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 seafoods or the exposure of wounds to warm seawater.

Aim: Therefore, this research work was designed with the aim of assessing *the* microbiological quality of the water bodies as well as the seafoods consumed in Cross River State (CRS).

Study Design: The Study was designed using the completely randomized block design and the data was analyzed using of two-way analysis of variance, Generalized Linear Model Univariate analysis. Significant means were separated using the Least significant difference (LSD).

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

Methodology: we evaluated a variety of seafoods viz; crayfish, blue crabs, Periwinkles, apple nails, red lobsters etc. collected from major Beaches, markets and other sale points and water sources (rivers streams sea and gutters) in Calabar, CRS 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 (seawater)-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 (Seawater)-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 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 (NSD) ranged from 2.81± 3.49 (Ogoja)- 3.14 ±4.07CFU/mL (Obudu). For the Central (CSD) the range was from 3.34 ±4.20 (Boki)- 9.89 ±5.15 (Ikom), while for the Southern (SSD) 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 (V. cholerae)* (both O1 and non-O1 serotypes) 1155 (31.61%), *Vibrio parahaemolyticus* (V. *parahaemolyticus)*, 752 (20.58%), *Vibrio fluvialis (V. fluvialis)* 480 (13.14%), *V. vulnificus* 473 (12.94%) *Vibrio mimicus (V. mimicus)* 400 (10.95%) and Other Vibrios 394 (10.78%). Out of the 3654 Vibrio isolates, the greatest number 663±3.31 (18.14%) were from Seawater, 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 NSD had the least number 327 (8.95%), followed by the CSD with 570 (15.59%) and then the SSD with 2757 (75.45%) as the highest number of isolates.

Conclusion: The presence of these pathogenic bacterial species in common seafoods 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.

Keywords: *Vibrio species,* Seafoods, water sources, Cross River State, Nigeria

1. INTRODUCTION

Cross River state (CRS), is naturally blessed with large bodies of water surrounding the state. The inhabitants of this state depend on the seafoods and their products, as well as the surface and seawater 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. This 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 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 vegetables 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; *Vibrio cholerae* (*V. cholerae)* and Vibrio *parahaemolyticus* (*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 the microbiological quality of the water bodies as well as the seafoods consumed in CRS.

2 Materials and Methods

2.1 Study Area: This study was done in CRS, Nigeria, between 2016-2019. The State is made up of three Senatorial Districts viz; Northern Senatorial District (NSD) with screening centers at Ogoja, Obudu, and Obanlikwu. The Central Senatorial District (CSD) with the centers at Boki, Ikom and Etung. The Southern Senatorial District (SSD) 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 samples were prepared according to the method described by Dixit *et al*. [18], with slight modifications. 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 was 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 samples were to be used.

2.4 Determination of Viable Counts and Isolation of Vibrio Strains

About 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 an 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 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 cultures of the strain. The pure isolates were then stored as stock cultures on nutrient agar slants pending when they were to be used. The growth of yellow /or green colonies were presumed to be those 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 were read using a 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(seawater)-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 (Seawater)-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).

Statistically, significant differences were observed between the different sources; F- value of 16.36 at p=.000, but none were observed between the seasons as well as 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 NSD ranged from 2.81± 3.49 (Ogoja)- 3.14 ±4.07CFU/mL (Obudu). For the CSD, the range was from 3.34 ±4.20 (Boki)- 9.89 ±5.15 (Ikom), while for the SSD, 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 NSD, the total Percentage mean was 3.01±3.77 CFU/mL, 7.05±5.79 CFU/mL from the CSD and 13.49± 5.72 CFU/mL from the SSD (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
Seawater	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 CRS 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).

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 Seawater, 642±1.66 (17.57%) from Crayfish, 460±1.82 (12.59%) from pple 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

V. cholerae (43.23 ±35.79% from River/Stream water), 41.27±19.91% (Crayfish), 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% (seawater).

V. parahaemolyticus had a mean count of 23.01±14.84% in Fish, 21.78±14.53% (Crayfish), 14.46±23.77% (River/Stream water), and the least 3.31±6.60% (seawater).

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

V. fluvialis was 11.22±14.88% in Fish, 9.85±14.42% (Crayfish), 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 (seawater).

