



Prevalence Rate of *Vibrio cholerae* and other *Vibrio* Species Isolated from Stool Samples in Andoni Community of Rivers State

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CKW and EGN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AA and DTC managed the analyses of the study. Authors VNA and DTC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study aims at determining the prevalence of *Vibrio cholerae* among the people of Andoni Local Government. One hundred stool samples were collected after administering well-structured questionnaire and were analysed with standard microbiological techniques which includes; macroscopy, microscopy, culture on thiosulphate-citrate-bile salts-sucrose agar, Gram staining and biochemical tests (indole, motility, catalase and oxidase tests). Results showed a prevalence of 30% of *Vibrio cholerae*. *Vibrio parahaemolyticus* was also isolated with a prevalence rate of 42%. Among the predisposing factors, the prevalence rate of vibrio species based on demographic data showed positive with 77.8% of those tested within the age range 11-15years, 80% of those tested within the age range 16-20years while 6-10years and 26-30years had 0%. Among the sexes, 78.2% of the females and 64.4% of males tested were positive. In the religion category, 75.8% positive among Christians tested and 33.3% among other religious groups tested. Based on educational qualification, the primary students 42.9% of those tested were positive, 85.2% for secondary and 0% among tertiary students. Among the occupational status, 83.3 of those doing

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business, 20% of civil servants, 73.3% of self-employed and 75% of students were positive among each category tested. Based on the different communities, 100% of those from Ajakajak, 100% from Apahia, 100% of those from Dema, 71.4% of those from Ibotirem, 66.7% of those from Udung-Ama and 0% of those from Ngo were positive among each category tested. Most of the cholera infection observed were because of a bad water source and contaminated sea food consumption

Keywords: Cholera; vibrio; prevalence; stool, Andoni.

1. INTRODUCTION

In Nigeria, cholera is an endemic and seasonal disease, occurring annually mostly during the rainy season and more often in areas with poor sanitation, with the first series of cholera outbreaks reported between 1970 and 1990 [1]. Major epidemics also occurred in 1992, 1995-1996, and 1997. The Federal Ministry of Health reported 37,289 cases and 1,434 deaths between January and October 2010, while a total of 22,797 cases of cholera with 728 deaths and case-fatality rate of 3.2% were recorded in 2011. Outbreaks were also recorded in 2018 with the Nigeria Centre for Disease Control (NCDC) reporting 42,466 suspected cases including 830 deaths with a case fatality rate of 1.95% from 20 out of 36 States from the beginning of 2018 to October 2018 [2].

Andoni kingdom is an area largely occupied by the presence of water bodies, it is evident that one of their primary sources of food would be largely dependent on sea food, and there might be a possibility of exposure to the vector and causative organism.

The aquatic environments, including fresh, marine and brackish water bodies, have been identified as the natural reservoirs for cholera pathogen. A definite pattern of regular seasonal resurgence is characteristic of cholera [3]. Although studies have proven the existence of vibrio in association with copepods, shellfish, and in biofilm stage [4], a clear and established understanding about the pathogen's inter-epidemic reservoir is not yet known. Practice of poor hygiene in most water sides of Rivers state is now a norm, disposal of faeces in large water bodies, these water bodies serve as source for drinking water and as a source for food. The practice of proper hygiene is an important factor to the prevalence of cholera in a community.

On the 8th of January 2015, the state epidemiologist announced the report of suspected cholera outbreak in Andoni Local

Government Area of Rivers State. This was following a report from the Medical Officer of Health in the L.G.A and a confirmation of a case by the State WHO [5]. The outbreak was said to have started in Ukwa community, and was spreading to neighboring communities.

