International Journal of Innovative Biochemistry & Microbiology Research 7(4):10-16, Oct.-Dec., 2019

© SEAHI PUBLICATIONS, 2019 **www.seahipaj.org** **ISSN: 2467-8554**

Incidence of *Vibrio cholerae* from Different Borehole Water Tanks In Alakahia Community, Obio/Akpor Local Government Area, Rivers State, Nigeria

Austin A. Okwelle[1] & Precious Baridilo Kpea[2]

Department of Biological Sciences (Microbiology Unit)
Faculty of Natural Applied Science
Ignatius Ajuru University of Education
Rumuolumeni, P. M. B 5047, Port Harcourt, Rivers State, Nigeria

[1]Email: okwelleaa@yahoo.com, austin.okwelle@iaue.edu.ng; Tel: 234-8030985524

[2]Email:preciousbaridilokpea@gmail.com; Tel: 234-8063764075

ABSTRACT
Cholera is an epidemic disease that affects different regions of the world, causing significant morbidity and mortality and economic losses to many developing third world countries including Nigeria. The aim of this study is to investigate the incidence of *Vibrio cholerae* in different borehole water tanks in Alakahia community, Obio/Akpor Local Government Area, Rivers State, Nigeria. Five boreholes tanks in the community were randomly selected for the study. The water samples for analysis were collected from each of the boreholes in two months (November and December) in 100ml sterile plastic containers with screw caps and appropriately labeled. The isolation and enumeration of total aerobic heterotrophic bacteria count of water samples was done using the standard dilution pour plate method and nutrient agar medium, while total and faecal coliforms were estimated using the Most Probable Number (MPN) method and Eosin Methylene Blue (EMB) medium. The total *V*ibrio count was enumerated using Thiosulphate Citrate Bile Salts Sucrose (TCBS) medium. The results indicated that total heterotrophic bacteria count ranged from 17.5 to 2.5 cfu/ml, while total coliform count of 33MPN Index/100ml was observed. *Staphylococcus aureus, Escherichia coli, Vibrio, and Pseudomonas aeruginosa* species were identified. *Staphylococcus aureus* had the highest percentage frequency of occurrence (38.9%), followed by *Escherichia coli* and *Pseudomonas aeruginosa* (22.2%), and *Vibrio sp* (16.7%).The analysis of variance shows no significant difference between the heterotrophic bacteria count, while there was significant difference in total coliform count at $p< 0.05$ level of significance. The microbial load can be reduced to minimum levels or eliminated through appropriate siting of boreholes and adherence to proper water treatment regimes.
Keywords: *Vibrio cholera*, borehole water tanks, humans, cholera

INTRODUCTION

According to Lippi and Gotuzzo (2013), Filippo Pacini was the first to discover the causative agent of cholera in humans in 1854, while examining the corpses of the patients who died in Santa Maria Nuova public hospital in Florence when the city experienced a devastating outbreak of cholera. Filippo Pacini called the millions of elements which he identified from the faeces and intestinal mucosa of the patients Vibrions (Nardi, 1954). However, the observations of Filippo Pacini remained unknown until 1966 when

the International Committee on Nomenclature formally accepted the denomination '1854 Pacini's cholera's Vibrio' as the causative agent of cholera (Feeley, 1966).

V. cholerae is known to belong to the family *Vibrionaceae*. It is also a facultative anaerobe, gram-negative, non-spore forming curved rod, with comma shape and size range of 1.04–1.06um long. *V. cholerae* is capable of utilizing both respiratory and fermentative pathways for metabolism. The incorporation of 1% sodium chloride (NaCl) in a medium is required to stimulate the growth of *V. cholerae*. It possesses both flagellar (H) and somatic (O) antigens in its structure. According to Tyagi and *Agarwal*, (2014), the organism is better defined on the basis of the biochemical tests and DNA homology studies. The DNA homology study is used to differentiate the organism into pathogenic and non-pathogenic strains, and currently the organism is classified into 206 'O' serogroups. *V. cholerae*, is classified into two serotypes: O1 and non O1 (Chatterjee and Maiti, 1984). The O1 serogroup of *V. cholerae* is further classified into two biotypes, namely , the classical and El Tor biotypes. In 1993, *V. cholerae* serogroup O139 made an explosive appearance and caused a severe epidemic in the Indian continent (Ramamurthy *et al*., 1993). The *V. cholerae* 01 and 0139 are the major strains identified with cholera disease epidemics. All strains that were identified as *V. Cholerae* on the basis of biochemical tests but did not agglutinate with "O" antiserum are generally grouped as non 01 *V. cholerae.* The EL Tor biotype of *V. cholerae* is the most common cholera pathogen.

