically, *Campylobacter jejuni* and *Campylobacter coli* are the primary global causes of bacterial gastroenteritis. The study's findings of *Campylobacter* species from prawns may point to contamination from environmental or animal sources. These bacteria are frequently seen in the gastrointestinal tracts of animals and birds. In order to minimize outbreaks of foodborne illness, Nguyen *et al.* (2019) emphasized the possible health risks connected to shrimp infected with *Campylobacter* and recommended stringent surveillance and control methods.

Vibrio species such as *Vibrio parahaemolyticus* and *Vibrio cholera* are frequently linked to infections related to seafood. According to Janecko *et al.* (2021), the existence of antibiotic resistance genes in *Vibrio* isolates from prawns raises questions concerning treatment efficacy and the possibility of transmission across the food chain.

Rahman *et al*. (2021) emphasized the possibility of foodborne outbreaks and the frequency of *Vibrio* species in prawns. In order to reduce the danger of diseases brought on by these pathogens, strict hygiene regulations must be followed throughout the seafood supply chain, as demonstrated by the isolation of *Vibrio* species from prawns.

The result of this study reveals observable changes in counts during the wet and dry seasons. Generally, *Campylobacter* and *Vibrio* species counts were highest in the wet months of May and September and lowest in the dry months od December and January for both Oyster and Prawn samples while the reverse was the case for the shrimp samples where counts of *Campylobacter* was highest in December in the dry season and lowest in September in the wet season. On the other hand, counts of *Vibrio* in the shrimp samples was in January and lowest in December which are both dry season months.

This indicates the impact of weather and season on the bacterial contamination of the oysters sold in various markets in Port Harcourt. Odekunle (2004) noted that on average the season of rainfall begins in April lasting till October in tropical areas of Nigeria which includes Port Harcourt. The increase in flow of water during rainfall through bushes and various dumpsites carrying waste materials due to erosion or run off due to torrential rain falls which are dumped in major water bodies where these oysters are harvested could have been the reason for the increased bacterial counts throughout the period of study. Garba *et al.* (2021), also reported high microbial loads in processed sea foods sold openly in markets could be associated with the location of the market due to various anthropogenic activities that could result to pollution of rivers and lack of proper drainage system, indiscriminate refuse dumps and lack of proper treatment/adequate processing by smoking of the fish and improper hygienic and poor handling procedures adopted by the smoked fish sellers.

Vibrio species from shrimps underscores the urgent need for robust quality control measures and stringent seafood safety regulations to prevent the transmission of *Vibrio*-associated infections through shrimp consumption. The isolation of these bacterial species underscores the importance of implementing stringent hygiene practices on sea foods production and supply chain to mitigate health risks to consumers. Proper handling will help reduce the incidence of these pathogenic contaminants.

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Campylobacter species, such as *Campylobacter jejuni* and *Campylobacter coli*, pose significant public health risks and are commonly associated with bacterial gastroenteritis. *Campylobacter* species from shrimps signifies potential contamination from animal or environmental sources. Lozano-Leon *et al* (2021) highlighted the prevalence of *Campylobacter* species in shellfish samples. Similar results were obtained as recorded in this study, while the genome sequencing with all isolates particularly *C. lari* were detected during the colder months (February and March) Caron *et al*., 2021). The source of *Campylobacter* species could be attributed to the fact that the coastal environment is a receptacle of agricultural and urban wastewater effluents. Also because of their filter-feeding activities/habits, these seafoods can concentrate and retain pathogenic microbes present in their environment.

In conclusion, this study has shown that, *Vibrio* and *Campylobacter* species burden and magnitude are significantly associated with seafood. The presence and high prevalence rate of *Vibrio* and *Campylobacter* species in the chosen seafood is regarded as a serious public health threat that could endanger consumers and public health. This should raise concerns about public health. The route of spread, alongside other factors like environmental and demographic factors, the non-pathogenic environmental strains (their invasiveness, virulence and pathogenicity characteristics), may reflect the unhygienic measures and unsuitable environmental conditions during processing and handling. This may help in the prediction of future outbreaks of the disease and for the preparation of better prevention and intervention strategies. Therefore it is recommended to establish programs on employee education and training in proper food handling and food protection principles that stress the dangers of poor personal hygiene and unsanitary practices as well as insufficient storage system.

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