Original Research Article

Prevalence of *Vibrio* species in Sea foods and water sources in Cross River State

ABSTRACT

In the coastal areas of the world, most *Vibrio species* have been incriminated as notorious agents causing foodborne, wound and other infections. These pathogens are known to be associated with the consumption of raw or undercooked sea foods or the exposure of wounds to warm seawater.

Aim: Therefore, this research work was designed with the aim of assessing microbiological quality of the waterbodies as well as the sea foods consumed in CRS.

Study Design: The completely randomized block design was used and data analyzed using of two

way analysis of variance, Generalized Linear Model Univariate analysis. Significant means were separated using Least significant difference.

Place and Duration of Study: This study was done in the Department of Microbiology,
University of Cross River State, Calabar, Cross River State, Nigeria, between 2016-2019.

Methodology: we evaluated a variety of sea foods viz; Crayfish, blue Crab, Periwinkle, apples nail, red Lobster etc. collected from major Beaches, markets and other sale points and water sources (rivers streams sea and gutters) in Calabar, Cross River State of Nigeria, using standard bacteriological techniques, for the prevalence of *Vibrio species*.

Results: The mean percentage mean viable cell counts obtained ranged from 1.79±3.45 (sea water)-9.15±4.79CFU/mL (gutter water) and 7.68±7.58 (Blue Crab)- 11.37±4.82 CFU/g (Fish) in the Rainy season. The counts for the Dry season Ranged from 1.79 ±3.42 (Sea Water)-8.94± 4.51(gutter water), and 5.83 7.21 CFU/g (Apple Snail) -12.64 5.95 CFU/g (Fish). The total percentage mean counts obtained were 8.09±6.91 CFU/mL in the Rainy Season to 7.61±6.58 CFU/mL in the dry Season. From the both seasons, the overall total mean count was 11.09±5.94 CFU/ml. From the nine locations evaluated in this study, it was observed that the Mean percentage counts for the Northern Senatorial District ranged from 2.81± 3.49 (Ogoja)- 3.14 ±4.07CFU/mL (Obudu). For the Central the range was from 3.34 ±4.20 (Boki)- 9.89 ±5.15 (Ikom), while for the Southern it was from12.01± 6.52 (Akamkpa)- 14.47 ±5.44 (Calabar). The overall Total percentage mean counts from all the three Senatorial Districts was 14.03±4.86 CFU/mL. From the Northern Senatorial District, the total Percentage mean was 3.01±3.77 CFU/mL, 7.05±5.79 CFU/mL from the Central and 13.49± 5.72 CFU/mL from the Southern Senatorial District. *The Vibrio* pathotypes isolated include *Vibrio cholerae* (both O1 and non-O1 serotypes) 1155 (31.61%), V. *parahaemolyticus***,** 752 (20.58%), *V. fluvialis* 480 (13.14%), *V. vulnificus* 473 (12.94%) *V. mimicus* 400 (10.95%) and Other Vibrios 394 (10.78%). Out of the 3654 Vibrio isolates, the greatest numbrn663±3.31 (18.14%) were from Sea water, while the least 133±.84 (3.64%) were from the Gutter Water. Also, the highest number 1245±2.61 (34.07%) came from Calabar, and the least 102±.65 (2.79%) from Obanlikwu. The Northern Senatorial District had the least number 327 (8.95%), followed by the Central Senatorial District with 570 (15.59%) and then the Southern Senatorial District with 2757 (75.45%) as the highest number of isolates.

Conclusion: The presence of these pathogenic bacterial species in common sea foods in this area is of great public health concern. It is therefore important that serious emphasis be laid on proper cooking of these seafoods as well as the establishment of regular hygiene surveillance strategies in the state.

Key words: *Vibrio species,* Sea foods, water sources, Cross River State, Nigeria

1. INTRODUCTION

Cross River state is naturally blessed with large bodies of water surrounding the state. The inhabitants of this state depend on the sea foods and their products, as well as the surface and sea water for their sources of proteins and daily activities.

Vibrio species have virtually been known for their autochthonous habitation of marine and surface and brackish waters worldwide [1, 2, 3, 4**,** 5, 6]. The spatial distribution of these *Vibrio species* has not been associated with the location and or environment because they have been found to be highly endowed with so many survival strategies and characteristics. These gives them the ability to flourish luxuriantly, irrespective of the location.

Vibrio species have been documented as causative agents of either acute, watery diarrhea (cholera disease), which is a severe life-threatening infection [7] or vibriosis (noncholera disease), which could manifest as a self-limiting gastroenteritis or a severe life-threatening septicemia with necrotizing fasciitis, wound and ear infections [6].

The global occurrence of Vibrio-related ailments has continued to be on the rising side [8, 9], and some of these illnesses are acquired through swimming/bathing in coastal waters [10, 11, 12, 13], consumption of seafoods and vegetable from irrigated farms [14] especially by those inhabiting low hygienic and over populated coastal areas. Infections due to *Vibrio species* are becoming a global public health menace. The species most commonly involved in human infections include; *V. cholerae* and *V. parahaemolyticus* [15, [16].

The presence of these *Vibrio species* in the environmental water bodies is often associated with the improper management of wastes from local communities and rural settlements, leading to the contamination of surface run-off, streams, rivers, wells, ponds and seawater with defecate [17]. These potential pathogens if found to be in the environmental water bodies, render them unfit for home and recreational use. Therefore, this research work was designed with the aim

of assessing microbiological quality of the waterbodies as well as the sea foods consumed in CRS.

2 Materials and Methods

2.1 Study Area: This study was done in Cross River State, Nigeria, between 2016-2019. The State is made up of three Senatorial Districts viz; Northern Senatorial District with screening centers at Ogoja, Obudu, and Obanlikwu. The Central Senatorial District with the centers at Boki, Ikom and Etung. The Southern Senatorial District with centers covering Akamkpa, the Calabar Municipality and Akpabuyo.

2.2 Study Materials: The materials used for the study include samples of seafoods (Blue crab (*Callinectes sapidus)*, Crayfish (*Pacifastacus leniusculus)*, Apple snail (*Pomacea Paludosa)*, red Lobster (*Homarus gammarus)*, Fish) and Periwinkle (*Tympanotonus fuscatus var radula*) and water samples from (the Sea, Streams Rivers and gutters).

2.3 Collection and preparation of Environmental Samples

The crabs, crayfish etc ware collected alive from the harvesting sources and sale points in the area of study in sterile plastic bags. The specimens were washed thoroughly with distilled water to remove sand and other dirt and then the gut removed into a sterile mortar by the use of a sterilized knife. These were macerated to paste in 45 ml of Alkaline Peptone Water (APW+ 1MNaOH pH-8.4) and incubated for 4–8 h, at 37^{o}C before storing in a sterile corked container pending when the sample were to be used.

2.4 Determination of Viable Counts and Isolation of Vibrio Strains

10ml of the macerated sample were aspirated using a sterile pipette into 90 ml of sterile Alkaline Peptone Water (APW+ 1MNaOH pH-8.4) which is enrichment medium. Serial dilutions were carried out on the original sample of the gut homogenate from the initial tube 10^{-1} to 10^{-5} containing 9 ml of alkaline peptone water. The test tubes were agitated vigorously to ensure equal distribution of microbial cells from the gut homogenate. Approximately 0.1 ml aliquot from each test tube was then aseptically sub-cultured onto Thiosulphate Citrate Bile Salt Agar

(TCBS) agar plates in duplicate using the pour plate method. The agar plates were then be incubated at 37^{o}C for 24 h. After incubation, viable counts were determined. The discrete colonies were isolated and sub-cultured twice to obtain pure culture of the stain. The pure isolates were then stored as stock cultures on nutrient agar slants pending on when they were to be used. The growth of yellow /or green colonies were presumed to be that of *V*. *cholerae/or other V. species* [18]

2.5 Identification and characterization of *V. cholerae* Strains Using Conventional Methods and 20 E; BioMerieux, Charbonnieres-Les-Bains, France

The isolates were identified and characterized by cultural, morphological and biochemical or physiological characteristics. Culturally, each isolate was examined for shape, elevation, colour and colony size. Morphologically, each isolate was examined by its Gram's reaction and distilled water motility test. Biochemically, each isolate was identified based on various biochemical tests such as Catalase test, Sugar utilization test, Citrate utilization, Starch hydrolysis test, Hydrogen sulphide production, Motility, Urease and Indole production (using MIU Medium), Salt tolerance test at 0, 3, 6, 8 and 10% concentration. colonies presumptively identified based on cultural and morphological characteristics on the TCBS agar plate.

The presumptively identified *V. cholerae* isolates were then confirmed using the Analytical Profile Index (API 20 E; BioMerieux, Charbonnieres-Les-Bains, France, following the Manufacturer's instructions. About 2 mL of API saline (0.85% NaCl) was inoculated with pure colonies from an 18-24hour culture of a presumptively identified isolate. This was then standardized by comparing with the 0.5 McFarland standard. Then about 56-60 (µL) of the reagents (Arginine dihydrolase (ADH) Lysine decarboxylase (LDH) urea test (UREA) Arabinose fermentation (LARL) Ornithine dehydrogenase etc.) were dispensed using the teat pipettes and smeared with two drops of mineral oil. They were then covered with the lid provided and incubated an atmosphere of oxygen at 35 ^{o}C ± 2^{o}C for 24 h (±2 h). After this, one drop of JAMES reagent was added in the microtube for indole (IND) reaction and the results read using mini-API app (BioMerieux, Charbonnieres-Les-Bains, France) and interpreted using the API identification software (BioMerieux, France).

3. RESULTS

3.1. Seasonal Mean Percentage *Vibrio* Counts Obtained from various Sampled Sources (x10^{10})

From the various environmental sources examined for the presence of *Vibrio species,* the mean percentage counts ranged from 1.79±3.45(sea water)-9.15±4.79CFU/mL (gutter water) and 7.68±7.58 (Blue Crab)- 11.37±4.82 CFU/g (Fish) in the Rainy season. The counts for the Dry season Ranged from 1.79 ±3.42 (Sea Water)-8.94± 4.51(gutter water), and 5.83 7.21 CFU/g (Apple Snail) -12.64 5.95 CFU/g (Fish). The total percentage mean counts obtained were 8.09±6.91 CFU/mL in the Rainy Season to 7.61±6.58 CFU/mL in the dry Season. From the both seasons, the overall total mean count was 11.09±5.94 CFU/ml (Table 1).

When the total percentage mean counts were compared statistically, it was observed that there were significant differences between the different sources examined; F- value of 16.36 at p=.000. No significant differences were observed between the seasons as well as the in the interactions between the seasons and the sources (P>.05).

3.2 Log10 Mean Percentage Vibrio Counts Obtained from Various Locations

From the nine locations evaluated in this study, it was observed that the Mean percentage counts for the Northern Senatorial District ranged from 2.81± 3.49 (Ogoja)- 3.14 ±4.07CFU/mL (Obudu). For the Central the range was from 3.34 ±4.20 (Boki)- 9.89 ±5.15 (Ikom), while for the Southern it was from12.01± 6.52 (Akamkpa)- 14.47 ±5.44 (Calabar). The overall Total percentage mean counts from all the three Senatorial Districts was 14.03±4.86 CFU/mL (Table: 2).

From the Northern Senatorial District, the total Percentage mean was 3.01±3.77 CFU/mL, 7.05±5.79 CFU/mL from the Central and 13.49± 5.72 CFU/mL from the Southern Senatorial District (Table: 2)

Table 1: Seasonal Means of Percentage *Vibrio* Counts Obtained from various Sampled Sources ($x10^{10}$)

Source	Rainy Season Mean	Std. Deviation	Dry Season Mean	Std. Deviation	Total Mean	Total Std. Deviation	N	Total Nx2
Crayfish	11.26	5.93	10.91	6.20	11.09	6.03	36	72
Fish	11.37	4.82	12.64	5.95	12.00	5.41	36	72
River/ Stream Water	7.16	2.45	6.17	1.96	6.67	2.26	36	72
Gutter Water	9.15	4.79	8.94	4.51	9.04	4.62	36	72
Blue Crab	7.68	7.58	6.95	6.92	7.32	7.21	36	72
Periwinkle	9.21	8.71	7.91	7.40	8.56	8.05	36	72
Apple Snail	7.06	8.51	5.83	7.21	6.45	7.86	36	72
Lobsters	8.15	8.29	7.39	7.41	7.76	7.82	36	72
Sea Water	1.79	3.45	1.79	3.42	1.79	3.41	36	72
Total	8.09	6.91	7.61	6.58	7.85	6.74	324	648

Table 2: Log10 Mean Percentage Vibrio Counts Obtained from Various Locations

Location	Senatorial Districts	N	Mean	Std. Deviation	Total mean	Total Std Deviation	Total N
Ogoja	Northern	72	2.81	3.49	3.01	3.77	216
Obudu	Northern	72	3.14	4.07			
Obanlikwu	Northern	72	3.07	3.79			
Boki	Central	72	3.34	4.20	7.05	5.79	216
Ikom	Central	72	9.89	5.15			
Etung	Central	72	7.93	5.88			
Akamkpa	Southern	72	12.01	6.52	13.49	5.72	216
Calabar	Southern	72	14.47	5.44			
Akpabuyo	Southern	72	14.03	4.86			
	Total		14.02	4.86	7.85	6.74	648

3.3 Cumulative Number of Different species of Vibrio Isolated in the Cross River State Environment

A cross-sectional study of the three Senatorial Districts of Cross River State for the presence of *V. species* in the Environment revealed the presence of *V. cholerae* 1155/3654 (31.61%), V. *parahaemolyticus***,** 752 (20.58%), *V. fluvialis* 480 (13.14%), *V. vulnificus* 473 (12.94%) *V. mimicus* 400 (10.95%) and Other Vibrios 394 (10.78%) (Table:3).

A multiple comparison of the percentage means of the Vibrio species showed that the number of *V. fluvialis* isolated had no statistically significant difference from *V. vulnificus* and other

Vibrio species (Sig values of .971 and .631>.05 Respectively). The number of *V. cholerae,* V. *parahaemolyticus***,** and *V. mimicus* were significantly different from each other as well as from *V. fluvialis, V. vulnificus* and other *Vibrio species* (P<.05)

3.4 Overall number of species of Vibrio in the Various Sources Examined

Out of the 3654 Vibrio isolates, 663±3.31 (18.14%) were from Sea water, 642±1.66 (17.57%) from Cray Fish, 460±1.82 (12.59%) from Apple snail, 441±1.81(12.07%) from Periwinkle, 421±1.09(11.52%) from Fish, 406±1.48 (11.11%) from Lobsters, 297±1.53 (8.13%) from Blue crab and the least 133±.84 (3.64%) from Gutter Water (Table:4)

3.4.1 Distribution of Different species of Vibrio in the Various Sources Examined

The most abundant isolate was the *V. cholerae* with mean percentage abundance of 43.23 ±35.79% (River/Stream water), 41.27±19.91% (Cray fish), 36.10±40.83% (Gutter water), 32.65±18.71% (Fish), 17.97±22.97% (Periwinkle), 17.68±29.97% (Blue crab), 18.21±20.94 (Lobsters), 15.65±21.85% (Apple snail), 5.79±11.74% (sea water).

V. parahaemolyticus showed its highest percentage mean count of 23.01±14.84% in Fish, 21.78±14.53% (Cray fish), 14.46±23.77% (River/Stream water), and the least 3.31±6.60% (sea water).

V. vulnificus showed 11.92±15.17% (Cray fish), 11.69±13.79% (Fish), 9.76±17.00% (River/Stream water), 2.80 ±5.645% (sea water).

V. fluvialis was 11.22±14.88% in Fish, 9.85±14.42% (Cray fish), 8.55±19.76% (River/Stream water), 806±16.98 (Periwinkle), 7.78± 21.69% (Blue crab), 7.15±10.92% (Lobsters), 5.68±13.46 (Apple snail), 3.59±7.46 (sea water).

V. mimicus were 8.61±11.19% (Cray Fish), 8.52±12.68% (Fish), 6.61±10.46% (Lobsters), 5.25±12.80% (Apple snail), 4.78±7.59% (Periwinkle), 4.09±12.87% (Blue crab), 1.77±6.22% (River/Stream water), 0.69±5.89 (Gutter water) as the lowest.

The proportion of other Vibrios were as follows: 18.53±32.73% (Gutter water), 9.200±14.87% (Cray Fish), 9.09±20.62% (River/Stream water), 6.26±10.87% (Fish), 512±14.24% (Periwinkle), and the least proportion were from the blue crab (1.78±5.759%) (Table:5)

3.4.2 Total percentage mean of Vibrio species from each Source Examined

Looking at the mean percentages obtained for each species of Vibrio from the sources examined, it was observed that the Cray fish sources were the most contaminated sources with a total percentage mean abundance of 17.11%, followed by Fish sources with 15.56, River/Stream Water 14.48, Gutter water 11.13, Lobsters 9.39%, Periwinkle 9.05%, Blue Crab 8.37%, Apple snail 7.82%, and Sea Water 3.69% (Fig:1)

Statistically, there were significant differences observed between the sources examined, the species of Vibrio isolated and in the interactions between the sources and the *Vibrio species* ($P<.05$).

A multiple comparison of the isolates from the various sources showed that the isolates from the Cray fish were not significantly different from those from Fish (Sig-value .19). Those from Fish were not also significantly different from those isolated from River/Stream Water (Sig-value .354). The same observations were seen when the isolates from Gutter Water were compared with those from Periwinkle and Lobster, and Blue Crab with Periwinkle, Apple snail and Lobster (Sig values .56, .64 and .38 respectively), as well as when Lobster sources were compared with Gutter Water, Blue Crab, Periwinkle, Apple snail (Sig values .14, .38, .78 and .18respectively). The above values were all greater than the P-value of .05

Table 3: Cumulative Number of Different species of Vibrio Isolated in the Cross River State Environment

Vibrio species Isolated	Mean	N	Std. Deviation	Sum
V. parahaemolyticus	1.16	648	1.818	752
V. mimicus	.62	648	1.413	400
V. vulnificus	.73	648	1.376	473
V. fluvialis	.74	648	1.552	480
Other Vibrios	.61	648	1.491	394
V. cholerae	1.78	648	2.487	1155
Total	.94	3888	1.781	3654

Table: 4 Overall Number of Different Vibrio species from Various Source

Source	Mean	N	Std. Deviation	Sum
Cray Fish	1.49	432	1.655	642
Fish	.97	432	1.091	421
River/Stream Water	.44	432	.759	191
Gutter Water	.31	432	.837	133
Blue Crab	.69	432	1.533	297
Periwinkle	1.02	432	1.805	441
Apple Snail	1.06	432	1.818	460
Lobsters	.94	432	1.476	406
Sea Water	1.53	432	3.307	663
Total	.94	3888	1.781	3654

Table: 5a Distribution of Different species of Vibrio in the Various Sources

Source	Vibrio species	Mean	Std. Deviation
Cray Fish	*V. parahaemolyticus*	21.78	14.53
	V. mimicus	8.61	11.19
	V. vulnificus	11.92	15.17
	V. fluvialis	9.85	14.42
	Other Vibrios	9.20	14.87
	V. cholerae	41.27	19.91
Fish	*V. parahaemolyticus*	23.01	14.85
	V. mimicus	8.52	12.68
	V. vulnificus	11.69	13.79
	V. fluvialis	11.23	14.89
	Other Vibrios	6.26	10.87
	V. cholerae	32.65	18.71
River/Stream	*V. parahaemolyticus*	14.47	23.78
Water	*V. mimicus*	1.77	6.22
	V. vulnificus	9.76	17.00
	V. fluvialis	8.55	19.76
	Other Vibrios	9.09	20.62
	V. cholerae	43.23	35.79
Gutter Water	*V. parahaemolyticus*	4.29	15.06
	V. mimicus	.69	5.89
	V. vulnificus	3.05	13.69
	V. fluvialis	3.91	14.38
	Other Vibrios	18.73	32.73
	V. cholerae	36.10	40.83
Blue Crab	*V. parahaemolyticus*	12.45	25.56
	V. mimicus	4.09	12.87
	V. vulnificus	6.44	17.75
	V. fluvialis	7.78	21.69
	Other Vibrios	1.78	5.76
	V. cholerae	17.68	29.97
Periwinkle	*V. parahaemolyticus*	11.74	16.96
	V. mimicus	4.78	7.59
	V. vulnificus	6.67	14.10
	V. fluvialis	8.06	16.98
	Other Vibrios	5.12	14.24
	V. cholerae	17.96	22.96
	Total	9.05	16.69

Table: 5b Distribution of Different species of Vibrio in the Various Sources Continued

Source	Vibrio species	Mean	Std. Deviation
Apple Snail	*V. parahaemolyticus*	10.57	15.02
	V. mimicus	5.25	12.80
	V. vulnificus	6.47	13.49
	V. fluvialis	5.68	13.46
	Other Vibrios	3.27	5.71
	V. cholerae	15.65	21.85
Lobsters	*V. parahaemolyticus*	13.42	17.03
	V. mimicus	6.61	10.46
	V. vulnificus	6.70	9.57
	V. fluvialis	7.15	10.92
	Other Vibrios	4.23	8.70
	V. cholerae	18.21	20.94
Sea Water	*V. parahaemolyticus*	3.31	6.60
	V. mimicus	2.91	6.45
	V. vulnificus	2.80	5.65
	V. fluvialis	3.59	7.46
	Other Vibrios	3.71	8.12
	V. cholerae	5.79	11.74
Total	*V. parahaemolyticus*	12.78	18.40
	V. mimicus	4.80	10.27
	V. vulnificus	7.28	14.07
	V. fluvialis	7.31	15.52
	Other Vibrios	6.82	16.43
	V. cholerae	25.39	28.83
	Total	10.73	19.47

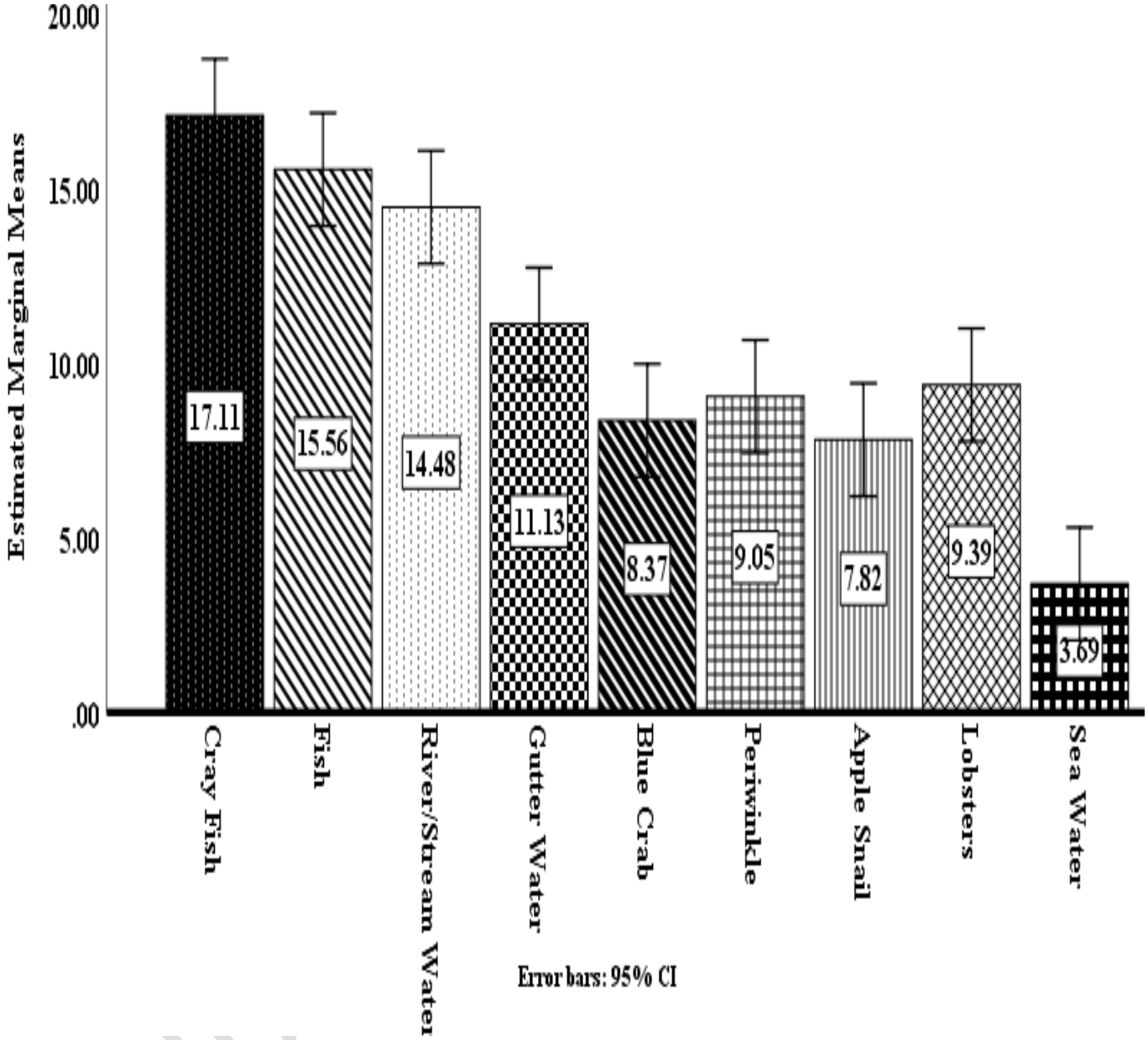

Figure 1: Total percentage mean of *Vibrio species* from each Source Examined

3.5 Cumulative Number of species of Vibrio in the various Locations Examined

Out of the 3654 isolates, 1245±2.61 (34.07%) were from Calabar, 1104±2.76 (30.21%) from Akpabuyo, 408±1.33 (11.17%) from Akamkpa, 269±.98 (7.36%) from Ikom, 189±.87 (5.17%) from Etung, 113±.76 (3.09%) from Obudu, 112±.781 (3.07%) from Boki, 112±.68 (3.07%) from Ogoja and the least 102±.65 (2.79%) from Obanlikwu (Table:6).

Looking at the total mean percentages obtained for each species of Vibrio from the Locations examined, it was observed that the Akpabuyo was the most contaminated Location with a total percentage mean abundance of 16.69±16.99%, followed by Calabar with 16.49±14.10%, then by Ikom with 14.69±24.86%, Akamkpa 14.39±20.73%, Etung 11.03±23.37%, Ogoja 5.94±17.51%, Obudu 5.79±16.11%, Boki 5.78±18.17%, and lastly by Obanlikwu, with 5.76±16.1% (Table:6)

3.5.1 Distribution of Different species of Vibrio in the Various Locations Examined

According to the locations examined, Ogoja had *V. cholerae as* the most abundant isolate with a mean percentage abundance of 19.82%, followed by *V. parahaemolyticus* with 5.57, *V. vulnificus* 3.59%, *V. fluvialis* 2.82%, *V. mimicus* 2.04%, Other Vibrios 1.8%.

Obudu had *V. cholerae as* the most abundant isolate with a mean percentage abundance of 17.06%, followed by *V. parahaemolyticus* with 4.51%, Other Vibrios 4.45%, *V. fluvialis* 4.35, *V. vulnificus* 2.67%, *V. mimicus* 1.69%.

Obanlikwu had *V. cholerae* 15.46%, Other Vibrios 5.81%, *V. parahaemolyticus* 5.06%, *V. vulnificus* 3.65%, *V. fluvialis* 3.27%, *V. mimicus* 1.33%.

In Boki *V. cholerae* was 20.65%, Other Vibrios 5.95%, *V. parahaemolyticus* 3.09%, *V. fluvialis* 2.82%, *V. vulnificus* 1.91%, *V. mimicus* 0.78%.

From Ikom *V. cholerae* was 36.96%, *V. parahaemolyticus* 18.52%, *V. fluvialis* 10.69%, Other Vibrios 8.47%, *V. vulnificus* 7.33%, and *V. mimicus,* 6.21%.

Etung showed a mean percentage abundance of 26.22% for *V. cholerae, V. parahaemolyticus* 17.08% *V. fluvialis* 9.58%, *V. vulnificus* 7.48%, *V. mimicus* 3.53% and Other Vibrios 2.28%.

The order from Akamkpa was as follows: 33.39% for *V. cholerae, V. parahaemolyticus* 19.25% *V. vulnificus* 11.59%, *fluvialis* 9.15%, Other Vibrios 6.54% and *V. mimicus* 6.45%.

From Calabar, *V. cholerae* was 31.25%, *V. parahaemolyticus* 19.76%, *V. fluvialis* 13.21%, *V. vulnificus* 12.28%, Other Vibrios 11.99% and *V. mimicus* 10.46%.

The location Akpabuyo had 27.72% for *V. cholerae, V. parahaemolyticus* 22.21% *V. vulnificus* 14.99%, Other Vibrios 14.07%, *V. mimicus* 10.74% and *fluvialis* 10.41% (Fig:2)

Statistically, there were significant differences observed between the Locations examined, the species of Vibrio isolated and in the interactions between the Locations and the *Vibrio species* (Sig values were .00 respectively (P<.05).

When the isolates from Ogoja, Obudu, Obanlikwu and Boki were compared, there were no statistically significant differences among them. This was also the case with those from Akamkpa, Calabar and Akpabuyo (P>.05). Statistically significant differences were observed among the rest of the locations examined (P<.05).

3.6 Seasonal Distribution of species of Vibrio in the Environment

A total sum of 3654 Vibrios were isolated, and out of this multitude, 1882±1.83 were in the Rainy Season, while 1772±1.73 were in the Dry Season (Table :7).

The mean percentage occurrence showed that 24.45%, (*V. cholerae*), 12.62%, (*V. parahaemolyticus*), 7.61% (Other Vibrios), 6.87% (*V. vulnificus)*, 6,85% (*V. fluvialis)*, and 5.04% *(V. mimicus),* were isolated in the Rainy season. While 26.34%, (*V. cholerae*), 12.95%, (*V. parahaemolyticus*), 7.77% (*V. vulnificus)*, 7.68% (*V. fluvialis)*, 6.03% (Other Vibrios), and 4.56% *(V. mimicus),* were isolated in the Dry season (Fig:3).

Statistically, Significant differences were observed between the *Vibrio species* isolated in both the rainy and dry seasons (P=.00<.05), but no significant differences were observed between the *Vibrio species* isolated during the rainy and dry seasons (Sig.-value .59). Same was seen in the interaction between the seasons and the *Vibrio species* (Sig.-value .61) (P>.05).

3.7 Overall Number of Different species of Vibrio from the Various Senatorial Districts.

The distribution of the *Vibrio species* according to the Senatorial Districts in Cross River state was such that the Northern Senatorial District had the least number 327 out of 3654 (8.95%), followed by the Central Senatorial District with 570 (15.59%) and then the Southern Senatorial District with 2757 (75.45%) (Table:8).

The mean percentage occurrence showed that 17.45%, (*V. cholerae*), 5.05%, (*V. parahaemolyticus*), 4.02% (Other Vibrios), 3.48% (*V. fluvialis)*, 3.61% (*V. vulnificus)*, and 1.68% *(V. mimicus),* were isolated in the Northern Senatorial District. While 27.94%, (*V. cholerae*),

12.89%, (*V. parahaemolyticus*), 7.53% (*V. fluvialis)*, 5.57% (*V. vulnificus* and (Other Vibrios), and 3.51% *(V. mimicus),* were isolated in the Central Senatorial District. From the Southern Senatorial District, 30.79%, (*V. cholerae*), 20.41%, (*V. parahaemolyticus*), 12.95% (Other Vibrios), 10.92% (*V. fluvialis)*, 10.87 % (*V. vulnificus)*, and 9.22% *(V. mimicus),* were isolated (Fig:4).

Statistically, Significant differences were observed between the *Vibrio species* isolated in Senatorial Districts (P=.00<.05), Same was seen in the interaction between the Senatorial Districts and the *Vibrio species* (P<.05).

A multiple comparison of the different Senatorial Districts revealed that there were statistically significant differences observed between the North and the Central, the north and the Southern Senatorial District. When the Central was compared to the Southern, the same trend was observed at sig. values of .000<P=.05.

Table:6 Total Number of Different Vibrio species from various Locations

Location	Sum	Mean	Std. Deviation	% Sum	%Mean	%Std. Deviation	N
Ogoja	112	.26	.683	2566.67	5.94	17.15	432
Obudu	113	.26	.761	2500.00	5.79	16.11	432
Obanlikwu	102	.24	.650	2489.05	5.76	16.10	432
Boki	112	.26	.781	2498.05	5.78	18.17	432
Ikom	269	.62	.982	6550.00	14.69	24.86	432
Etung	189	.44	.865	4764.23	11.03	23.37	432
Akamkpa	408	.94	1.330	6219.74	14.39	20.73	432
Calabar	1245	2.88	2.611	7124.74	16.49	14.10	432
Akpabuyo	1104	2.56	2.758	7256.24	16.69	16.99	432
Total	3654	.94	1.781	41968.71	10.7310	19.47	3888

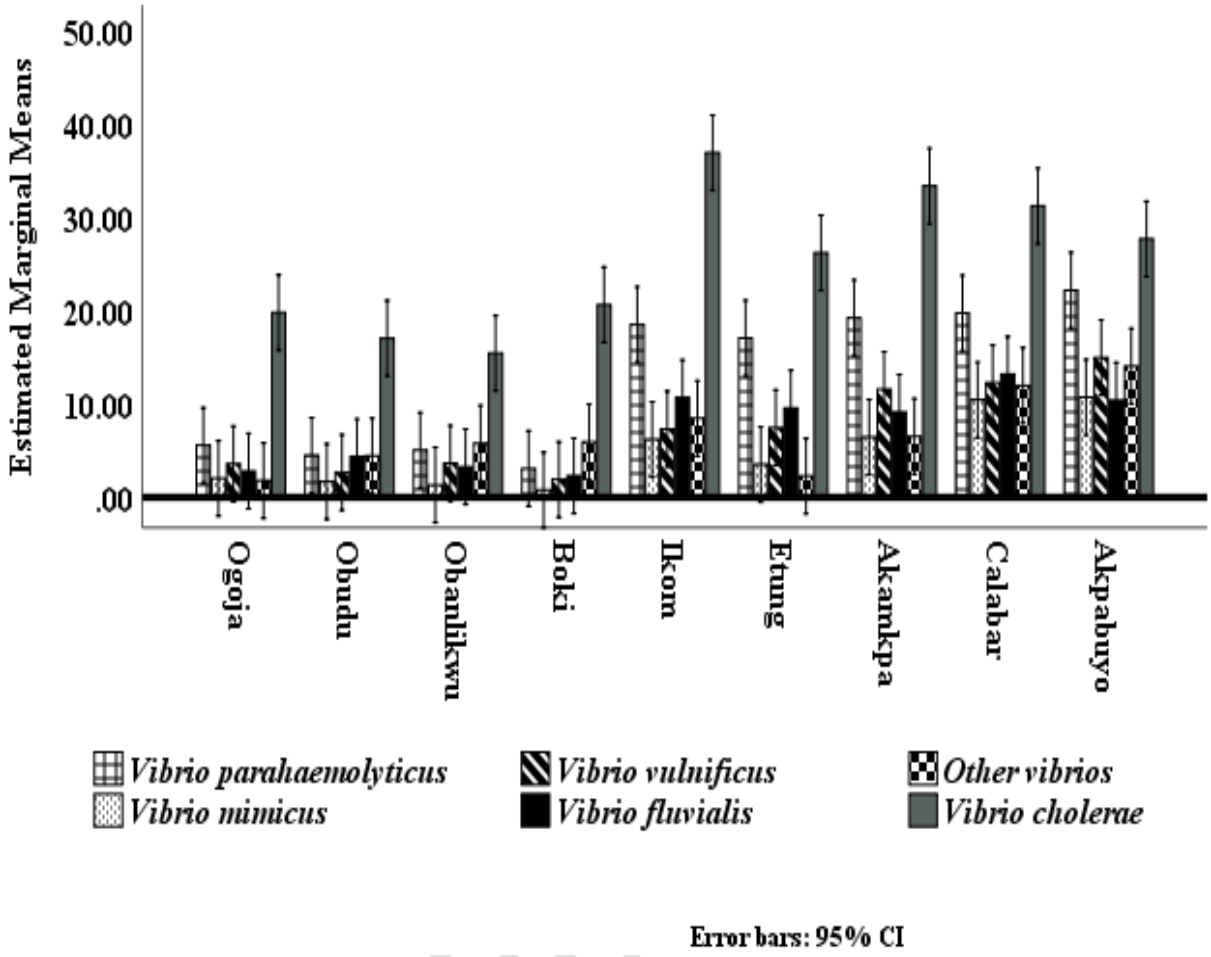

Fig:2 Distribution of Different species of Vibrio in the Various Locations Examined

Table:7 Number of Different Vibrio species from Various Seasons

Season	Mean	N	Std. Deviation	Sum
Rainy Season	.97	1944	1.833	1882
Dry Season	.91	1944	1.729	1772
Total	.94	3888	1.781	3654

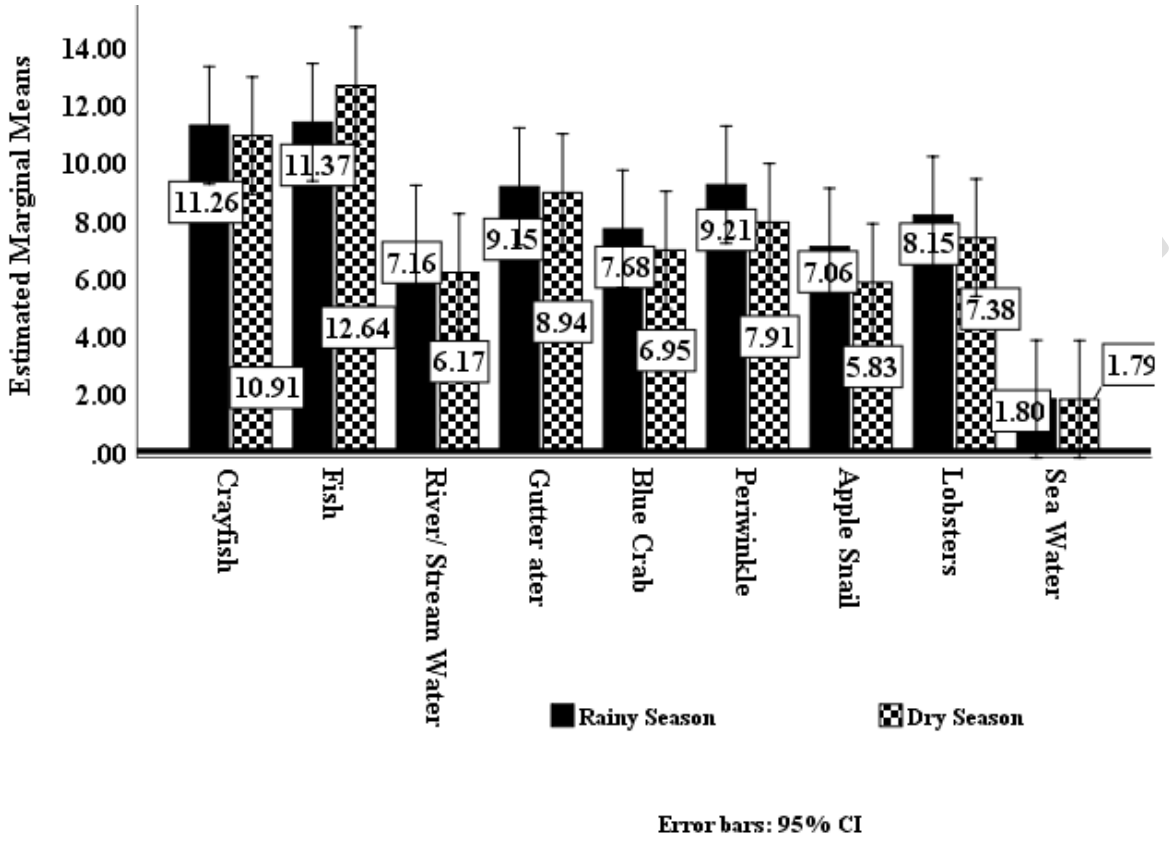

Figure 3: Mean Percentage Seasonal Distribution of species of Vibrio in the Environment

Table:8 Number of Different Vibrio species from Various Senatorial Districts

Senatorial Districts	Mean	N	Std. Deviation	Sum
Northern Senatorial District	.25	1296	.699	327
Central Senatorial District	.44	1296	.892	570
Southern Senatorial District	2.13	1296	2.471	2757
Total	.94	3888	1.781	3654

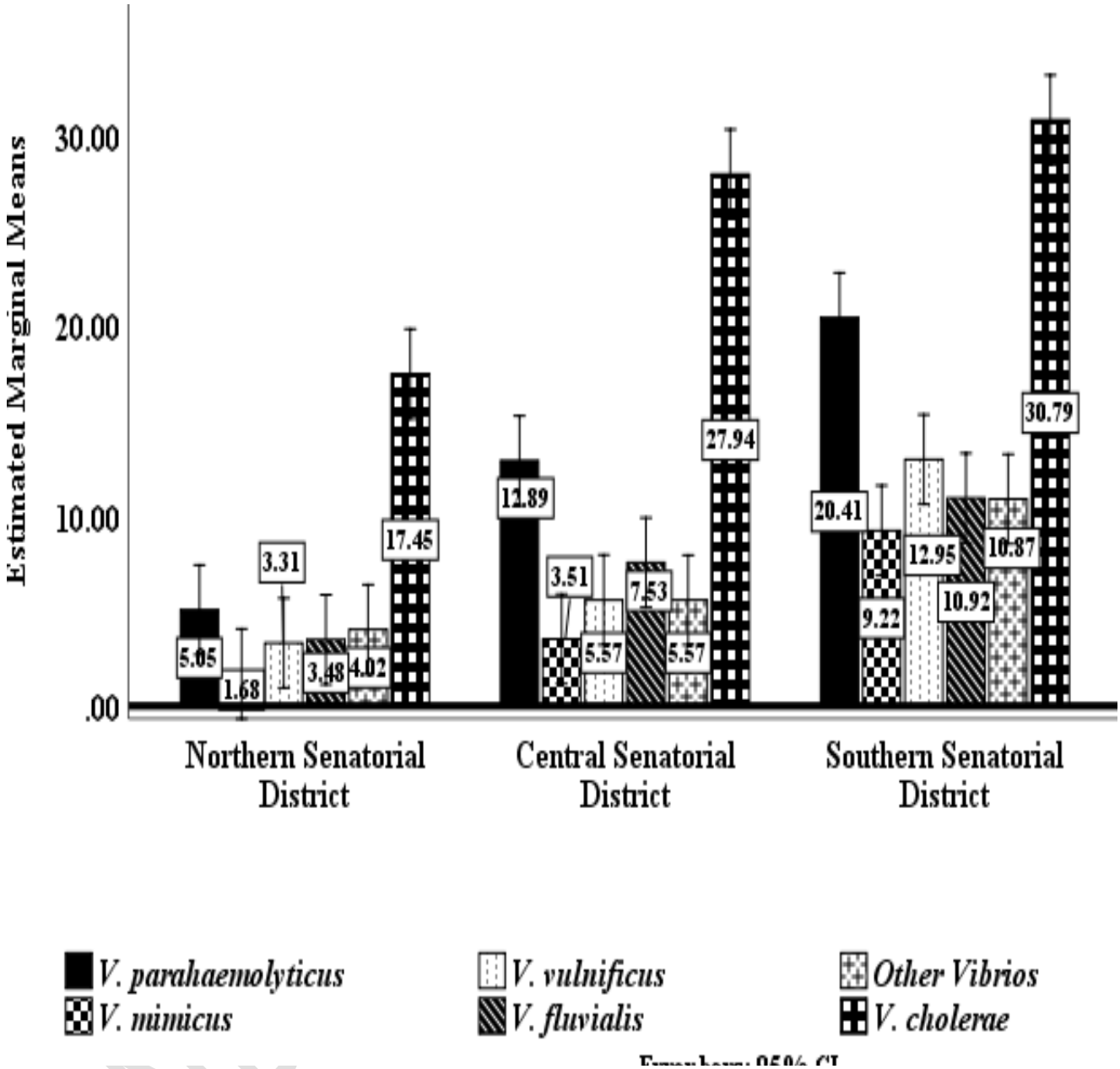

Figure 4: Mean Percentage Occurrence of *V. species* from the Various Senatorial Districts

4. DISCUSSION

A cross sectional study of the Cross River State Environment, conducted between 2017-2019, showed that from the various environmental sources examined for the presence of *Vibrio species,* the total percentage mean counts ($\times 10^{10}$) obtained ranged from 8.09±6.91 CFU/mL in the Rainy Season to 7.61±6.58 CFU/mL in the dry Season. The least percentage total mean counts obtained were from sea water, followed by apple snail, then river/stream water etc.

When the total percentage mean counts were compared statistically, it was observed that there were significant differences between the counts from the different sources examined; F-value of 16.36 at p=.000. No significant differences were observed between the counts from rainy and dry seasons as well as the in the interactions between the seasons and the sources (P>.05).

The total percentage mean counts from the seasons were in corroboration with the results obtained by Eyisi *et al*. [19], from the Calabar Estuary, though the counts in this study were much higher.

The seafoods and water samples evaluated in this study were heavily infested with *Vibrio* species. Since the marine, fresh and brackish waters have been marked as the natural habitats for these organisms, they therefore, serve as natural sources of shellfish contamination. Moreover, these seafoods feed from planktonic species and contaminated faeces which sometimes is defecated directly into these bodies of water by the population living around them. All these avenues together with some of the surface wash offs from human activities, running into the rivers and seas, could serve as the direct contributors to contamination of the water sources themselves and indirectly, the seafoods which live in them. The more the effects of such activities are on a particular location, the more the contamination of the sources, hence accounting for the differences observed in the *Vibrio* counts obtained in this study.

It was also observed that the sea water had the lowest total mean percentage counts of the Vibrio species. This could be justified by the fact that only two locations in this study (Calabar and Akpabuyo had sea water sources. However, the percentage mean counts per location showed that out of the 3654 Vibrio isolates, 663±3.31 (18.14%) were from Sea water, 642±1.66

(17.57%) from Cray Fish, 460±1.82 (12.59%) from Apple snail, 441±1.81(12.07%) from Periwinkle, 421±1.09 (11.52%) from Fish, 406±1.48 (11.11%) from Lobsters, 297±1.53 (8.13%) from Blue crab and the least 133±.84 (3.64%) from Gutter Water. This showed that sea water, although from two locations only, still had the greatest number of *vibrio species*; being the natural habitat of these species.

This study also revealed the presence of some known pathogenic strains of vibrio, namely; *V. cholerae* and *V. parahaemolyticus vulnificus*, *fluvialis* and *mimicus.* In this study, a total sum of 3654 Vibrio organisms were isolated, and out of this multitude, 1882±1.83 were in the Rainy Season, while 1772±1.73 were in the Dry Season from different seafood and water sources.

The mean percentage occurrence showed that 24.45%, (*V. cholerae*), 12.62%, (*V. parahaemolyticus*), 7.61% (Other Vibrios), 6.87% (*V. vulnificus)*, 6,85% (*V. fluvialis)*, and 5.04% *(V. mimicus),* were isolated in the Rainy season. While 26.34%, (*V. cholerae*), 12.95%, (*V. parahaemolyticus*), 7.77% (*V. vulnificus)*, 7.68% (*V. fluvialis)*, 6.03% (Other Vibrios), and 4.56% *(V. mimicus),* were isolated in the Dry season.

According to current information from research data, the global incidence of Vibrio-associated ailments has continued to be on the rising side ([8, 9). And since the bacteria (*Vibrio species*) have virtually been known for their autochthonous habitation of marine and surface and brackish waters worldwide [12, 3, 4, 5, 6), some of these illnesses are acquired through swimming/bathing in coastal waters [10, 11, 12; 13), consumption of seafoods and vegetable from irrigated farms [20]

Thus, the isolation of the above-named pathogenic *Vibrio* species from the sea-water, surface water

and shellfish from CRS environment is a serious public and environmental health challenge. This is because the inhabitants of this state depend on the sea foods and their products as well as the surface and sea water for their sources of proteins and daily activities. Some of these sea foods are eaten uncooked at the point of harvest by the fisher men, young and new born babies are even submerged into these bodies of water as a tradition and custom of some of these people while swimming in theses rivers is a hubby and the only means by which some off the population can take their bath.

Two categories of infection by these Vibrio species have been documented; acute, watery diarrhea (cholera disease), which is a severe life-threatening infection [7] and vibriosis (noncholera disease), which could manifest as a self-limiting gastroenteritis or a severe life-threatening septicemia with necrotizing fasciitis, wound and ear infections (6). The species most commonly involved in human infections include; *V. cholerae* and *V. parahaemolyticus* ([15, 16]. *Vibrio parahaemolyticus* is responsible for acute diarrheal illness and Gastroenteritis in humans and ranks next to *Vibrio cholerae* in incidence [21]. Infections with *V. cholerae* non-O1 or *V. parahaemolyticus* have most often been associated with or linked to a history of seafood consumption and the most common manifestation of the *V. parahaemolyticus* gastroenteritis is bloody and mucus stools [22].

However, some *tdh* and *trh* or *ctxAB*, *zot*, *flrA*, and *vpsR* virulence genes have been identified in strains of *vulnificus*, *fluvialis* and *mimicus*, etc and these have now been ranked among the clinically relevant re-emerging Vibrio pathogens of humans [23, 24, 5], causing gastroenteritis. Although *V. mimicus* to a certain extent has been shown to have some similarity to *V. cholerae* [25], there have only been a global record of high morbidity and mortality due to infections with *Vibrio parahaemolyticus* and *Vibrio vulnificus* [26]. However, *V. cholerae* O1 and *V. parahaemolyticus* serotype O3:K6 have been noted for their formidable pathogenicity and significant ability to cause bacterial pandemics [27, 28, 29]. *Vibrio vulnificus* also has been incriminated in wound infections, while epidemic cholera is associated with *V. cholerae* [19].

The prevailing species in this study have also been implicated in shrimp and sea-food pathogens being able to cause enteric, systemic or external ear infections [30, 31, 32, 33].

The presence of these *Vibrio species* in the environmental water bodies is often associated with the improper management of wastes from local communities and rural settlements, leading to the contamination of surface run-off, streams, rivers, wells, ponds and seawater with defecate [17]. These potential pathogens in the environmental water bodies render them unfit for home and recreational use. There is therefore, a need to assess and treat these wastes and waterbodies for microbial pathogens and improve the quality of water [34].

It was observed that the Cray fish were the most contaminated sources with a total percentage mean abundance of 17.11%, followed by Fish sources with 15.56, River/Stream water 14.48,

Gutter water 11.13%, Lobsters 9.39%, Periwinkle 9.05%, Blue Crab 8.37%, Apple snail 7.82%, and Sea Water 3.69%.

The results of this study showed that *V. cholerae* and *V. parahaemolyticus* were the most abundant species isolated in all the locations examined in Cross River State. This is in agreement with [35, 19] who also evaluated the Cross River estuary and isolated *Vibrio cholerae* and *V. parahaemolyticus.* They also noted that the shellfish (crayfish and lobster) harvested from waters of the estuary were heavily contaminated with *Vibrio* species just like we observed in this study.

Arab *et al*. [36], evaluated farmed fishes and isolated the following strains; *V. alginolyticus* (48%), *V. cholerae* (36%), *V. fluvialis* (12%), and *V. hollisae* (4%). Also, in accordance with our study, 64 (67%) *V. cholerae*, 30 (31%) for *V. alginolyticus*, and 2 (2%) for *V. parahaemolyticus* strains were detected in treated wastewater, soil and groundwater by [4]. *V. parahaemolyticus* have also been proven to be abundant in fish [37], bivalves [38], wastewater 39, 40], seawater samples ([20], river water [41]. Saad *et al*. [42], also reported the presence of *V. parahaemolyticus*, *V. vulnificus*, *V. fluvialis*, *V. mimicus*, *V. alginolyticus*, and *V. damsel* in marine, fresh and farm water fish, n farm water fish.

The locations found in this study to be contaminated with these pathogens were as follows:

Akpabuyo was the most contaminated location with a total percentage mean abundance of 16.69±16.99%, followed by Calabar with 16.49±14.10%, then by Ikom with 14.69±24.86%, Akamkpa 14.39±20.73%, Etung 11.03±23.37%, Ogoja 5.94±17.51%, Obudu 5.79±16.11%, Boki 5.78±18.17%, and lastly by Obanlikwu, with 5.76±16.1%. The incidence of *Vibrio* was higher in the Southern Senatorial District than in the Central and Northern Senatorial Districts.

The recovery of *Vibrio* spp. was also affected by the seasonal changes as observed in the study. The differences in distribution of the species from different locations and in the two seasons were statistically significant ($p<0.05$) and the mean percentage distribution of each species varied with locations and season.

It is also worthy to note that Cross River State is situated along the Atlantic coastline of West Africa and Calabar is headquarters with a tropical monsoon climate. It has a lengthy wet season spamming up to ten months and a short dry season covering the remaining two months. The

harmattan, which significantly influences weather in West Africa, is noticeably less pronounced in the city. Temperature is relatively constant throughout the year, with average temperatures of about 25 to 28 degree Celsius. It is a well-known fact that high temperatures above 18°C, and lower salt concentrations below 25% favor the growth of the human pathogenic vibrios [43, 5]. Even though the optimum growth temperature for *V. vulnificus*, *V. cholerae,* and *V. parahaemolyticus* has been shown to be at 42°C [44], a temperature which can affect the recovery of stressed cells [45], *V. parahaemolyticus* can still grow at temperature range between 37°C and 41.5°C. This may explain the abundance of the major human pathogenic *Vibrio* spp. isolated in this study.

The city of Calabar's economy is based on tourism and it is described as the tourism capital of Nigeria. These places are characterized by greater levels of anthropogenic contamination of the environment and seawater which is not found in the other locations. The contributing factors include the following: Rural to urban migration, the overcrowding, poor accommodation and social facilities and sewage disposal systems, nearness to source of seafoods etc. This high concentration of wastes is washed into the water environment from the surrounding polluted areas. It is natural that even the non-pathogenic species that habit the estuarine muddy environments could have been let loose by the rough river and water currents. This environment therefore, is suitable for the proliferation of *Vibrio species*. Arab *et al*. [36] detected, the largest numbers (n=28) of Vibrio strains during the summer and principally in August from the fishes.

5. CONCLUSION

In this study, a comprehensive epidemiological picture of the three senatorial districts of the Cross River state environment has been presented. Here, potential human pathogenic vibrio species like *V. cholerae* O1 and Non O1, V. *parahaemolyticus, vulnificus*, *fluvialis* and *mimicus* have been identified as major contaminants of the sea foods and water sources in the environment. The cray fish sources, carried the highest percentage, while blue crab carried the least percentage. Also, among the three water sources evaluated, the sea water sources were the most contaminated, while the gutters yielded the least percentage. Cumulatively, the

percentage abundance by location in decreasing order was as follows; Calabar, Akpabuyo Akamkpa, Ikom, Etung, Boki, Obudu, Ogoja, and Obanlikwu.

None of the three Senatorial Districts was free of the contaminating bacterium of interest and the bacteria were isolated both in the rainy and dry seasons of the year, indicating that infection can occur at any time of the year. This therefore, suggest that there exists a probable role of these variant strains in the development of Virulent toxigenic strains of *V. cholerae* in CRS. This result is of public health significance because, it will serve as a guide and provocatory stimulus towards the development of novel surveillance as well as, prevention and control strategies, that will help to curb the disease in case there is an eventual outbreak of cholera in the state.

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