V. cholorae exists in the marine environment in several forms. These include a free living state, particularly during high water temperature and nutrient concentrations; an epibiotic phase, association of *Vibrios* with specific substrates, such as chitin of shellfish and the viable but non-culturable state (Akubuenyi *et al.,* 2013). Faruque (2013) identified *V. cholerae* strains in fresh water, carrying both virulence factors and resistance to antimicrobial agents.

Cholera is closely connected with poverty, poor sanitation and lack of uncontaminated drinking water. *V. cholerae* is responsible for a large number of water borne outbreak (Agbugui and Deekae, 2016). Khan *et al.*, (1994), stated that out of 632 cholera reports, 65% originated in Sub Saharan Africa, 16.8% in south Asia, 7.1% in south and Central America and 5.7% in North Africa and west Asia. Lutendo *et al*, (2016) noted that approximately six to nine million people die annually, worldwide due to water-related diseases. According to Tyagi *et al.*, (2014), *Vibrio cholerae* 01 can survive in de-chlorinated drinking water for 10 days with the presence of oxide and organic materials prolonging its survival.

Seiyabor *et al.*, (2013) observed that cholera has unique epidemiologic characteristics. *Vibrio cholerae* O1 and O139 strains produce a cholera toxin (CTX) that causes cholera; illness caused by other *Vibrio* strains are referred to as Vibriosis. The toxigenic *V. cholerae* is a native flora of the aquatic environment which is transmitted through drinking water and still remains the leading cause of morbidity and mortality in many underdeveloped countries ((CDC, 2003). According to Yusuf (2007), there is no supply of potable water to most African communities, with many of the residents resorting to drill boreholes for their households, while others buy from water vendors in tanks. The issue with the use of water from these sources is that they are not treated and so do not meet the WHO approved standard for drinking water, hence the need for continuous monitoring water for the possible contamination with pathogenic bacteria species.

3.0 MATERIALS AND METHODS

3.1Description of Study Area

This study was carried out in five borehole tanks in Alakahia community, Obio/Akpor Local Government Area, Rivers State. The locations of the tanks are: Public Borehole Mission Road (longitude 4 53' 6.81N, longitude 6 55' 25. 3812" E), Ogbonda's compound (latitude 4 53'5.0208"N, longitude 6 55'20. 0388"), Silver's compound (latitude4 53'14. 856"N, longitude 6 55'17.0652"E), Woke's compound (latitude 4 53'10.23"N, longitude 6 55' 46.210" E), and Great Villa Lodge (latitude 4 52'58.35" N, longitude 6 55'46.210" E).

