



PREVALENCE OF *VIBRIO CHOLERA*E AND *VIBRIO* SPECIES FROM DIFFERENT SOURCES IN BAYELSA STATE, NIGERIA

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ABSTRACT: *The distribution of Vibrio cholerae and non-cholera Vibrio species from different sources from five localities in Bayelsa State, Nigeria was investigated in this study. A total of 44 stool samples, 22 freshwater samples, 60 brackish water samples and 64 seafood samples (crabs, shrimps and fishes) were collected from January to April, 2019 for the purpose of V. cholerae prevalence study. Samples were transported to the laboratory using Car-Blair's medium. This was followed by samples enrichment in 1% alkaline peptone water and pour plating on thiosulphate citrate bile-salt sucrose (TCBS) agar. Characteristic yellow colonies were subjected to further biochemical and physiological characterization to further identify V. cholerae. Antibiotics susceptibility patterns for isolated V. cholerae strains were investigated. Furthermore, water samples (fresh and brackish) and seafood samples were collected on a monthly basis to ascertain the effect of seasons (dry and wet months) on the distribution of Vibrio spp. A total of 16 (36.36%) stools samples were positive for V. cholerae. In addition, 12 (54.55%) of freshwater samples, 28 (46.67%) of brackish water samples and 22 (34.38%) of seafood samples were contaminated with V. cholerae. The monthly mean values of Vibrio spp. from environmental sources showed statistically significant difference ($P < 0.05$) between the dry months (low rainfall) and wet months (frequent rainfall). Higher average values were observed during the dry months. The result of the antibiotics sensitivity test showed all V. cholerae strains were susceptible to ciprofloxacin, ofloxacin and pefloxacin while varying degree sensitivities were observed in tetracycline and augmentin. Cholera and other non-cholera Vibrio spp gastrointestinal infections are still a major concern to the health of the public. Local and regional governments should enforce and promote the need for personal and communal hygienic practices.*

KEYWORDS: *Vibrio Cholerae, Vibrio Species, Bayelsa State, Nigeria*



INTRODUCTION

Vibrio cholerae is a potentially pathogenic Gram-negative bacterium. It is a facultatively anaerobic, oxidase positive and highly motile bacterium with the aid of polar flagellum. Regardless of a rich history of cholera with seven distinct pandemics since 1817 (Faruque *et al.*, 1998), the bacterium is still relevant in public health in many settlements where safe drinking water is inadequate or absent.

The cholera *vibrio* is primarily a waterborne infection (Idika *et al.*, 2000). The bacterium gets into the human system via faecal-oral route (Hutin *et al.*, 2003). The cholera *vibrio* is able to evade the acidic conditions of the stomach through movement into the small intestine using its flagellum. Once inside the small intestine, the bacterium under appropriate environmental indices secretes an enterotoxin known as cholera toxin, which alters the host's physiology through the alteration of the adenylate cyclase enzyme system. This results in excessive release of water and electrolytes into the intestinal lumen. The resultant effect of this massive fluid loss is diarrhea and vomiting (Miller, 2003; Siddique *et al.*, 1995).

The emergence of non-cholera *Vibrio* species associated with gastroenteritis is causing concerns to public health. *Vibrio parahaemolyticus*, which is a non-sucrose fermenting, oxidase positive and halophilic bacterium that could survive up to 6% salinity, is a prominent non-cholera *Vibrio* species with pathogenic potentials (Makino, 2003). Another non-cholera *Vibrio* species, *V. vulnificus*, infects humans through the ingestion of contaminated seafoods, mostly oysters (Miceli, 1993). *V. mimicus* is another non-cholera *Vibrio* species with epidemic potentials. This bacterium has been found to possess multiple copies of the ctx genetic element in its genome. This ctx genetic element is the lysogenic genome of the bacteriophage which confers toxigenic capabilities to *Vibrio cholerae* (Waldor & Mekalanos, 1996; Waldor & Raychaudhuri, 2000). Other non-cholera *Vibrio* spp are *V. metschnikovii*, *V. fluvialis*, *V. hollisae* and *V. alginolyticus* (Igbiosa *et al.*, 2009; Elhadi *et al.*, 2004; Rippey, 1994).