There were 77 cases of fever and acute watery diarrhea and 10 reported deaths [5] The Nigerian Field Epidemiology and Laboratory Training Program (NFELTP) residents in Rivers state informed the NFELTP program coordinator in Abuja about the unusual increase in reported cases and deaths from Gastro enteritis in the LGA. In response, four residents of the Nigeria Field Epidemiology and Laboratory Training Program (NFELTP) were mobilized to investigate the outbreak. The team worked in collaboration with the state WHO and the State Ministry of Health (SMOH) to investigate the outbreak and institute prevention and control measures [5]. The focus of this study is to determine the prevalence rate of *Vibrio cholerae* in the Andoni Community in South-East Senatorial district of Rivers State.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Andoni Local Government Area in Rivers State. The LGA is a riverine community made up of 12 wards and settlements with the wards. The major occupation of the people is Fishing and trading. Source of drinking water in most towns and villages is rain water collected into ponds or containers at home during the rainy season, and stream or well water in dry season. It has an area of 233 km² and an estimated population of 211,009 according to the 2006 census [5]. There are no toilet facilities in the homes of most of the people of Andoni and the source of excreta disposal is the overhung community latrine. There are 27 Primary health care centers and one general hospital in the LGA. Private hospital and Patent Medicine Vendors are also present in various towns in the LGA [5].

2.2 Study Population

The study population for this research work has a total number of 100 samples. These samples were collected from residents and students in government owned secondary schools in Andoni LGA.

2.3 Research Design

This research was carried out seasonally, from February to November 2020. The timeframe covers months before the rainy season, the middle of the rainy season up till the end of the rainy season. This is because *Vibrio cholerae* is influenced by season, hence seasonal determination is important. The study was designed to determine the prevalence rate of *Vibrio cholerae* isolated from stool samples. The research was done to examine the prevalence rate of cholera in Andoni L.G.A in Rivers State. Samples were collected and examined using standard bacteriological techniques.

2.3.1 Inclusion criteria for sample collection

- All participants who had diarrhea up to 3 months had their stool samples collected
- Participants who filled the questionnaire and gave their oral consent
- Those who passed out watery and soft stool had their samples collected
- Participants not on antibiotic had their stool samples collected
- Both male and female participants, between the ages of 1-60 had their stool samples collected.

2.3.2 Exclusion criteria for sample collection

- Participants on antibiotics medication were not included in the study
- Participants with constipation were not captured in the study to avoid wrong isolates
- Those who did not show interest did not have their stool samples collected

2.4 Sample Collection

A total of one hundred (100) diarrheic stool samples were collected from the elderly and students in Ibot-Ireme amongst other Neighborhood communities in Andoni L.G.A of Rivers State. All participants gave their consent before samples were collected and authorization was obtained from the school authorities. A comprehensive questionnaire was given to the

participants to fill in their personal details and demographic data. A clean dry wide-mouth screw capped universal container was given to each participant for sample collection. The containers were properly and correctly labeled with a laboratory number corresponding to each sample and individual. The samples were properly and correctly labeled. The samples were transported to the laboratory within three (3) hours after collection for analysis.

2.4.1 Experimental analysis

Samples were examined macroscopically, cultured on to Thiosulphate citrate bile sucrose agar (TCBS), examined for other pathogens in wet preparation. After 24hrs incubation at 37°C, the culture plates were examined for growth and morphological characteristics of *Vibrio cholerae* and other vibrio species. Gram stain and biochemical tests (oxidase, citrate, catalase, and indole) were carried out according to the standard procedures.

2.5 Statistical Analysis

Data collected from this study was statistically analyzed using descriptive and inferential statistical tool of Statistical Package for Social Science (SPSS) software package. The level of statistical significance was set at $\alpha=0.05$.

3. RESULTS

The prevalence of the isolates with *Vibrio cholerae* at thirty percent and *Vibrio parahaemolyticus* at forty-two percent.

Of the seventy-two plates, all were gram-negative rods, oxidase, catalase and indole positive and were all motile. Based on the colour on the culture medium and the biochemical tests carried out, two species of vibrio were isolated; *Vibrio cholerae* and *Vibrio parahaemolyticus* with a prevalence rate of 30% and 42% respectively.

The result shows that people within the age group of 16-20 years had the highest prevalence of 80% followed by age group 11-15 years which had a prevalence of 77.8% while age group 26-30 years and 6-10 years having 0% Prevalence respectively. Females showed a higher prevalence of 78.2% compared to men with a lower prevalence of 64.4%. The table also shows 72.0% prevalence in single people compared to 0% prevalence in married people. Students with secondary educational qualification had the

highest prevalence of 85.2%, the table also shows that persons who partook in businesses had the highest prevalence of 83.3% while civil servants had the lowest prevalence of 20.0%

under occupational status. Three communities; Ajakajak, Apahia and Dema had the highest prevalence of 100% respectively with Ngo community being the lowest with 0% prevalence.