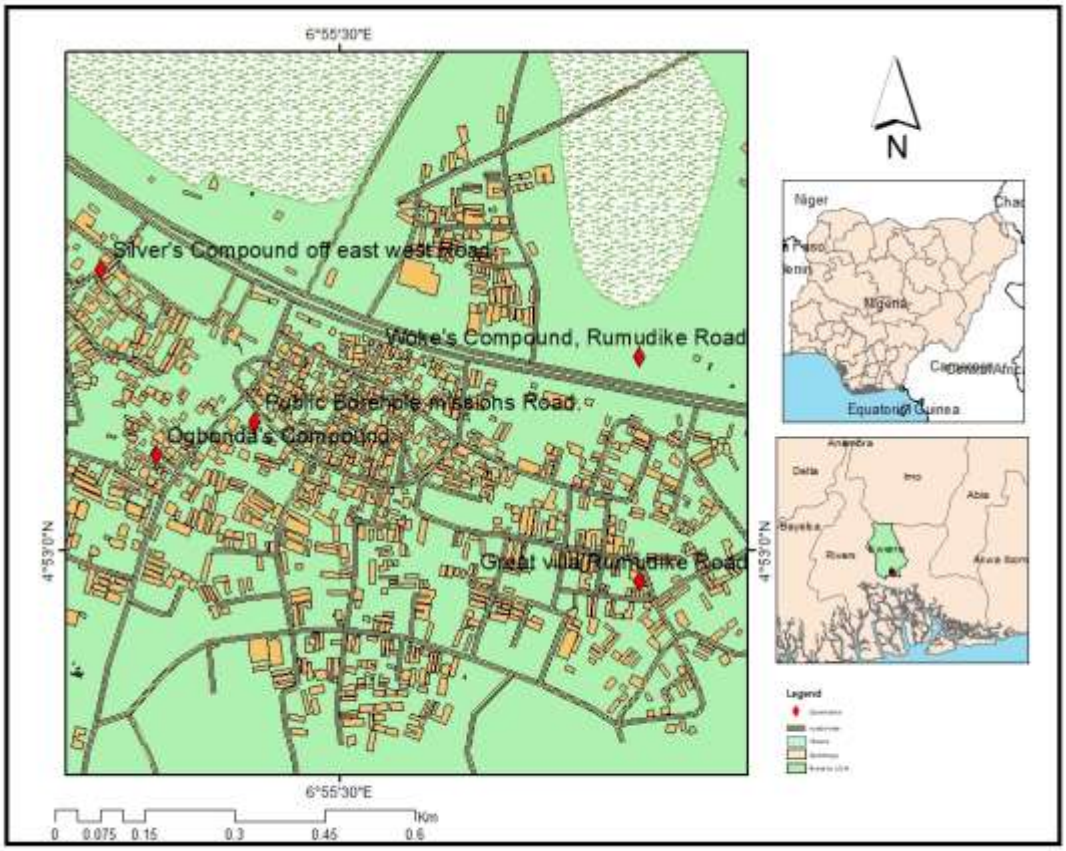

Figure 1: Map of Obio/Akpor Local Government Area showing study locations.

3.2 Collection of Samples

Samples for the water analysis were collected from each of the 5 boreholes located in the community between the months of (November and December). The samples were collected in appropriately labeled sterile plastic containers with screw caps and analysed within 24 hours. All the plastic containers were sterilized with 70% ethanol and initially rinsed with sterile distilled water and thereafter with the water samples from the boreholes. The APHA, (2005) sampling protocols was adopted for the sample collection

3.3 Enumeration of Total Aerobic Heterotrophic Bacteria Count

The total heterotrophic aerobic bacteria was isolated and enumerated using the standard dilution pour plate method in nutrient agar medium. Ten-fold serial dilution of (10^{-1} to 10^{-4}) was prepared using physiological saline and 1ml of the dilutions was transferred to sterile petri dish plates. The sterilised nutrient agar medium was melted by heating in boiling water and then allowed to cool in water bath to 45^{o}C. Approximately 20ml of the nutrient agar medium was poured into the petri dish plates containing the sample. The sample and agar were mixed thoroughly by rotating the plate several times. When the media has solidified, the plates were inverted and incubated at 37^{o} C for 24-48 hours. Following the appropriate length of incubation, 10^{-3} dilutions were selected and visible colonies were counted using a colony counter. The colonies were expressed as colony forming unit cfu/ ml of water.

3.4 Isolation and Enumeration of Total and Faecal Coliform Bacteria Counts

Total coliforms were estimated using the most probable number (MPN) method. MacConkey's lactose bile salt broth with neutral red as indicator was used for the presumptive tests. With a sterile pipette, 50ml of each of the water sample was aseptically dispensed into 50ml double strength broth another 10 ml of the sample into each of the five tubes containing 10ml double strength broth and another 1ml of the

sample was then inoculated into each of the second five culture tubes containing 5 ml single strength MacConkey broth with Durham's tubes. Inoculated tubes of MacConkey broth were incubated at 37^{o}C for 24 to 48 hours. After 24-48 hours of incubation, the cultures were observed for the presence of acid production and gas formation. A sterile pipette was used to transfer 1ml of the culture from the positive presumptive fermentation tubes into tubes containing 5ml MacConkey broth aseptically and incubated for 24-48 hours at 37^{o}C. Following incubation, culture positive tubes were inoculated into MacConkey agar for total Coliform and Eosin Methylene Blue agar for faecal coliform and incubated at 37 ^{o}C and 44 ^{o}C respectively. The MPN was then determined from the MPN table for the three set of tubes as described by ALPHA (2005) and Dhawale and LaMaster (2003). On Eosin Methylene Blue (EMB) agar, *E. coli* strains shows a greenish metallic sheen colonies and this was further confirmed by the ability of the organism to ferment lactose at 44.5oC, while *Aerobacter aerogenes* appeared as large pinkish mucoid colonies (Edema *et al*., 2001 and Adogo *et al*., 2016). Mannitol salt was used for the isolation of *Staphylococcus aureus* while *Salmonella* species was isolated on Salmonella-Shigella agar (Adogo *et al*., 2016).

3.5 Characterization and identification of bacteria isolates

The characterization and identification of the isolated bacteria species was done using the Gram staining techniques and biochemical tests.

4.0 RESULTS

Table 1: Total Heterotrophic bacteria count

Bore hole sample	**10^3**	**10^3**	Mean value
A1	25	20	22.5
A2	15	20	17.5
B1	35	30	32.5
B2	25	23	24.5
C1	35	37	36.5
C2	40	43	41.5
D1	25	24	24.5
D2	35	40	37.5
E1	36	30	33.0
E2	40	45	42.5

The total heterotrophic bacteria count (THB) ranged from 17.5 to 42.5 cfu/ml. The density of THB from the water samples from the study area were highest at location five (Great villa lodge, Rumudike Alakahia) with a mean density of 42.5cfu/ml and least at location one (Mission Road, Alakahia) with a density of 17.5cfu/ml. Also, the total heterotrophic bacteria count (THB) of 41.5cfu/ml, 37.5 cfu/ml and 32.5 cfu/ml were observed at location 3 (Silver's compound Choba extension Alakahia), location 4 (Woke's Rumudike Alakahia) compound and respectively Location 2 (Ogbonda's compound, Rumudamaya Alakahia).

Table 2: Total MPN of coliform count

Bore hole sample	[1]	2	3	MPN INDEX/100mL
A1	0	0	0	0
A2	0	0	0	0
B1	1	0	5	25
B2	1	0	0	1
C1	1	0	0	1
C2	0	0	0	0
D1	1	0	0	1
D2	0	1	3	5
E1	0	0	0	0
E2	0	0	0	0
TOTAL	0	0	0	33 MPN INDEX/100mL

The total coliform count was 33MPN Index/100mL, no coliform count was observed for the two samples taken from location1 (Mission Road). The highest coliform count was observed for the sample taken from location 2 (Ogbonda's compound).

Table 3: Bacteria species identified from borehole water

Bacteria Isolates	**Locations**										**Total(%)**
	A1	A2	B1	B2	C1	C2	D1	D2	E1	E2	
Staphylococcus aureus	1	1	1	-	1	1	1	1	-	-	**7(38.9)**
Escherichia coli	-	-	1	1	-	-	-	1	-	1	**4(22.2)**
Vibro sp	-	-	1	1	-	-	1	-	-	-	**3(16.7)**
Pseudomonas aeruginosa	-	-	-	-	-	-	1	1	1	1	**4(22.2)**
Total(%)	**1**	**1**	**3**	**2**	**1**	**1**	**3**	**3**	**1**	**2**	**18(100%)**

Table 4: One-way ANOVA of heterotrophic bacterial count for the five samples

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	936.3	234.07	7.37	0.002
Error	15	476.2	31.75		
Total	19	1412.5			

Since the p-value is less than the level of significance (0.05), we accept the null hypothesis and conclude that the test is not statistically significant. Thus, the result in Table 4 shows that no significant difference exist between the heterotrophic bacteria count for the five samples at 0.05 level of significance.

Table 5: One-Way ANOVA for total MPN of coliform count for the five samples

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	6.867	1.717	1.62	0.201
Error	25	26.500	1.060		
Total	29	33.367			

Table 5 shows that the total coliform count differs significantly between the various water samples at 0.05 level of significance since the p-value (0.201) is greater than the level of significance.

Table 6: One-Way ANOVA of bacterial isolates for the samples

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	9	2.4	0. 2667	1.07	0.414
Error	30	7.5	0.2500		
Total	39	9.900			

In Table 6, the p-value (0.414) is greater than the level of significance (0.05). Thus, a significant difference exist between the numbers of bacteria isolates from the various water samples at 0.05 level of significance.

5.1 DISCUSSION

The study was carried out to determine the incidence of *Vibrio cholerae* in boreholes used as drinking water sources in Alakahia community, Port Harcourt. The findings of the study reveals that the density of total aerobic heterotrophic bacteria count from the water were highest at location five (Great villa lodge, Rumudike, Alakahia) with a mean density of 42.5cfu/ml. Four genera of bacteria were identified from the water samples studied including; *Staphylococcus, Escherichia coli, Vibrio,* and *Pseudomonas* species. *Staphylococcus aureus* had the highest percentage frequency of occurrence (38.9%), followed by *Escherichia coli Pseudomonas aeruginosa* (22.2%), and *Vibrio sp* (16.7%). Uzoigwe (2012), also identified *Vibrio* species from borehole water with prevalence of 33.3%. Nkwachukwu (2013) reported that 33% of borehole water samples analysed had greater than 100cfu/ml for total viable counts and from 0 to 3 MPN/100ml for faecal coliform respectively. Azunwo *et al*., (2017) also stated that the average microbial load from borehole water was 1.78 × 103 with total coliform and faecal coliform count of (4.6/100 mL) and (0) respectively.

The high count of these pathogenic bacteria observed in the borehole water samples could be attributed to many factors such as, sitting of boreholes close to septic tanks and dumpsites, extraction of ground water from shallow aquifers, indiscriminate dumping of sewage and wastewater from domestic activities, and the discharge from septic tanks and latrines close to the bore holes. This is in line with the position of Nwachukwu and Otokunefor (2006) who stated that there is a positive correlation between high bacterial count in borehole water supplies and discharges from septic tanks and waste materials from close dumpsites. Similarly, Uzoigwe and Agwa (2012) attributed the high incidence of coliform and other pathogenic bacteria in borehole water to seepages from septic tanks into household drinking water supply, poor latrine systems which have exceeded their expected life span or post treatment contamination along the distribution line.

The results of this study shows that most of the parameters analysed in the water samples from the areas under study were not within the acceptable water quality standards. This reveals that these drinking water sources are polluted. High total and faecal coliform count in the water samples may be as a result of contamination of water from the sewerages and other pollutants. WHO (2003) and the Nigerian Standard for Drinking Water Quality (NSDWQ, 2007) recommends that no faecal coliform should be found in any water meant for drinking.

5.2 CONCLUSION

Based on the WHO standards, the borehole water samples analysed in this study are contaminated, hence not suitable for human use due to the incidence of *Vibrio cholerea* and high heterotrophic bacteria count. The presence of indicator feacal coliform in the borehole water samples suggests the possibility of waterborne infection to the unsuspecting consumers. The microbial load can be reduced to minimum levels or eliminated through proper water treatment regimes.

REFERENCES

Agbugui, M. O. & