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Prevalence of *Vibrio cholerae* and *Vibrio* species from clinical and environmental sources in Rivers State, Nigeria

***Aladese, M.A. and Omeje, F.I.**
Department of Microbiology Federal University Otuoke, Nigeria

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Abstract

This study investigated the prevalence of *Vibrio cholerae* and other *Vibrio* species from clinical and environmental sources from five localities; Ebukuma, Onne, Okoli'ile, Ikuru and Opobo all in Rivers State, Nigeria. A total of 61 faecal samples and anal swabs, 26 fresh waters, 60 brackish waters and 73 seafoods (crabs, shrimps and fishes) were collected for the purpose of prevalence of *Vibrio cholerae* from January to April, 2017. Samples were transported to the laboratory using Cary-Blair's medium. This was followed by enrichment in 1% alkaline peptone water and plating on thiosulphate citrate bile sucrose (TCBS) agar. Characteristic yellow colonies were subjected to further identifications using physiological and biochemical characterization. In addition, water samples and seafoods samples were collected separately for duration of 12 months from January to December, 2017 with aim to determine the monthly distribution patterns of *Vibrio* spp in these sources. Antibiotics susceptibility for isolated *V.cholerae* strain was investigated. The cholera bacterium was isolated from 31(50.82%) in clinical samples, 13(50%) in fresh water samples, 34(56.67%) in brackish waters and 21(28.77%) in seafoods. The monthly mean values of *Vibrio* spp is less prevalent in the wet months where rainfall is relatively higher in the region. There is statistically significant difference ($P<0.05$) in low mean values observed in the wet months when compared to average values in the dry months. Ciprofloxacin, ofloxacin and pefloxacin were recommended as the best line of drug treatment in that all strains were sensitive to these therapeutic compounds.

***Corresponding Author**: **Aladese, M.A.**: aladesema@gmail.com

Introduction

Vibrio cholerae is the agent associated with cholera. It is a Gram negative, oxidase positive and highly motile bacterium (Nandi *et al*., 2003). The cholera bacterium continues to maintain its relevance in public health with pockets of outbreaks in many localities where basic amenities especially potable water is lacking (Aladese *et al*., 2015). The cholera bacterium is primarily transmitted by water (Idika *et al*., 2000) while consumption of contaminated seafoods like shrimps, lobsters and crabs could also lead to infection.

The pathology of cholera is the excessive outflow of fluids from the host characterized by profuse diarrhea accompanied with vomiting. The resultant effect leads to dehydration and elevated blood proteins leading to cardiac arrest in many patients (Faruque *et al*., 2003). Cholera disease has recorded seven different global epidemics with the most recent being the seventh pandemic originating from Island of Sulawesi in Indonesia in 1961 (Faruque *et al*., 1998). Although, *V. cholerae* serogroup O1 which has been associated with seventh pandemic is still epidemiologically active but the emergence of newer serogroups especially *V. cholerae* serogroup O139 from Madras, India in 1992 epidemic (Agarwal *et al*., 1994) and non-O1 and non-O139 serogroups of *V. cholerae* with epidemic potentials has been of serious concerns in recent times (Dutta *et al*., 2006).

Apart from the cholera vibrio, there have been reported cases of non-cholera *Vibrio* spp which have been implicated in gastroenteritis as a result of ingestion of contaminated water and seafoods (Rippey, 1994). Prominent among these potentially pathogenic non-cholera *Vibrio* spp is *Vibrio parahaemolyticus* (Makino, 2003). Others include *V.alginolyticus*, *V.mimicus*, *V.vulnificus*, *V.hollisae*, *V.fluvialis* and *V.metschnikovii*, an oxidase negative

non-cholera *Vibrio* specie (Ramamurthy *et al*., 1994; Elliott *et al*., 1998). Ingestion of contaminated raw or undercooked seafoods has been pointed as a major predisposing factor for non-cholera *Vibrio* spp infections (Elhadi *et al*., 2004; Hau and Ho, 2011).

The study on the prevalence of these potentially pathogenic *Vibrio* spp among different sources will provide appreciable insights in which of the sources will pose the highest predisposing factor of infections. In addition, the investigation of distribution pattern through total vibrio count from different sources on a monthly basis will provide appreciable information on the basis of outbreaks of *Vibrio cholerae* and non-cholera *Vibrio* spp in some months especially in some parts of sub-Saharan African communities.

This study is aimed at the distribution of *V. cholerae* from clinical, water samples (fresh and brackish water) and seafoods obtained from five (5) localities in Rivers States, South-south region of Nigeria. Furthermore, this study will also investigate the monthly distribution of *Vibrio* spp counts from fresh waters, brackish water and seafoods from these five areas of Rivers State.

Materials and methods

Study area: Rivers State (4.84^0 N; 6.91^0 E) is one the states which constitute the oil-rich region of the Niger-Delta. Rivers State is bordered by Bayelsa State in the west, Imo and Abia in the north, Akwa-ibom in the east and the Atlantic Ocean in the south. Rivers is home to many indigenous ethnic groups which are; Ijaw, obolo (andoni people), ogoni, ikwere and kalabari among others. The terrain of Rivers State can be grouped into three (3) major zones namely; fresh water swamps, mangrove swamps and coastal sand ridges. Port Harcourt is the most populous city in the state with the population size of over 1 million inhabitants. Other major towns and localities are Bori, Ogoni, Onne, Khana and Opobo. The primary occupation of most of the local inhabitants outside major cosmopolitan cities are fishing, agriculture and brewing local gins. Five (5) areas which are Ebukuma, Onne, Okoli'ile, Ikuru and Opobo were selected in this study (figure 1).

Ebukuma, Okoli'ile (which could also be referred to as Okolo'ile) and Ikuru are suburban communities located in Andoni region of Rivers State. The primary activity of the local population is fishing, farming and trading. These Andoni settlement are commercially relevant because they are the link between the Port Harcourt and other southern areas of the state. Onne (also referred to as Onne-Eleme) is harbor town and it is bordered with coastal towns like Alode, Ebubu and a tributary of Bonny river known as Ngololo creek. Opobo town which could also be called Opubo is a southern town in the heart of Opobo kingdom. It is a coastal town where the inhabitants engage primarily in fishing activities.

Clinical samples

A total 61 faecal samples (rice watery stool) and anal swabs were collected from January-April 2017 from patients at the different primary health centres; Ebukuma, Onne, Okoli'ile, Ikuru and Opobo in Rivers State. The samples were collected using sterile swabs sticks and were inoculated into Cary-Blair medium for transport purposes.

Fresh and Brackish water samples

A total of 26 fresh water and 60 brackish samples were collected from January-April 2017 during the duration of the outbreak. Fresh water samples were collected from hand-dug wells while brackish waters were sampled from nearby rivers. Samples were inoculated into Cary-Blair medium (Oxoid, UK) for transport purposes to the laboratory for identification of *V.cholerae*.

Sea-foods sample

A total of 73 sea-foods (Crabs, shrimps and fishes) were collected for 4 months (January-April 2017) from the five selected locations. Samples were collected from major markets in these localities and these were placed in sterile polythene bags, kept on ice and were transferred to laboratory for immediate analysis.

Enrichment and isolation of *V.cholerae* from clinical and water samples

Clinical and water samples (fresh and brackish) from Cary-Blair medium were inoculated into 1% alkaline peptone water for enrichment purposes. They were plated out on Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar (Oxoid, England), by the pour plate method and cultures were incubated at 37^0C for 24 hrs. Characteristic yellow colonies were considered for further identification.

Enrichment and isolation of *V.cholerae* from sea-foods

Crabs were dissected and the chitinous external layer were discarded. Shrimps, fishes and the internal components of the dissected crabs were placed in blender for grinding to achieve homogenization. Samples were inoculated into 1% alkaline peptone water for enrichment purposes. Plating was then done on Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar (Oxoid, England), using pour plate method. Cultures were thereafter incubated at 37^0C for 24 hrs.

Characteristic yellow colonies were considered for further identifications.

Determination of viable plate count of *Vibrio* spp from water samples

Water samples were collected for the purpose of *Vibrio* species enumeration on a monthly basis from the selected settlements in this study for the duration of 12 months. One millilitre each fresh and brackish water samples were serial diluted ten-fold down to 10^{-6}. From each water samples, 0.1ml was inoculated by pour plate method on a freshly-prepared thiosulphate citrate bile-salt sucrose (TCBS) agar (Oxoid, England) plate at 37^0C for 24 hrs. The plate was examined for characteristic *Vibrio* spp colonies.

Determination of viable plate count of *Vibrio* spp from sea-foods

Seafoods samples were collected for the purpose of *Vibrio* species enumeration on a monthly basis from the selected settlements in this study for the duration of 12 months. Crabs were dissected and the chitinous external layer was discarded. Shrimps, fishes and the internal components of the dissected crabs were placed in blender for grinding to achieve homogenization. One gram (1g) of the homogenized tissue was subsequently serially diluted ten-fold, down to 10^{-6}. Thereafter, 0.1ml was inoculated by pour plate method on a freshly-prepared thiosulphate citrate bile-salt sucrose (TCBS) agar (Oxoid, England) plate at 37^0C for 24 hrs. The plate was examined for characteristic *Vibrio* spp colonies (Adebayo-Tayo *et al*., 2011).

Data representation

All data obtained were represented in the forms of means with their standard deviations and pictorial representation using Microsoft excel version 2016. The statistical comparisons of means were also determined using Microsoft excel version 2016. The analysis of variance (ANOVA) and post hoc analysis were determined using IBM SPSS (version 23.0).

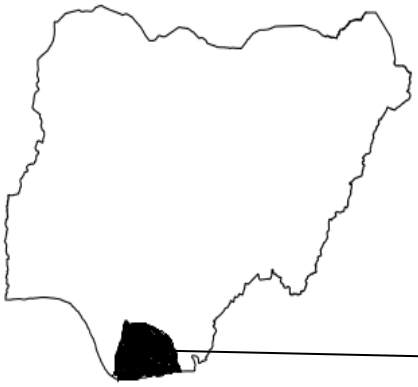

Figure 1a. Map of Nigeria showing the location of Rivers State

Figure 1b. Map of the section of Rivers State showing the selected localities of study (Source: Google map Inc.)

Results

Cholera bacterium were isolated from 31 (50.82%) of the total 61 patients examined (table 1). The highest prevalence was observed in Ebukuma (80%) followed by Okoli'ile (72.22%). Out of the 26 fresh water samples investigated 13(50%) were contaminated with the cholera vibrio with Okoli'ile (100%) and Ebukuma (87.5%) having the highest distribution (table 2). The investigation of the presence of *V.cholerae* in surface waters from the localities showed Ebukuma and Ikuru having all its samples contaminated with the bacterium (table 3). A total of 21 (28.77%) out of 73 seafoods had *V.cholerae* contamination. The highest distribution was found in crabs (table 4). Table 5 shows the prevalence of *V.cholerae* from sampled sources in Rivers State. The highest prevalence was observed in brackish waters (56.67%) with least distribution in seafoods. The summary of the distribution of *V.cholerae* in all the sources (table 5) showed highest percentage of contamination in brackish waters (56.67%) followed by clinical (50.82%) and fresh water samples (50%). The lowest percentage of contamination was observed in seafoods samples.

The monthly variation of total vibrio counts for brackish water samples (figure 2) showed significant of *Vibrio* spp counts in the months of April-September. Similar, trend was observed brackish water investigation for *Vibrio* spp (figure 3). The pictorial representation of the monthly mean values of *Vibrio* spp count for crabs (figure 4) showed higher plate counts in months of January-March and October-

December. The analysis of variance (ANOVA) showed statistically significant difference in these high months compared to the counts in months with lower *Vibrio* spp counts. The monthly average distribution *Vibrio* spp in shrimps showed lower *Vibrio* spp load in the months of May-September (figure 5). The ANOVA also showed statistically significant differences in these months with higher counts using the post hoc analysis. The *Vibrio* spp counts in fishes had lower values were observed in the months of June-September (figure 6). The overall analysis of variance comparison of all mean values obtained from crabs, shrimps and fishes (table 6) revealed statistically significant differences ($P<0.05$) in the values of *Vibrio* spp counts in crabs when compared to seafoods. The result of the antibiotics susceptibility pattern of strains showed total sensitivity (100%) of isolated strains to ofloxacin, pefloxacin and ciprofloxacin while, resistant strains were observed in antibiotics like tetracycline and Augmentin (table 7)

Table1. Distribution of pathogenic *Vibrio cholerae* isolated in patients from different localities in Rivers State.

Localities	Total numbers of patients examined	Number of patients with cholera
Ebukuma	20	16 (80.00)
Onne	5	0 (0.00)
Okoli'ile	18	13 (72.22)
Ikuru	10	2 (20.00)
Opobo	8	0 (0.00)
Total	61	31 (50.82)

*Numbers in parenthesis represents percentage

Table 2. Distribution of *Vibrio cholerae* isolated from fresh water in different localities in Rivers State

Localities	Total numbers of Fresh water examined	Number of wells with *V. cholerae*
Ebukuma	8	7 (87.50)
Onne	5	0 (0.00)
Okoli'ile	5	5 (100.00)
Ikuru	3	1 (33.33)
Opobo	5	0 (0.00)
Total	26	13 (50.00)

*Numbers in parenthesis represents percentage

Table 3. Distribution of *V. cholerae* isolated from brackish waters in different localities in Rivers State

Localities	Total numbers of Brackish waters examined	Number of Brackish with *V.cholerae*
Ebukuma	12	12 (100.00)
Onne	12	1 (8.33)
Okoli'ile	12	8 (66.67)
Ikuru	12	12 (100.00)
Opobo	12	1 (8.33)
Total	60	34 (56.67)

*Numbers in parenthesis represents percentage

Table 4. Distribution of *Vibrio cholerae* isolated from sea-foods in Rivers State

Localities	Total numbers of Sea food examined	Number of wells with *V. cholerae*
Crabs	30	11(36.67)
Shrimps	25	5(20.00)
Fishes	18	5(27.78)
Total	73	21(28.77)

*Numbers in parenthesis represents

Table 5. Prevalence of *Vibrio cholerae* from different sources in Rivers State, Nigeria.

Source	Number examined	Number positive for *V.cholerae*
Clinical	61	31(50.82)
Fresh Water	26	13 (50.00)
Brackish	60	34 (56.67)
Sea-Foods	73	21 (28.77)
Total	220	99(45.00)

*Figure in parenthesis represents percentages

Table 6. Distribution of *Vibrio* species colony forming units count in sea-foods

Sea-foods	Log_{10} mean *Vibrio* count ± SD
Crabs	(8.18 ± 1.45)b
Shrimps	(6.07 ± 1.33)a
Fishes	(5.47 ± 1.42)a

* Values with different alphabets are significantly different at $P<0.05$

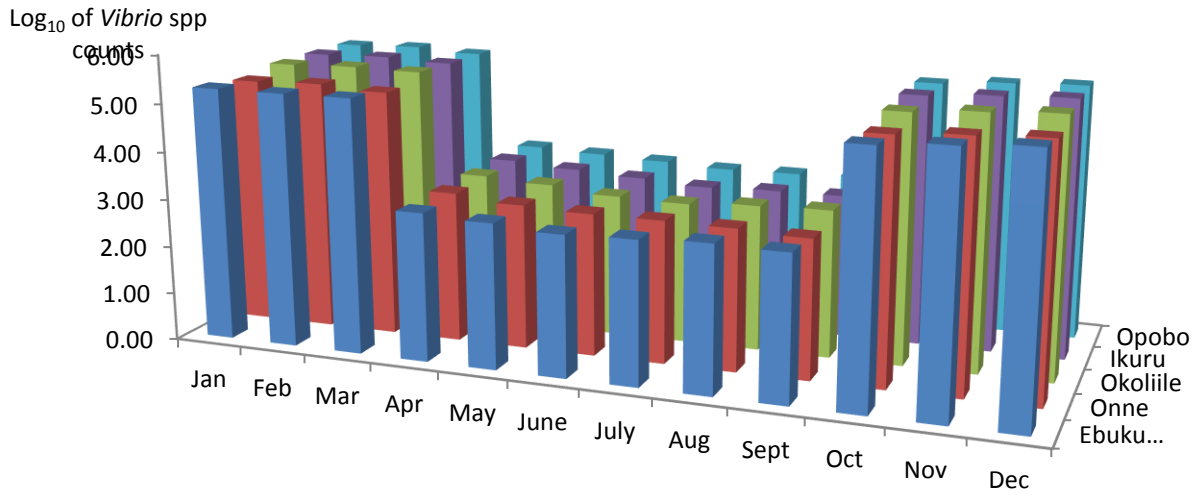

Figure 2: Average monthly distribution of *Vibrio* species counts in fresh samples collected from different localities in Rivers State.

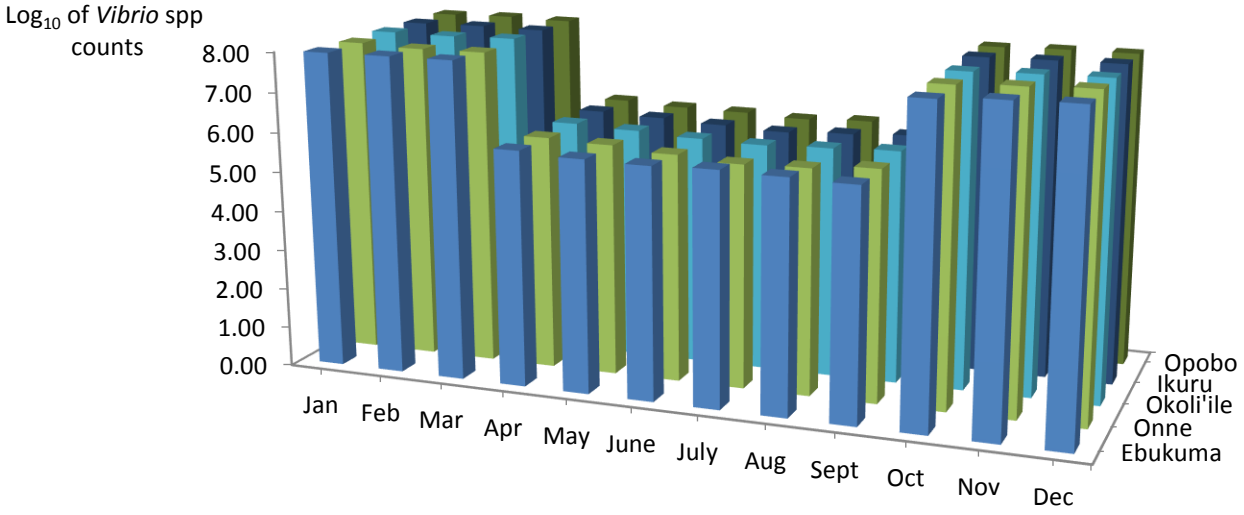

Figure 3: Average monthly distribution of *Vibrio* species counts in brackish samples collected from different localities in Rivers State

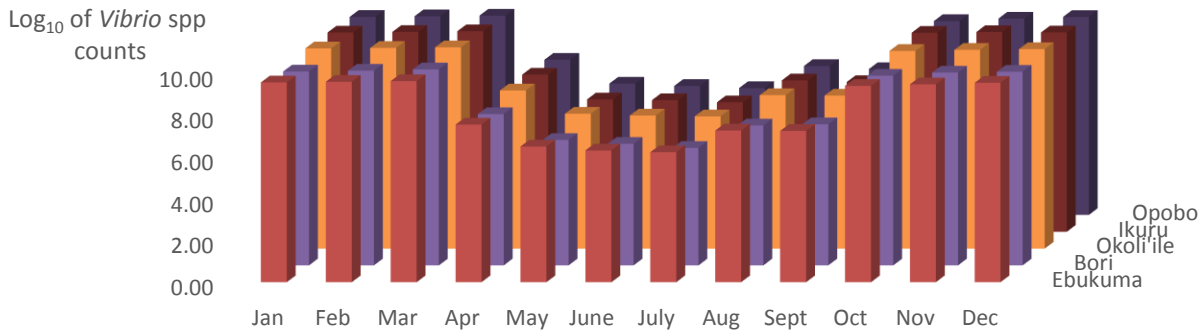

Figure 4: Average monthly distribution of *Vibrio* species counts in crabs from different localities in Rivers State

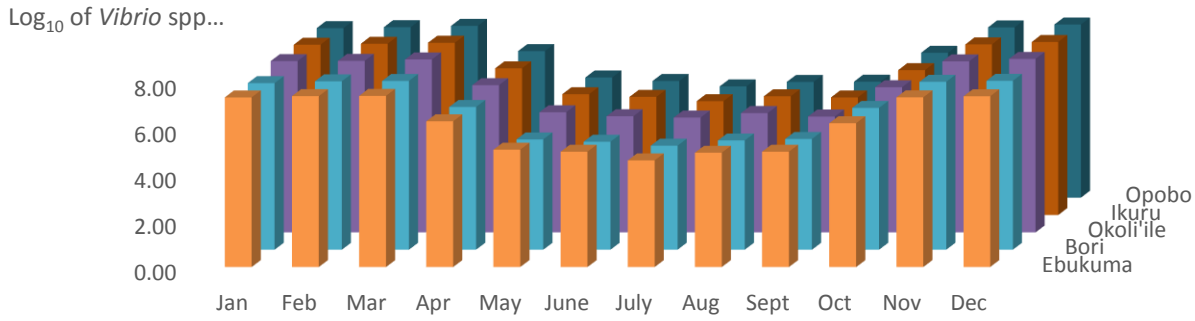

Figure 5: Average monthly distribution of *Vibrio* species counts in shrimps from different localities in Rivers State

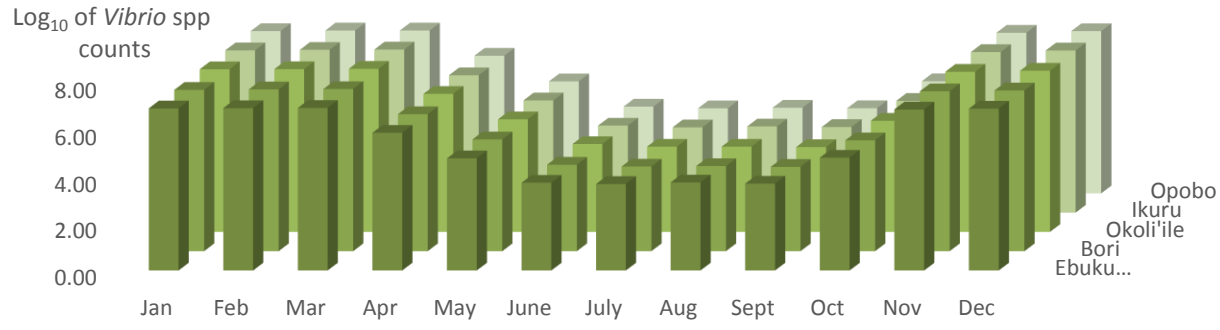

Figure 6: Average monthly distribution of *Vibrio* species counts in fishes from different localities in Rivers State

Table 7. Antibiotics susceptibility of *Vibrio cholerae* from different sources in Rivers State

	Clinical		**Fresh**		**Brackish**		**Seafoods**	
Antibiotics	Sensitive of strains	Number Examined	Sensitive of strains	Number Examined	Sensitive of strains	Number Examined	Sensitive of strains	Number Examined
Amx	38 (62.30)	61	17 (65.38)	26	36 (60.00)	60	49 (67.12)	73
Aug	43 (70.49)	61	19 (73.08)	26	40 (66.67)	60	56 (76.71)	73
Cot	39 (63.93)	61	18 (69.23)	26	38 (63.33)	60	50 (68.49)	73
Tet	53 (86.89)	61	23 (88.46)	26	54 (90.00)	60	68 (93.15)	73
Pef	61 (100.00)	61	26 (100.00)	26	60 (100.00)	60	73 (100.00)	73
Ofl	61 (100.00)	61	26 (100.00)	26	60 (100.00)	60	73 (100.00)	73
Cpx	61 (100.00)	61	26 (100.00)	26	60 (100.00)	60	73 (100.00)	73

Amx = Amoxicillin, Aug= Augmentin, Cot = Cotrimoxazole, Tet = Tetracycline, Pef = Pefloxacin, Ofl = Ofloxacin, Cip = Ciprofloxacin

Discussion

The distribution of *V.cholerae* in patients from different localities in Rivers State (table 1) showed Ebukuma and Okoli'ile having high percentage of prevalence. Similar trend was observed in fresh waters, with the two settlements showing high percentage of *V.cholerae* contamination of fresh water sources. The high contamination of *V.cholerae* in fresh water samples from Ebukuma and Okoli'ile, both communities located in the heart of Andoni-land was not surprising. A similar study on the bacteriological qualities of hand-dug wells as source of drinking water in Ebukuma revealed some physicochemical and indicator bacteria exceeding the World Health Organisation (WHO) standards for safe drinking water (Aladese and Ariyo, 2017). The reason for this high contamination from these two localities could be due to the fact that there is relatively lower development in terms of availability of basic amenities in these two localities when compared to others. It was observed that there is gross lack of potable water as many of the inhabitants results into drinking from hang-dug wells many of which were too close to nearby rivers where domestic and municipal wastes are dumped.

Ikuru town in addition with the other two Andoni settlements showed high percentage of contamination of *V.cholerae* from samples in brackish waters (table 3). The attributable reason for these high contaminations of surface waters in Ebukuma, Okoli'ile and Ikuru could be as a result of the practice of the local population directly channeling their excreta into the nearby rivers. Past reports have shown the existence of high correlation between indiscriminate and uncontrolled disposal of human faeces into water bodies and proportionate increase in the bacterial population in an aquatic environment. This unhealthy practice is as a result of ineffective or total absence of municipal waste treatment and disposal systems in many of these creek settlements around the Niger-Delta region (Lawson, 2011; Amangabara and Egenma, 2012; Onyema, 2013; Olorode *et al*., 2015). The prevalence of cholera bacterium in seafoods showed highest percentage of contamination in crabs (table 4). This shows the epidemiological importance of these seafoods especially crabs as reservoirs for cholera vibrio. The result obtained in the distribution of *V.cholerae* in these seafoods is in concordance with past reports (Aladese and Enabulele, 2014; Oramadike and Ogunbanwo, 2015).

The monthly mean values of *Vibrio* spp distribution in fresh waters (figure 2), brackish waters (figure 3), crabs (figure 4), shrimps (figure 5) and fishes (figure 6) showed a continuous trend where lower average values of distribution *Vibrio* spp counts were consistently observed in the months where rainfalls are relatively frequent in these local communities and all over tropical rainforests climate of Africa. These period of the year where rainfall is relatively abundant is collectively referred to as wet seasons or wet months. The statistically significant difference observed in these wet months could be due to the continuous discharge of fresh waters mostly through rainfalls to these aquatic environments.

In table 7 the 12 months' comparisons of all mean values of *Vibrio* spp counts showed crabs to be statistically significantly higher when compared to others using post hoc analysis of variance. This affirms the continuous epidemiological relevance of these seafoods especially crabs as major predisposing

factors of infection potentially pathogenic *Vibrio* spp (Rippey, 1994; Aladese and Enabulele, 2014; Oramadike and Ogunbanwo, 2015).

The treatment of *V.cholerae* is effective through constant epidemiological surveillance of emergence of resistant strains to antibiotics. Although, some antibiotics like Augmentin and tetracycline showed varying efficacies in treatment but the emergence of resistant strains has been reported in various studies in the past notably from Horns of Africa (Coppo *et* al., 1995), India (Amita *et al*., 2003) and Italy (Ottaviani *et al*., 2001). Adeleye *et al*. (2008) asserted that the emergence of antibiotics resistant strains could be due to over-exposure of these traditional drugs especially tetracycline. These has resulted into strains acquiring resistance capabilities to these antibiotics. Their submission had earlier been demonstrated by Amita *et al*. (2003) on the presence of plasmid-mediated antibiotics resistance capabilities among *V.cholerae* strains. The result which shows ofloxacin, pefloxacin and ciprofloxacin as best line of treatment is in agreement with past study (Aladese *et al*., 2015).

Conclusion

Vibrio cholerae and other non-cholera *Vibrio* spp remain threats to public health. However, with the practices of personal and community hygiene through prevention of contaminating water-bodies could be one of the major steps to prevent pockets of sporadic outbreaks of cholera and cholera-like epidemics.

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