The prevalence of *V. cholerae* from different sources gives considerable information on the predisposing dangers. Also, the monthly prevalence of *Vibrio* spp generates indicative patterns of seasonal survival and persistence of these species from different environmental sources. The data obtained from the monthly distribution pattern of *Vibrio* spp is expected to give appreciable insights into the reasons cholera and other non-cholera *Vibrio* spp outbreaks are predominant in some months.

This study is aimed at the prevalence of *V. cholerae* from clinical and environmental samples from 5 (five) locations in Bayelsa State, South-South region of Nigeria. The study will also investigate the monthly distribution of total *Vibrio* spp counts in environmental samples to ascertain the seasonal effects on the survival and persistence of *Vibrio* species in fresh waters, brackish waters and seafoods.

MATERIALS AND METHODS

Study area

Bayelsa State is geographically located at 4.45° N, 6.05° E and its name was derived from three major towns within the boundary of the state—Brass (BA), Yenagoa (YEL) and Sagbama (SA). Bayelsa State is located in the South-South region of Nigeria (Figure 1a) with eight (8) local government areas. There are four spoken dialects in the state which include: Izon, Nembe, Epie-Atissa and Ogbia. The primary occupations of most of the local populations in the suburban and rural communities are fishing, agriculture and brewing of local gins. Five locations were selected in Bayelsa State for this study; they are: Brass, Southern-Ijaw, Ogbia, Sagbama and Yenagoa (Figure 1b).

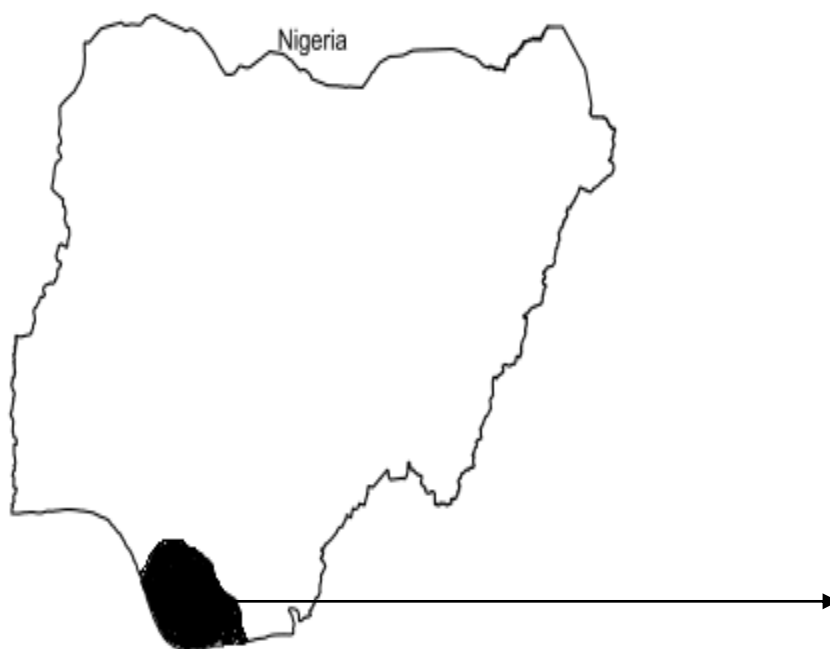


Figure 1a. Map of Nigeria showing the location of Bayelsa State.

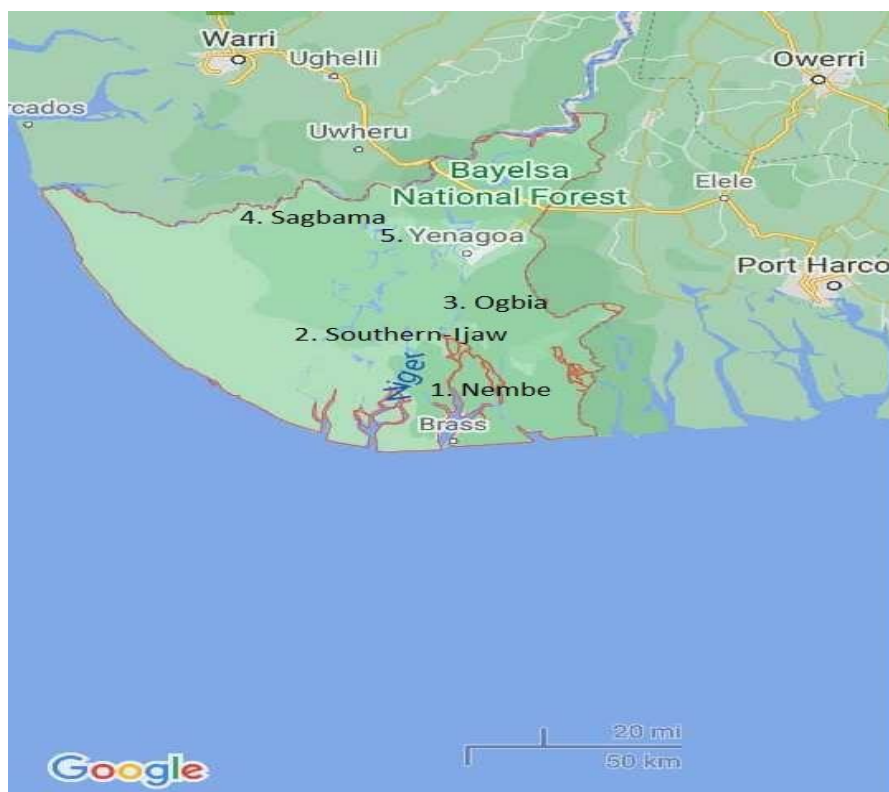


Figure 1b. Map of Bayelsa showing the selected localities of study.

Source: Google maps Inc.

Clinical samples

A total 44 stool samples were collected from January to April, 2019 from patients at the different primary health centres in Bayelsa. The samples were collected using sterile swabs sticks and inoculated into Cary-Blair medium for transport purposes.

Water samples

A total of 22 fresh water samples from wells and 60 brackish water samples, for the purpose of *Vibrio cholerae* detection, were collected between January and April, 2019 in different localities in Bayelsa State. The samples were inoculated into Cary-Blair medium for transport purposes.

Seafood samples

A total of 64 seafood samples were collected from major markets and in partnership with local fishermen in these localities. Samples were collected in sterile polythene bags, kept on ice and transferred to the laboratory for immediate analysis. Crabs were dissected and the chitinous external layers were discarded. Shrimps, fishes and the internal components of the dissected crabs were placed in a 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 a pour plate method. Cultures were thereafter incubated at 37⁰C for 24 hrs.

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 37⁰C for 24 hrs. Characteristic yellow colonies were considered for further identification based on the methods described by Canada Communicable Disease Report (1998).

Determination of viable plate count of *Vibrio* spp for samples

Water samples and seafood 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 serially diluted down to 10⁻⁶. From each water sample, 0.1ml and 1g of homogenized tissue of seafoods were inoculated by pour plate method on a freshly prepared thiosulphate citrate bile-salt sucrose (TCBS) agar (Oxoid, England) plate at 37⁰C for 24 hrs. The plate was examined for characteristic *Vibrio* spp colonies and enumerated.

Data representation

All statistical calculations involving mean values, standard deviations, bar charts and pictorial representations were determined using the Microsoft Excel version 2016 and SPSS (version 23.0).

RESULT

The examination of clinical samples from patients for cholera bacterium showed highest prevalence in Southern-Ijaw with 75% of examined patients (table 1). All the freshwater samples were contaminated in Brass and Southern-Ijaw while Sagbama/Kaiama and Yenagoa did not show presence of *V. cholerae* (table 2). In the same vein, all the samples of brackish waters from Southern-Ijaw (100%) were found to be contaminated with cholera vibrio (table 3). The distribution of *V. cholerae* from seafood showed highest prevalence in crabs with 40.91% of test samples with *V. cholerae* contamination (table 4). Table 5 shows the summary of prevalence of *V. cholerae* from all the sources. The highest percentage of occurrence was recorded in freshwater samples (54.55%) followed by samples from brackish waters (46.67%).

The average monthly distribution of *Vibrio* spp counts in freshwater samples from the different localities showed higher counts in the month of October to December and January to March (figure 2). Similarly, higher distribution of *Vibrio* spp counts were observed in the month of January to March and October to December, in samples of brackish waters (figure 3). The mean values of monthly distribution of *Vibrio* spp in crabs showed lower counts from the month of May to September (figure 4). The total viable *Vibrio* spp counts from shrimp samples had the lowest average values from the month of June to August (figure 5). Figure 6, which represents the mean monthly values of total *Vibrio* spp counts from fishes, showed lower distribution in



the month of June to August. There are statistically significant differences ($P < 0.05$) in the mean values of total *Vibrio* spp counts in months with recorded higher mean values.

The result of the antibiotics sensitivity patterns of *V. cholerae* showed all isolated strains were susceptible to the actions of ciprofloxacin, pefloxacin and ofloxacin while tetracycline, cotrimoxazole and augmentin showed different ranges of efficacies on isolated strains.

Table 1. Distribution of pathogenic *Vibrio cholerae* isolated in patients from different localities in Bayelsa State.

Localities	Total numbers of patients examined	Number of patients with cholera
Nembe/Brass	7	3 (42.86)
Southern Ijaw	12	9 (75.00)
Ogbia	10	4 (40.00)
Sagbama/Kaiama	7	0 (0.00)
Yenagoa	8	0 (0.00)
Total	44	16 (36.36)

*Numbers in parenthesis represents percentages of the positive cases of the test numbers

Table 2. Distribution of *Vibrio cholerae* isolated from freshwater in different localities in Bayelsa State, Nigeria

Localities	Total numbers of fresh examined	Number of wells with <i>V. cholerae</i>
Brass	3	3 (100.00)
Southern Ijaw	3	3 (100.00)
Ogbia	7	6 (85.71)
Sagbama/Kaiama	4	0 (0.00)
Yenagoa	5	0 (0.00)
Total	22	12 (54.55)

* Numbers in parenthesis represents percentages of the examined cases of the test numbers in each locality

Table 3. Distribution of *Vibrio cholerae* isolated from brackish waters in different localities in Bayelsa State, Nigeria

Localities	Total numbers of brackish waters examined	Number of brackish waters with <i>V. cholerae</i>
Brass	12	4 (33.33)
Southern Ijaw	12	12 (100.00)
Ogbia	12	8 (66.67)
Sagbama/Kaiama	12	0 (0.00)
Yenagoa	12	4 (33.33)
Total	60	28 (46.67)

*Numbers in parenthesis represents percentages of the examined cases of the test numbers in each locality

Table 4. Distribution of *Vibrio cholerae* isolated from seafood in Bayelsa State

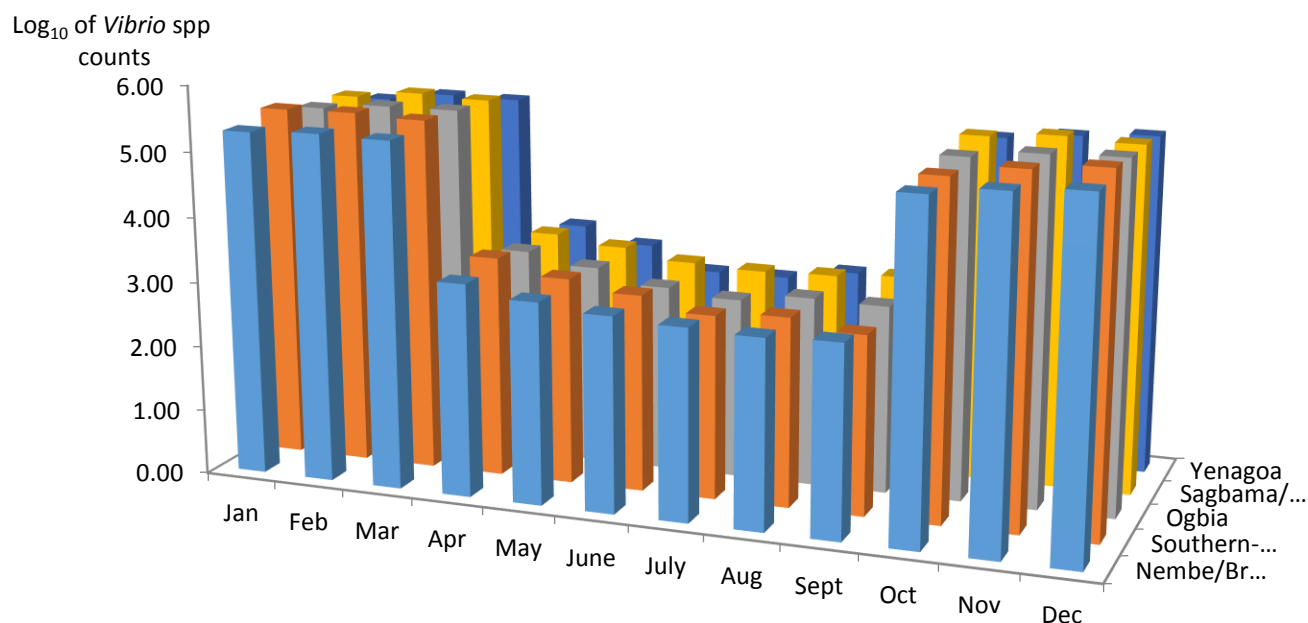
Seafood	Total numbers of seafood samples examined	Number of seafood samples with <i>V. cholerae</i>
Crabs	22	9 (40.91)
Shrimps	18	6 (33.33)
Fishes	24	7 (29.17)
Total	64	22 (34.38)

*Numbers in parenthesis represents percentage of examined seafood samples

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

Source	Number examined	Number positive for <i>V. cholerae</i>
Clinical	44	16 (36.36)
Fresh Water	22	12 (54.55)
Brackish	60	28 (46.67)
Seafood	64	22 (34.38)
Total	190	78 (41.05)

*Figure in parenthesis represents percentages

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

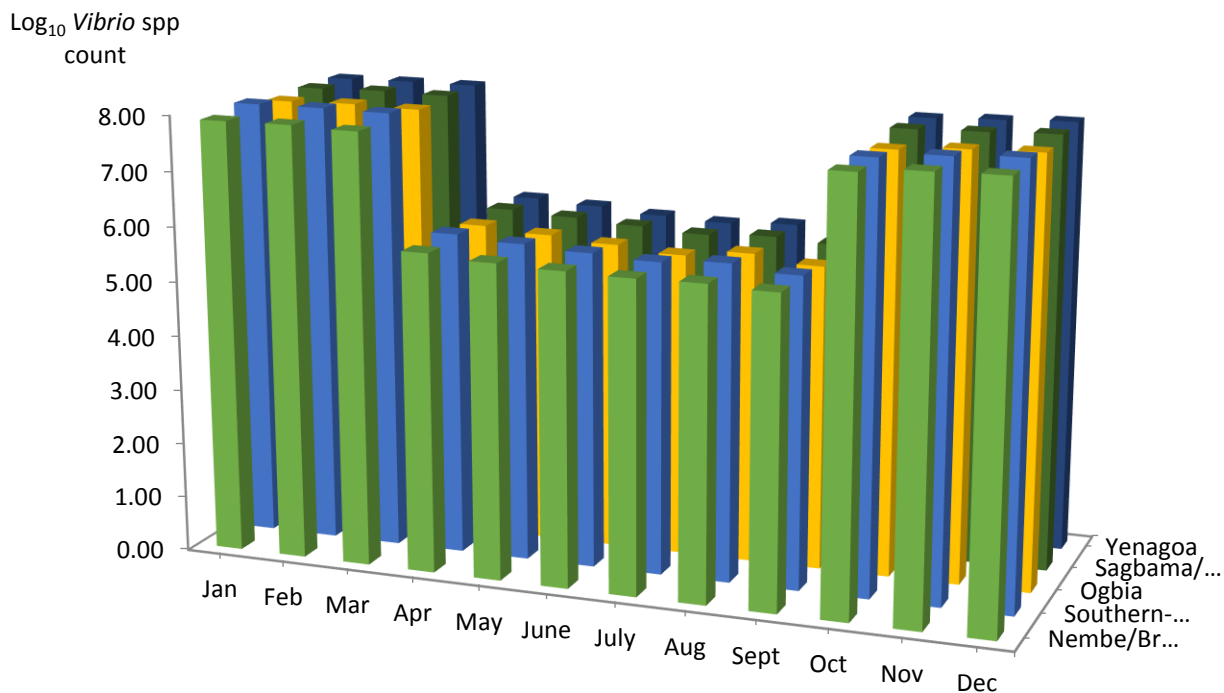


Figure 3: Average monthly distribution of *Vibrio* species counts in brackish water samples collected from different localities in Bayelsa State.

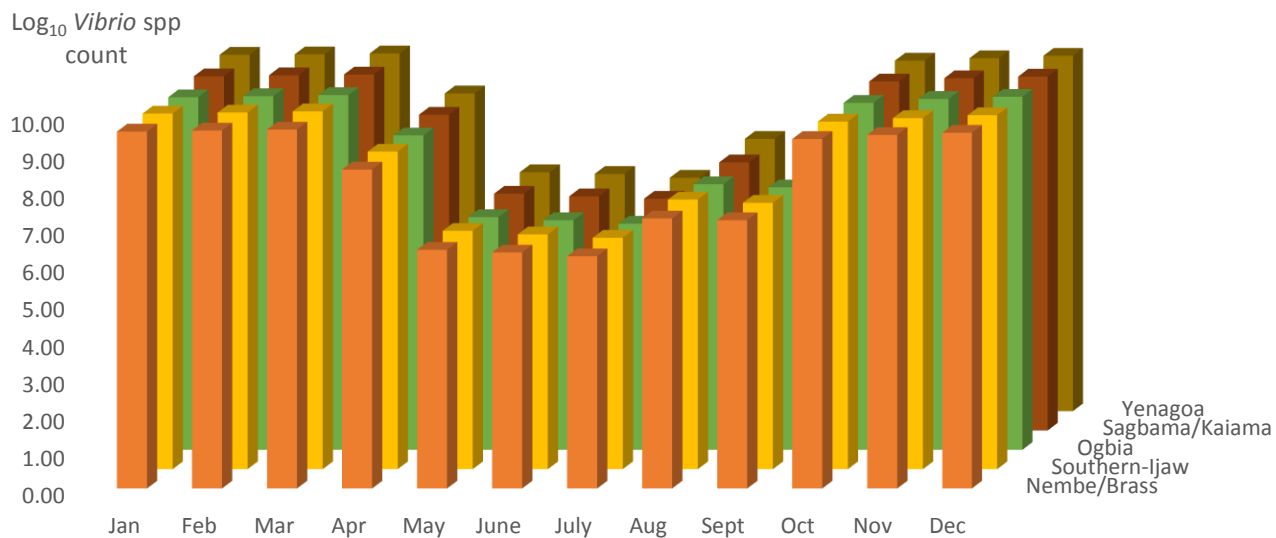


Figure 4: Average monthly distribution of *Vibrio* species counts in crabs from different localities in Bayelsa State.