V. mimicus were 8.61±11.19% (Crayfish), 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.

Other Vibrios were 18.53±32.73% (Gutter water), 9.200±14.87% (Crayfish), 9.09±20.62% (River/Stream water), 6.26±10.87% (Fish), 512±14.24% (Periwinkle), and the blue crab (1.78±5.759%) (Table:5)

3.4.2 Total percentage mean of Vibrio species from each Source Examined

Crayfish sources were the most contaminated sources with a total percentage mean abundance of 17.11%, Fish 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 Seawater 3.69% (Fig:1)

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

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
Crayfish	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
Seawater	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
Crayfish	*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
Seawater	*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

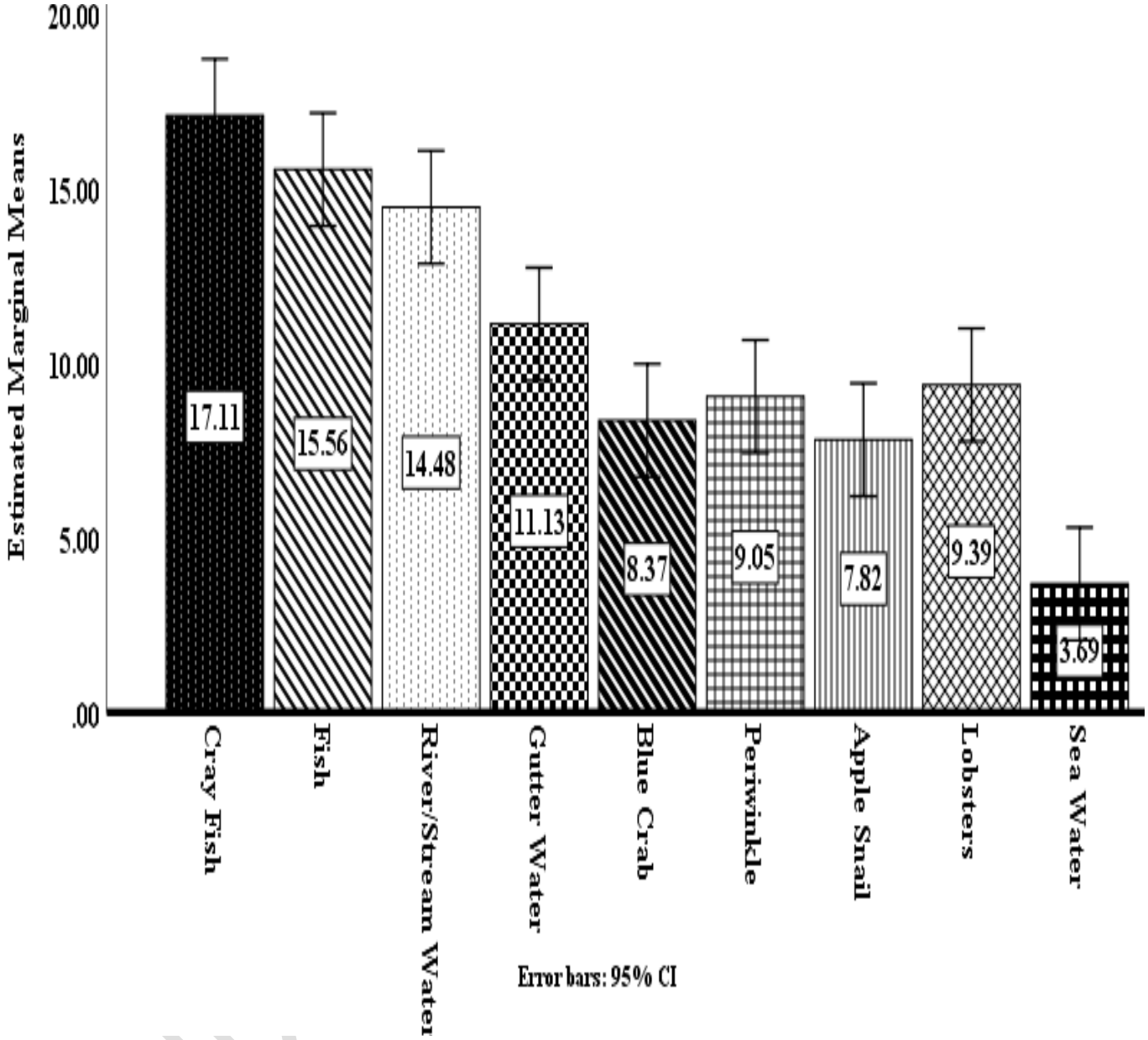

Figure 1: Total percentage mean of *Vibrio species* from each Source Examined

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).

From the total mean percentages Akpabuyo was the most contaminated Location (16.69±16.99%), Calabar with 16.49±14.10%, Ikom 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 Obanlikwu, with 5.76±16.1% (Table:6)

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

From, Ogoja *V. cholerae* was the most abundant isolate with 19.82%, *V. parahaemolyticus* 5.57, *V. vulnificus* 3.59%, *V. fluvialis* 2.82%, *V. mimicus* 2.04%, Other Vibrios 1.8%.

From Obudu, *V. cholerae* 17.06%, *V. parahaemolyticus* 4.51%, Other Vibrios 4.45%, *V. fluvialis* 4.35, *V. vulnificus* 2.67%, *V. mimicus* 1.69%.

Obanlikwu, *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 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%.

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).

3.6 Seasonal Distribution of species of Vibrio in the Environment

A total of 1882±1.83 were isolated in the Rainy Season, while 1772±1.73 were in the Dry Season (Table :7). 24.45%, of *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).

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

The NSD had the least number 327 out of 3654 (8.95%), the CSD 570 (15.59%) and then the SSD with 2757 (75.45%) (Table:8).

From the NSD, 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).* From the CSD*,* 27.94%, (*V. cholerae*), 12.89%, (*V. parahaemolyticus*), 7.53% (*V. fluvialis)*, 5.57% (*V. vulnificus* and (Other Vibrios), and 3.51% *(V. mimicus)*. From the SSD, 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), and in the interaction between the Senatorial Districts and the *Vibrio species* (P<.05).

Multiple comparisons 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

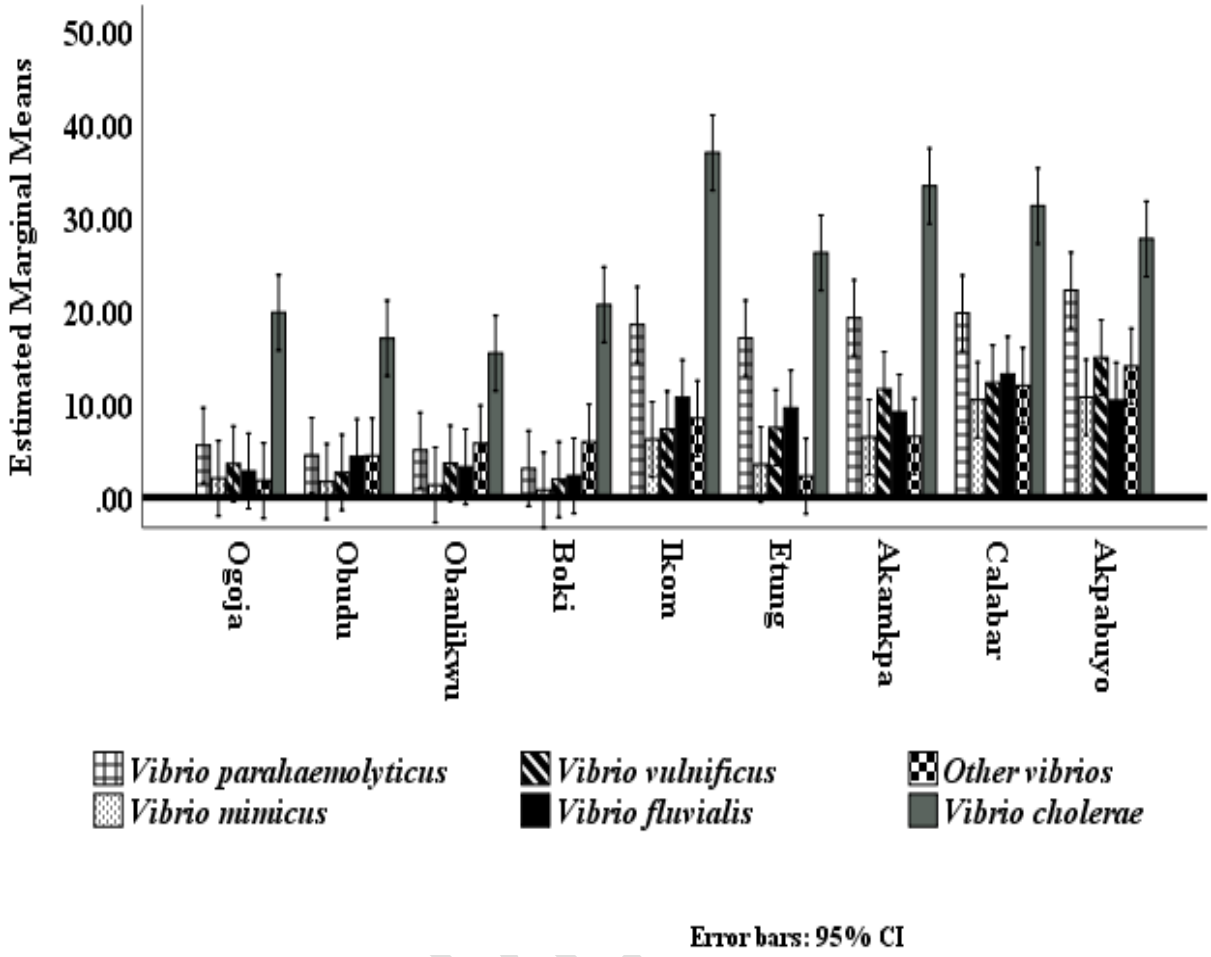

Fig:2 Distribution of Different species of Vibrio in the Various Locations Examined