Table 1. Prevalence rate of isolates

Isolates	Number Tested	Number Positive	Prevalence Rate (%)
<i>V. Cholerae</i>	100	30	30.0
<i>V. Parahaemolyticus</i>	100	42	42.0
Total Prevalence	-	-	72

Table 2. Biochemical tests of cultures that yielded growth

Test	Positive	Negative	Total
Gram	0	72	72
Oxidase	72	0	72
Catalase	72	0	72
Indole	72	0	72
Motility	72	0	72

Table 3. Prevalence rate of vibrio species based on demographics

Parameter	Number Tested	Number Positive	Prevalence (%)
Age			
11-15yrs	72	56	77.8
16-20yrs	20	16	80
26-30yrs	4	0	0.0
6-10yrs	4	0	0.0
Sex			
Female	55	43	78.2
Male	45	29	64.4
Marital Status			
Married	0	0	0.0
Single	100	72	72.0
Religion			
Christian	91	69	75.8
Muslim	0	0	0
Others	9	3	33.3
Educational Qualification			
Primary	7	3	42.9
Secondary	81	69	85.2
Tertiary	12	0	0
Occupational Status			
Business	36	30	83.3
Civil servant	10	2	20.0
Self Employed	38	28	73.7
Student	16	12	75.0
Community			
Ajakajak	13	13	100
Apahia	4	4	100
Dema	6	6	100
Ibotirem	63	45	71.4
Ngo	8	0	0
Udung-Ama	6	4	66.7

Table 4. Percentage distribution of predisposing factors

Parameter		Frequency (%)
Drinking Water Source	Bore Hole	7 (7)
	Tap Water	18 (18)
	Well Water	75 (75)
	Total	100 (100)
Cooking Water Source	Bore Hole	7 (7)
	Tap Water	12 (12)
	Well Water	81 (81)
	Total	100 (100)
Bathing Water Source	Bore Hole	7 (7)
	Tap Water	8 (8)
	Well Water	85 (85)
	Total	100 (100)
Sea Foods Consumed	Crabs, Periwinkle, Fish	4 (4)
	Fish	32 (32)
	Periwinkle	10 (10)
	Prawns, Crabs, Periwinkle, Fish	54 (54)
	Total	100 (100)

Table 5. Association between cholera and other variables

	Variable Description	Chi Square	df	p-value	Interpretation
Socio-demographic	Age	37.087	8	.000	Significant
	Sex	3.319	2	.190	Not Significant
	Education	42.177	4	.000	Significant
	Occupation	17.023	6	.009	Significant
	Community	67.879	10	.000	Significant
Knowledge/Practices	Drinking Water Source	20.608	4	.000	Significant
	Cooking Water Sources	16.436	4	.002	Significant
	Bathing Water Source	25.173	4	.000	Significant
	Sea Foods Consumed	44.123	6	.000	Significant
	Knowledge of Cholera	22.052	2	.000	Significant
	Had Diarrhoea and Dehydration	18.633	2	.000	Significant
	If Yes, How Long Ago	5.637	2	.060	Not Significant
	Visit to Hospital	20.730	4	.000	Significant
	Had Laboratory Test	17.183	4	.002	Significant
	Antibiotic Administration	31.624	6	.000	Significant
	How Long for Antibiotic Administration	64.217	6	.000	Significant
	Are you still on Antibiotics	7.311	4	.120	Not Significant
	Identification Technique	Macroscopy	99.259	24	.000
Microscopy		82.404	10	.000	Significant
TCBS		163.810	40	.000	Significant
MacConKey		190.204	38	.000	Significant
Oxidase		81.203	2	.000	Significant
Catalyse		81.203	2	.000	Significant
Motility		74.359	2	.000	Significant

It was observed that 75% of the study population get their drinking water from the well, 18% from tap and 7% from borehole. It also shows that

81% get their cooking water from well, 12% from tap and 7% from borehole, and their source of bathing water; 85% well water, 8% tap water and

7% borehole water. The results shows that the well water happens to be their major source of water usage. Results also show that 54% of the study population consume seafoods such as prawns, crabs, periwinkle and fish, 32% consumes just fish, 10% consumes periwinkle and 4% consumes crab, periwinkle and fish.

Significance was observed among all the socio-demographic factors except sex, also among identification techniques and antibiotic profiling. In the knowledge and practices category, significance was observed in all except, duration of diarrhoea and dehydration and if the person was still on antibiotic.

4. DISCUSSION

In this study one hundred (100) stool samples were collected from residents and students in government owned secondary schools in Andoni Local Government Area. Of the 100 samples used for the research 30 were positive for *Vibrio cholerae* which gave a prevalence rate of 30% (Table 1) and this is in agreement with the research carried out by Utsalo et al., [6] in Calabar which had a prevalence of 30.6%. In Utsalo et al., [6] it was also noted that the occurrence of cholera in Calabar occurred seasonally as seen in Andoni community of Rivers state. The low level of *Vibrio cholerae* in Andoni could be due to an increased awareness to consumption of properly cooked periwinkles, crabs and prawns which are natural hosts for the causative organisms. Forty-two samples out of the total population tested positive for *Vibrio parahaemolyticus* as shown in the culture growth carried out. A total of 28 samples yielded no growth on culture plate.

Biochemical characterization and identification of isolates presented showed that *Vibrio* species exhibited positive reaction to the biochemical tests used (catalase, oxidase, insole and motility test) carried out. Gram staining technique was also used which identified the isolates as gram positive organisms. The colonies appeared on Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar at 37°C for 24 hours as yellow colonies (30%) and green colonies (42%) of about 2-4mm in diameter.

The prevalence rate of *vibrio* species based on demographic data shows 77.8% of those tested within the age range 11-15years were positive, 80% of those tested within the age range 16-20years were positive while 6-10 years and 26-

30years had 0%. Among the sexes, 78.2% of the females tested were positive and 64.4% of males tested were positive. This study is in agreement with Elimian et al., [7], which showed that individuals aged 15 years or older (47.76%) were the most affected age group during the outbreak and with respect to gender, there was a slight dominance of females (50.74%) over males (49.26%). In the religion category, 75.8% positive among Christians tested, and 33.3% among other religious groups tested. Among the primary students 42.9% of those tested were positive, 85.2% for secondary and 0% among tertiary students. Among the occupational statuses, 83.3 of those doing business, 20% of civil servants, 73.3% of self-employed and 75% of students were positive among each category tested. Based on the different communities, 100% of those from Ajakajak, 100% from Apahia, 100% of those from Dema, 71.4% of those from Ibotirem, 66.7% of those from Udung-Ama and 0% of those from Ngo were positive among each category tested. No study has considered religion, a study in Bangladeshi by Colombara et al.,[8] identifies education as a predisposing factor, and according to this research those that have secondary educational qualification show higher frequency, this could be as a result of over population in classrooms, mode of food consumption and lifestyle in secondary schools in Andoni community of rivers state. A study conducted by Stoltzfus et al., [9] in India stated significance in occupational status as a predisposing factor, having a frequency distribution rate of 80% thereby agree to the research at Andoni which has a distribution of 81% for secondary schools. There might yet be a study for communities as predisposing factors.

Source of water for drinking, cooking and bathing and consumption of sea foods are major predisposing factors for contracting *Vibrio cholerae*. This study reveals that those that have borehole water as their source of drinking water had a 100% prevalence rate among those tested in that category, for those with tap water had 55.5% prevalence rate among those tested in that category, and those with well water as their source of drinking water had the highest number of those tested with a prevalence rate of 73.3%. People with bore- hole water as their source of cooking had a 100% prevalence rate among those tested in that category, for those with tap water, 33.3% prevalence rate among those tested in that category, and those with well water had the highest number of those tested with a prevalence rate of 75.3%. Those with borehole

water as source of bathing had a 100% prevalence rate among those tested in that category, for those with tap water, 0% prevalence rate among those tested in that category, and those with well water had the highest number of those tested with a prevalence rate of 76.5%. Tarh [10] described those who live in areas that lack potable water supply and good toilet systems and also drink from brooks, springs, streams and shallow wells which are easily polluted by sewage from the environments as one of the easiest ways of becoming infected with cholera. When it comes to sea foods consumed the prevalence rate among those that consume prawns, crabs, periwinkle and fish had the highest number of individuals tested with a prevalence rate of 92.6%, followed by those that consume just fish with a prevalence rate of 37.5, and then those that consume periwinkle with a prevalence rate of 60, and the least are those that consume crab, periwinkle and fish with a prevalence rate of 100% among those tested. Since the bacterium (*Vibrio cholerae*) is often excreted in stool and vomitus, it can easily be carried by vectors such as house flies to contaminate food most especially [10]. Vegetables contaminated by faeces carried by surface runoff can also serve as vehicles for the transmission of these pathogens to human if they are eaten raw or undercooked and this applies to sea foods also [10]. Result of bi-variate analysis for risk factors for cholera carried out by Kanu and colleagues [11], revealed that drinking tap water was protective of the disease with an odds ratio of 0.1 and Confidence Interval 0.0-0.7. Kanu and colleagues [11] carried out a physical assessment of affected communities in Andoni, which revealed very poor drinking water sources, inadequate refuse and sewage disposal methods, and the community latrine where majority of the inhabitants defecated was an overhung toilet which empties directly into the stream. The residents defecate, fish from and eat of the various sea foods from the same water body. This is in agreement with the studies carried out in Germany [12], France [13], and Calabar Nigeria [14] where it was reported that sea foods have been implicated in the risk factors for cholera.

It is evident in this study that well water has a greater distribution rate of 75% which agrees with a research carried out by Ranjbar et al., [15] that also proved that contaminated well water can be the major leading factors for the spread of cholera, due to the shallowness of some wells and their proximity to water and indiscriminate

dedication in water bodies can lead to contamination of wells in Andoni community of Rivers state. A study carried out by Rabbani et al., [16] identified food as a major means for the transmission of cholera, they also identified that using contaminated well water as source of bathing and cooking can also lead to the spread of cholera, this agrees with the study carried out in Andoni which stated out on table 4.8 that well water can be a major predisposing factor for the spread of *Vibro cholerae* in Andoni community of Rivers state.

When association between cholera and other variables were analysed, in the socio-demographic category, significance with p-value <0.0001 was observed with age, education (p-value <0.0001), occupation (p-value 0.0090), community (p-value <0.0001), sex was not significant (p value 0.1900) Sur et al. [17] associated cholera to young age and lower educational level as predisposing factors. In the knowledge and practices category, significance was observed in the drinking water source (p-value <0.0001), cooking water source (p-value 0.0020), bathing water source (p-value <0.0001), sea foods consumed (p-value <0.0001), knowledge of cholera (p-value <0.0001), those that had diarrhoea and dehydration (p-value <0.0001), visited the hospital (p-value <0.0001), had laboratory test (p-value 0.0020), antibiotic administration (p-value <0.0001), duration for antibiotic administration (p-value <0.0001). those that were still on antibiotics were not significant (p-value 0.1200) In a multivariate analysis by Stoltzfus et al. [9], water, lack of proximity to health facilities, lack of piped water, and religion were significantly associated with an increased risk of cholera. In the category of identification technique, macroscopy, microscopy, TCBS, indole, oxidase, catalase, and motility were all significant with p-value at <0.0001.

5. CONCLUSION

The investigation on cholera in Andoni local government area gave a percentage prevalence of 30% with well water being the most common source of exposure. Although limited epidemiologic information and studies existed regarding the extent of infection and characteristics of circulating strains in Nigeria, there is clearly a link to poverty, dirty environment, and lack of social amenities including provision of good water sources. These factors definitely imparted much on the frequency

and severity of the disease as well as its epidemic potential.

6. RECOMMENDATION

The Rivers State Ministry of Health should support to institute prevention and control measures by providing technical support, supply medical kits and community sensitization. There is need for continuous community health education on the critical times of hand washing, especially after defecation. Alternate sources of portable drinking water should be provided in affected communities of Andoni LGA of Rivers State.

CONSENT AND ETHICAL APPROVAL

Permission was obtained from the relevant school and community authorities before samples were collected. Participants also showed interest and gave their consent.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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