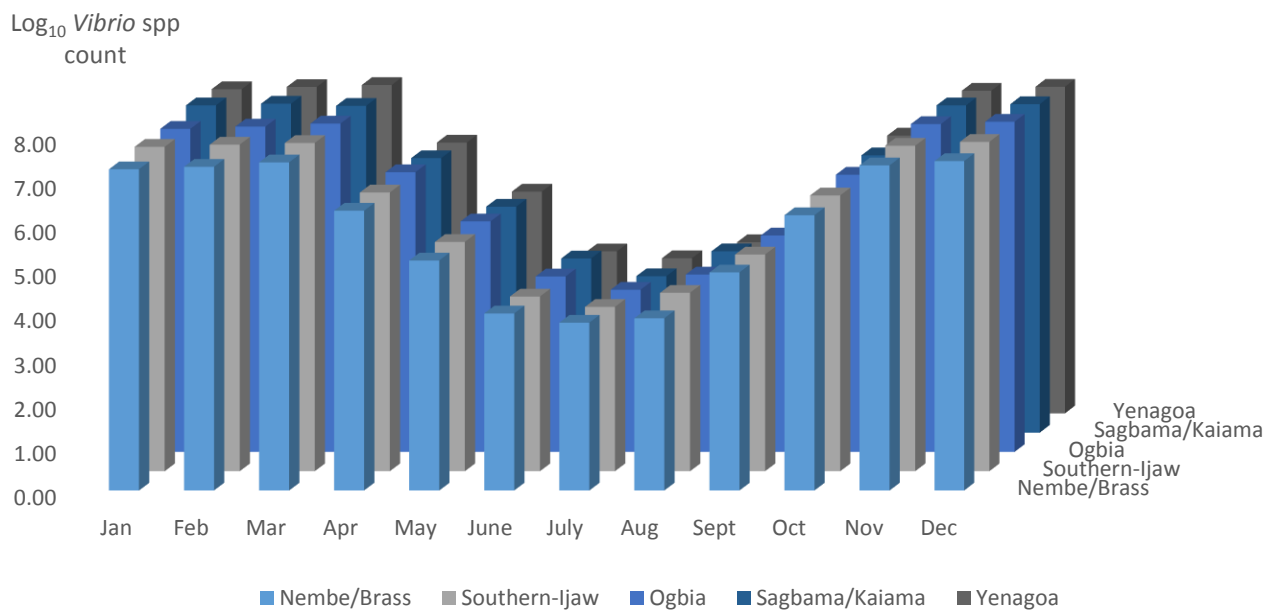


Figure 5: Average monthly distribution of *Vibrio* species counts in shrimps from different localities in Bayelsa State.

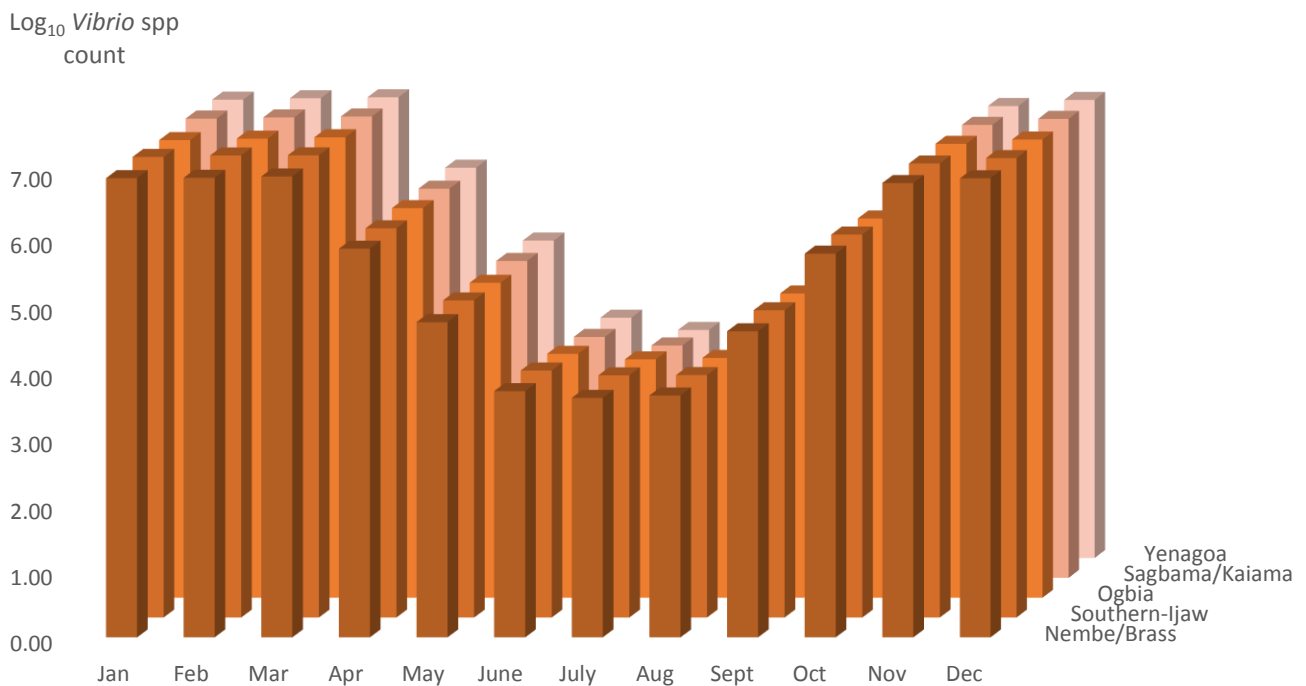


Figure 6: Average monthly distribution of *Vibrio* species counts in fishes from different localities in Bayelsa State.