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

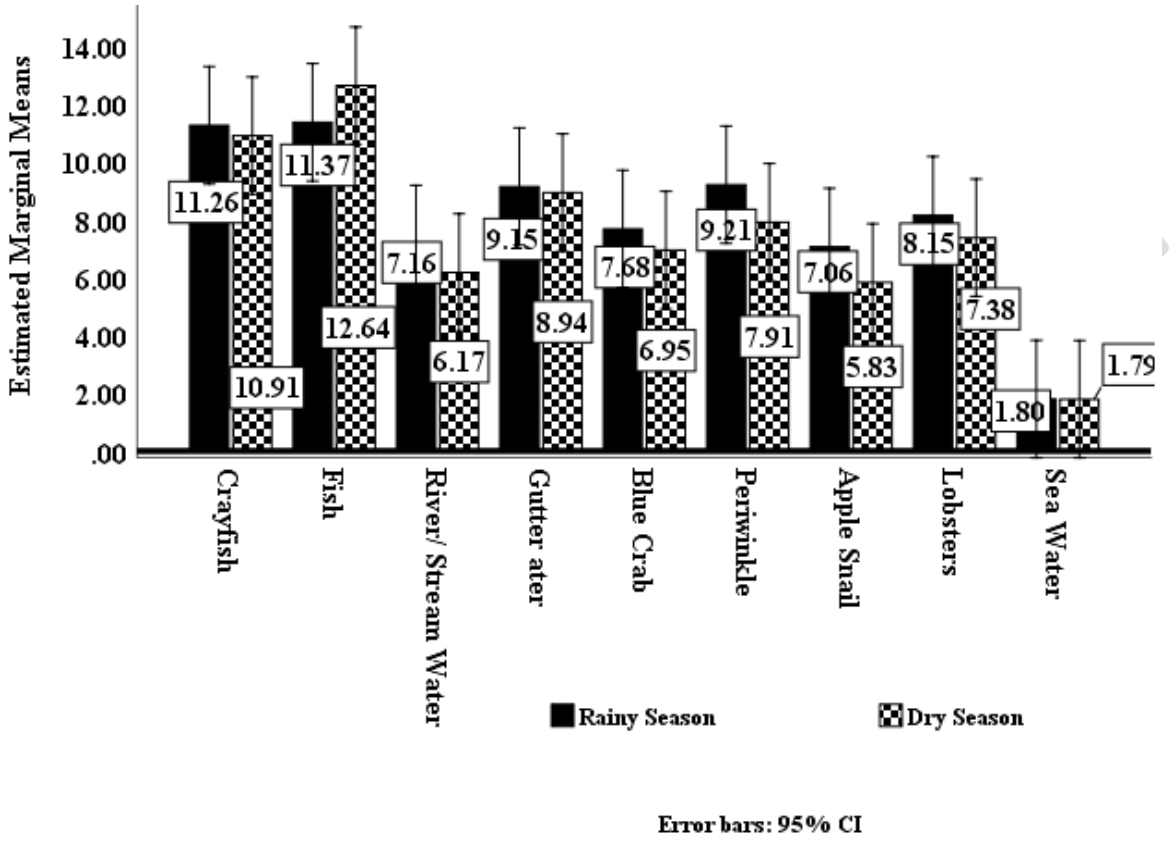

Figure 3: Mean Percentage Seasonal Distribution of species of Vibrio in the Environment

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

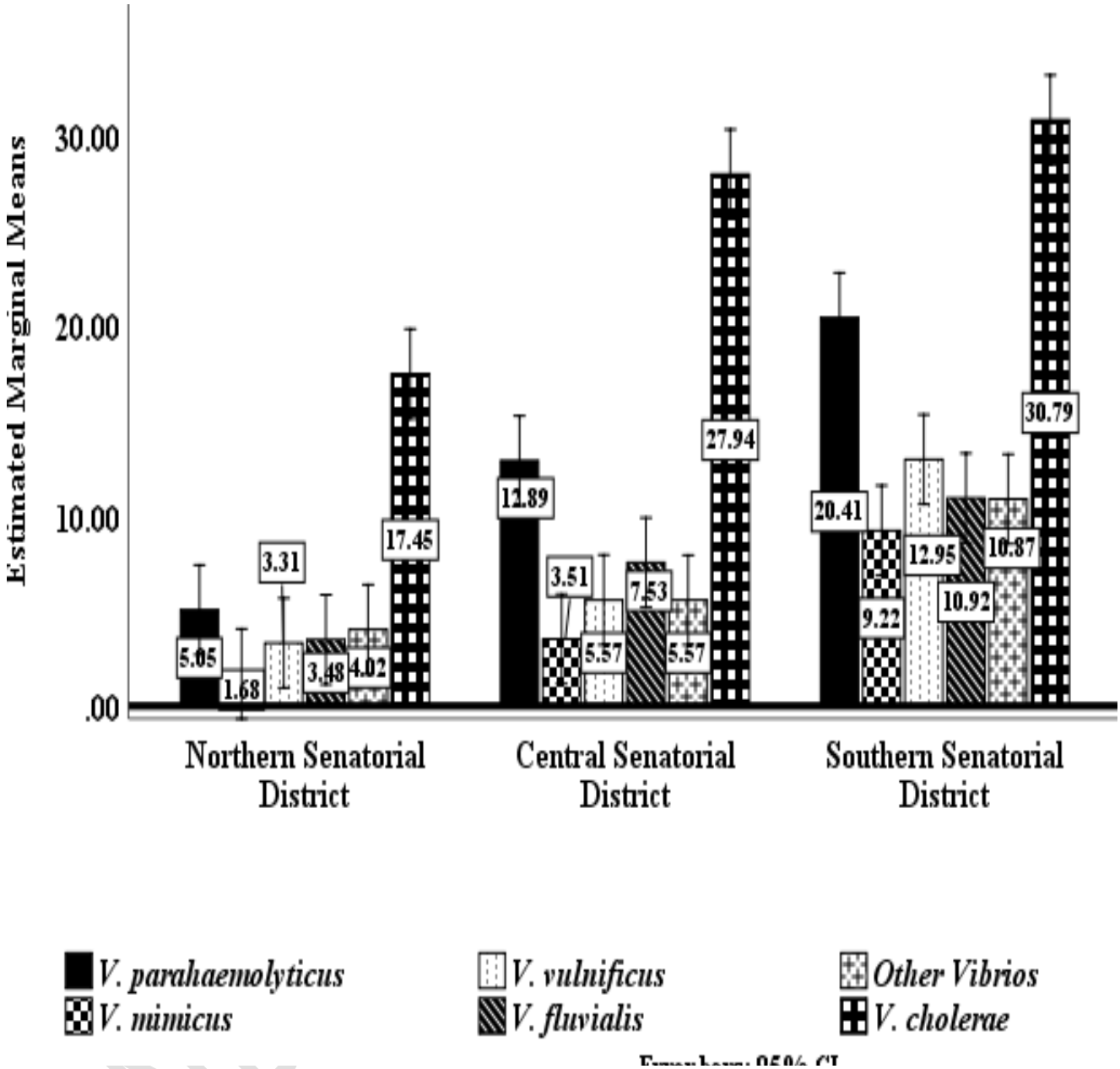

Figure 4: Mean Percentage Occurrence of *V. species* from the Various Senatorial Districts

4. DISCUSSION

A cross sectional study of the CRS 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 seawater, 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*. Contaminated faeces which sometimes is defecated directly into these bodies of water by the population living around them, together with some of the surface wash off 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 seawater 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 seawater sources. However, the percentage mean counts per location showed that out of the 3654 Vibrio isolates, 663±3.31 (18.14%) were from Seawater (the highest), 642±1.66 (17.57%) from Crayfish, 297±1.53 (8.13%) from Blue crab and the least 133±.84 (3.64%) from Gutter Water. This showed that seawater, although was from two

locations only, still had the greatest number of *Vibrio species*; being the natural habitat of these species [12, 3, 4**,** 5, 6].

This study also revealed the presence of some known pathogenic strains of vibrio, namely; *V. cholerae* and *V. parahaemolyticus vulnificus*, *fluvialis* and *mimicus.* These bacteria were isolated in both Seasons of the year, from different seafoods and water sources.

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 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 seawater for their sources of proteins and daily activities. During the course of the research, it was observed that some of these sea foods are eaten uncooked at the point of harvest by the fishermen, young and newborn babies are even submerged into these bodies of water as a tradition and custom of some of these people while swimming in these rivers is a hubby and the only means by which some of 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 water bodies for microbial pathogens and improve the quality of water [34].

It was observed that the Crayfish 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 Seawater 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 CRS. 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, lastly by Obanlikwu, with 5.76±16.1%. The incidence of *Vibrio* was higher in the SSD than in the CSD and NSD

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. Arab *et al*. [36] also, detected, the largest numbers (n=28) of Vibrio strains during the summer and principally in August from the fishes.

It is also worthy to note that CRS, which is situated along the Atlantic coastline of West Africa, has temperature range of about 25 to 28 degree Celsius. Moreover, temperatures above 18°C, and lower salt concentrations below 25% favor the growth of the human pathogenic Vibrios [43, 5]. The optimum growth temperature for *V. vulnificus*, *V. cholerae,* and *V. parahaemolyticus* is at about 42°C [44], which can affect the recovery of stressed cells [45], but *V. parahaemolyticus* can still grow between 37°C and 41.5°C. This may explain the abundance of the major human pathogenic *Vibrio* species isolated in this study.

Finally, since the city of Calabar's economy is based on tourism, greater levels of anthropogenic contamination due to rural to urban migration, overcrowding, poor accommodation, social facilities and sewage disposal systems, nearness to source of seafoods etc. abound. High concentrations of wastes are washed into the water environment from the surrounding polluted areas; letting loose even the non-pathogenic species that habit the estuarine muddy

environments favoring the proliferation of *Vibrio species*. The case is different with the other locations evaluated in this study.

5. CONCLUSION

In this study, a comprehensive epidemiological picture of the three senatorial districts of the CRS 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 Crayfish sources, carried the highest percentage, while blue crab carried the least percentage. Also, among the three water sources evaluated, the seawater sources were the most contaminated, while the gutters yielded the least percentage. Cumulatively, the percentage abundance by location in decreasing order was as follows; SSD>CSD>NSD.

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.

LIMITATIONS

The unprecedented long period of global lock down due to the COVID-19 pandemic, as well as the lack of funds, led to the restriction of movement and acquisition of some of the necessary requirements and consequently loss of viability by a great majority of the isolates. Thus, further investigations towards the molecular characterization could not be accomplished on these isolates.

6. REFERENCES

[1] Wang R, Huang J, Zhang W, Lin G, Lian J, Jiang L, et al.. Detection and identification of Vibrio parahaemolyticus by multiplex PCR and DNA-DNA hybridization on a microarray. J. Genet. Genomics. 2011; 38:129–135. Available on: https://doi.org/10.1016/j.jgg.2011.02.002

[2] Narracci M, Acquaviva MI, Cavallo RA, Mar Piccolo of Taranto. Vibrio biodiversity in ecotoxicology approach. *Env Sci Poll Res.* 2014; 2 (21): 2378–2385. Available on: https://doi.org/10.1007/s11356-013-2049-3

[3] Castillo D, D'Alvise P, Kalatzis PG, Kokkari C, Middelboe M, Gram L, e al. Draft Genome Sequences of *Vibrio alginolyticus* Strains V1 and V2, Opportunistic Marine Pathogens. *Gen Anno.* 2015; 3. Available on:https://doi.org/10.1128/genomea.00729-15

[4] Brahim B, Mohammed H, Fatima H, Aicha AA, Mohammed B, Asma L, et al. Treated Wastewater Reuse in Irrigation: Detection and Characterization of Potentially Pathogenic Vibrio Spp. *Amer J Inn Res Appl Sci.* 2020;11(5): 108-113.

[5] Håkonsholm F, Lunestad BT, Aguirre SJR, Martinez-Urtaza J, Marathe NP, Svanevik CS. Vibrios from the Norwegian marine environment: Characterization of associated antibiotic resistance and virulence genes. *Microbiology Open*. 2020;9:e1093. https://doi.org/10.1002/mbo3.1093

[6] Monsreal JF, Serralta–Peraza LEDS, Abuxapqui JJF. Species of the genus *Vibrio* of clinical–epidemiological importance. *MOJ Biol Med.* 2021; 6(3):116–125. DOI: 10.15406/mojbm.2021.06.00142

[7] Takahashi E, Ochi S, Mizuno T, Morita D, Morita M, Ohnishi M. Virulence of Cholera Toxin Gene-Positive *Vibrio cholerae* Non-O1/non-O139 Strains Isolated from Environmental Water in Kolkata, India. Front. Microbiol. 2021; 12:726273. doi: 10.3389/fmicb.2021.726273
http://dx.doi.org/10.1016/j.ijid.2018.11.083

[8] Pascual M, Rodó X, Ellner SP., Colwell R, Bouma MJ. Cholera dynamics and El Niño-Southern oscillation. Sci. 2000;289(5485):1766–1769.

[9] Martinez-Urtaza J, Bowers JC, Trinanes J, DePaola, A. Climate anomalies and the increasing risk of Vibrio parahaemolyticus and Vibrio vulnificus illnesses. Food Res Int 2010;43(7):1780–1790.

[10] Frank, C., Littman, M., Alpers, K., Hallauer, J. Vibrio vulnificus wound infections after contact with the Baltic Sea, Germany. *European Surveillance.* 2006;11(8): E060817.1.

[11] Schets, F. M. (2006) *Vibrio alginolyticus* infections in the Netherlands after swimming in the North Sea. *Euro Surveill*, 11(11): E061109.3.

[12] Semenza, JC. Climate change impact assessment of food- and waterborne diseases. *Critical Reviews on Environmental Science and Technology.* 2012; 42(8):857–890.

[13] Naseer U, Blystad H, Angeloff L, Nygård K, Vold L, Macdonald E. Cluster of septicaemia and necrotizing fasciitis following exposure to high seawater temperatures in southeast Norway, June to August 2018. *Interl J Infect Dis.* 2019; *79*, 28.

[14] Nigeria Centre For Disease Control Weekly Epidemiological Report Main Highlight of The Week. Prevention of Cholera Outbreaks I: Safe Water Use and General Hygiene Practices Volume 7 No. 25 7th July 2017. 17th August 2018 Volume 8 No. 31.

[15] Kirschner AKT, Schauer S, Steinberger B. Interaction of *Vibrio cholerae* non-O1/non-O139 with copepods, cladocerans and competing bacteria in the large alkaline lake Neusiedler See, Austria, *Microb Ecol*. 2011;61 (3): 496–506.

[16] Canellas ALB, Lopes IR, Mello MP, Paranhos R, de Oliveira BFR, Laport MS. *Vibrio species* in an Urban Tropical Estuary: Antimicrobial Susceptibility, Interaction with Environmental Parameters, and Possible Public Health Outcomes. Microorganisms. 2021; 9: 1007. https://doi.org/ 10.3390/microorganisms9051007

[17] Teklehaimanot GZ, Genthe B. Kamika IMN, Momba B. Prevalence of enteropathogenic bacteria in treated effluents and receiving water bodies and their potential health risks. *Sci Tot Envir* 2015;518-519: 441–449

[18] Dixit SM, Fatema-Tuz J, Sulochana M, Abdus S, Rajesh M R, Shahnewaj BM, Munirul A. Cholera outbreaks (2014) in three districts of Nepal reveal clonal transmission of Multi-Drug Resistant *Vibrio cholerae* O1. *BMC Infect Dis*. 2012; 14; 392. DOI: 10.1016/S2214-109X (16)30211-X.

[19] Eyisi OAL, Nwodo UU, Iroegbu CU. Distribution of *Vibrio species* in Shellfish and Water Samples Collected from the Atlantic Coastline of South-East Nigeria. J Health Pop Nut. 2013; *31*(3):1-7

[20] Schets FM, van den Berg HHJL, Marchese A, Garbom S, de Roda Husman AM. Potentially human pathogenic vibrios in marine and fresh bathing waters related to environmental conditions and disease outcome. *Int. J. Hyg. Environ. Health.* 2011; 214, 399–406. Available on: https://doi.org/10.1016/j.ijheh..05.003

[21] Pal D, Das N. Isolation, identification and molecular characterization of *Vibrio parahaemolyticus* from fish samples in Kolkata. *Eur Rev Med Pharmacol Sci.* 2010; 14: 545-549

[22] Ndon, J. A. Udo, S. M. and Wehrenberg, W. B. (1992). Vibrio-Associated Gastroenteritis in The Lower Cross-River Basin of Nigeria. *Journal of Clinical Microbiology*, 30, (10): 2730-2732.

[23] Gennari M, Ghidini V, Caburlotto G. and Lleo MM. Virulence genes and pathogenicity islands in environmental *Vibrio* strains nonpathogenic to humans," *FEMS Microbiol Ecol* 2012; 82(3): 563–573

[24] Ramamurthy T, Chowdhury G, Pazhani GP. and Shinoda S. *Vibrio fluvialis*: An emerging human pathogen. *Front. Microbiol*. 2014; 5(91): 1-9.

[25] Takahashi A, Miyoshi SI, Takata N, Nakano M, Hamamoto A, Mawatari,K, et al. Haemolysin produced by *Vibrio mimicus* activates two Cl– secretory pathways in cultured intestinal-like Caco-2 cells. *Cell Microbiol* 2007; 9(3): 583-595.

[26] Vezzulli L, Chiara G, Philip C. Reid PH, Martin E, Manfred G.H. Climate influence on Vibrio and associated human diseases during the past half-century in the coastal North Atlantic. PNAS 2016; E5062. E5071. www.pna.org/cgi/doi/10.1073/pnas.1609157113

[27] Nair GB Global dissemination of Vibrio parahaemolyticus serotype O3:K6and its serovariants. Clin Microbiol Rev 2007; 20(1):39–48.

[28] Nelson EJ, Harris JB, Morris JG, Jr Calderwood SB, Camilli A. Cholera transmission: The host, pathogen and bacteriophage dynamic. *Nature Review in Microbiology*. 2009; 7(10): 693–702.

[29] López-Hernández KM, Pardío-Sedas V, Lizárraga-Partida L, Williams J, Martínez-Herrera D, Flores-Primo A, et al; Seasonal abundance of Vibrio cholerae non O1/non O139 chxA+ in oysters harvested in a coastal lagoon of Mexico's Gulf coast: A seafood safety risk concern. *Food Contr.* 2015; 53: 46–54. Available on: https://doi.org/10.1016/j.foodcont.2015.01.004

[30] Mendes ES, Mendes PP., Góes Lmnb, Bezerra SS. and Vieira Kpba. OS víbrios na carcinicultura. Panor Aquic, 2005;26-29.

[31] Austin B. Vibrios as causal agents of zoonoses. *Vet Microbiol* 2010;140: 310–317.

[32] Fu K, Li J, Wang Y, Liu J, Yan H, Shi L, & Zhou L. An innovative method for rapid identification and detection of Vibrio alginolyticus in different infection models. *Front Microbiol*. 2016;7(651), http://dx.doi.org/10.3389/fmicb.2016.00651

[33] Baker-Austin C, Oliver JD, Alam M, Ali A, Waldor M K, Qadri F, & Martinez-Urtaza J. *Vibrio* spp. infections. *Natur RevDis Prim* 2018; *4*, 8.

[34] Mpho DM, Christ DKT, Madira CM, Justine F. and Collins NA. Characterization of *Vibrio species* from Surface and Drinking Water Sources and Assessment of Biocontrol Potentials of Their Bacteriophages *Hindawi Inter J Microbiol.* 2020; 1-15

[35] Chigbu LN, Iroegbu CU. *Vibrio species* from diarrhoeal stools and water environment in Cross River State, Nigeria. *International Journal of Environmental Health Research* 2000; 10:219-28.

[36] Arab S, Nalbone L, Giarratana F, Berbar A. Occurrence of *Vibrio* spp. along the Algerian Mediterranean coast in wild and farmed *Sparus aurata* and *Dicentrarchus labrax*, *Veterinary World*. 2020; 13(6): 1199-1208.

[37] Rodrigues SMA, Gonçalves EGR, Mello DM, Oliveira EG. and Hofer E. Pesquisa de bactérias do gênero *Vibrio* em feridas cutâneas de pescadores do município de raposa-MA. *Review Society of Bras Med Trop Res Jl* 2001; 34(5): 407-411.

[38] Bauer A, Ostensvik O, Florvag M, Ormen, O, & Rorvik LM. Occurrence of *Vibrio parahaemolyticus*, *V. cholerae*, and *V. vulnificus* in Norwegian Blue Mussels (*Mytilus edulis*). *Appl Envirt Microbiol* 2006;72: 3058–3061.

[39] Okoh AI, Sibanda T, Nongogo V, Adefisoye M, Olayemi OO, Nontongana N. Prevalence and characterization of non-cholerae Vibrio spp. in final effluents of wastewater treatment facilities in two districts of the Eastern Cape Province of South Africa: implications for public health. *Envir Sci Poll Res* 2015; 22: 2008–2017. Available on: https://doi.org/10.1007/s11356-014-3461-z

[40] Hounmanou YMG., Mdegela RH, Dougnon T V, Mhongole OJ, Mayila ES, Malakalinga J, et al. Toxigenic Vibrio cholerae O1 in vegetables and fish raised in wastewater irrigated fields and stabilization ponds during a non-cholera outbreak period in Morogoro, Tanzania: An environmental health study. *BMC Res. Notes* 2016; 9:466. Available on: https://doi.org/10.1186/s13104-016-2283-0

[41] Francisca GR, DeM Marina TTR., Fátima CT De C, Rosa H R, Renata AC, Oscarina De S, Ernesto H. and Regine HSFV. Pathogenic *Vibrio species* isolated from estuarine environments (Ceará, Brazil) - antimicrobial resistance and virulence potential profiles *Ann Braz Acad Sci.* 2017; 89(2): 1175-1188

[42] Saad MS, Maha MS, Hania ElS, Abd El Maksod Incidence of Vibrio species in fish with special emphasis on the effect of heat treatments Benha Vet Med J , 2015;. 29, (1):38-44

[43] Vezzulli L, Colwell RR. & Pruzzo C. Ocean warming and spread of pathogenic vibrios in the aquatic environment. *Microb Ecol*. 2013; *65*, 817–825.

[44] NMKL. (1997). Pathogenic Vibrio species. In Nordic Committee on Food Analysis (Ed.). D*etection and enumeration in foods*, 2nd edn. Espoo, Finland: NMKL.

[45] Huq A, Haley BJ, Taviani E, Chen A, Hasan NA, & Colwell RR. Detection, isolation, and identification of *Vibrio cholerae* from the environment. *Curr Prot Microbiol*. 2012; Chapter *6*, Unit6A.5. 1-58. DOI:10.1002/9780471729259.mc06a05s26.