Table 6. Antibiotics susceptibility of *Vibrio cholerae* from different sources in Bayelsa State

Antibiotics	Clinical		Fresh		Brackish		Seafood	
	Number examined	Number of sensitive strains	Number examined	Number of sensitive strains	Number examined	Number of sensitive strains	Number examined	Number of sensitive strains
Amx	44	29 (65.91)	22	15 (68.18)	60	37 (61.67)	64	41 (64.06)
Aug	44	34 (77.27)	22	16 (72.73)	60	41 (68.33)	64	44 (68.75)
Cot	44	32 (72.73)	22	14 (63.64)	60	39 (65.00)	64	42 (65.63)
Tet	44	38 (86.36)	22	18 (81.82)	60	52 (86.67)	64	54 (84.38)
Pef	44	44 (100.00)	22	22 (100.00)	60	60 (100.00)	64	64 (100.00)
Ofl	44	44 (100.00)	22	22 (100.00)	60	60 (100.00)	64	64 (100.00)
Cpx	44	44 (100.00)	22	22 (100.00)	60	60 (100.00)	64	64 (100.00)

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

DISCUSSION

The prevalence of pathogenic *V. cholerae* in patients from five (5) localities in this study (table 1) showed the highest percentage in patients from Southern-Ijaw (75%). The distribution of *V. cholerae* in freshwater samples was high in Brass, Southern-Ijaw and Ogbia (table 2). Similarly, cholera bacterium was recovered from all examined brackish water samples in Southern-Ijaw (100%) followed by Ogbia (table 3). The high percentage presence of *V. cholerae* in the water samples from these localities, especially Southern-ijaw, is expected. This could be attributed to the fact that there is an absence of effective sewage disposal system in many of these suburban creek settlements in Bayelsa State. The act of open defecation by local populations of these settlements, coupled with indiscriminate disposal of domestic wastes into water bodies, has been fingered as one of the predisposing epidemiological factors of infections and the resultant sporadic outbreaks in these communities. Aladese and Ariyo (2017) demonstrated a high correlation between indiscriminate disposal of human wastes and high microbial population in aquatic environments. Their assertion and our findings in this study further agree with other reported studies in the past (Edokayi, 2004; Onyema, 2011; Olorode *et al.*, 2015).

The average monthly values of *Vibrio* spp counts (\log_{10}) in freshwater samples (figure 2) showed a trend of lower mean values of prevalence in the months where rainfall is relatively higher. These months of profound increase in the amount of rainfall are collectively referred to as wet months or wet seasons of sub-Saharan tropical Africa. There is a statistically significant difference ($P < 0.05$) in the mean values obtained in dry months and wet months. Similar trends were observed in the \log_{10} values of *Vibrio* spp counts in brackish waters (figure 3). The reason for this trend could be as a result of increased discharge of freshwater into these surface waters during the wet seasons, due to increase in the amount of precipitation into the aquatic



environments. This assertion is in concordance with past studies (Lawson, 2011; Amangabara & Egenma, 2012).

The result of the monthly mean values of *Vibrio* spp counts (\log_{10}) in crabs (figure 4), shrimps (figure 5) and fishes (figure 6) underlie the continuous epidemiological importance of these seafood as environmental reservoirs and a major predisposing factor of infection, potentially pathogenic *Vibrio* spp (Rippey, 1994; Aladese & Enabulele, 2014; Oramadike & Ogunbanwo, 2015).

Effective treatment of cholera infection is predicated on constant epidemiological surveillance of emergence of antibiotic-resistant strains. Table 6 showed pefloxacin, ofloxacin and ciprofloxacin as the antibiotics recommended for treatment. The efficacy of these fluoroquinolone drugs in this study is not unexpected as it is in agreement with documented study in the past (Aladese *et al.*, 2015).

The presence of tetracycline-resistant strains in this study was not surprising. Amita *et al.* (2003) demonstrated the horizontal transfer of SXT genetic element among *V. cholerae* strains; this plasmid mediated transfer of SXT genetic element confers antibiotic resistance—especially of tetracycline—to a recipient *V. cholerae*. The presence of antibiotic-resistant strains in this study further agrees with reported findings in the past (Ottaviani *et al.*, 2001; Adeleye *et al.*, 2008).

CONCLUSION AND RECOMMENDATIONS

Incessant and indiscriminate disposal of wastes, especially excreta, into water bodies in these creek communities should be discouraged. Efforts should also be intensified by local and regional authorities on the importance of personal and communal hygiene through public awareness on the dangers of *V. cholerae* and non-cholera *Vibrio* spp infections so as to forestall possible epidemics in these communities.

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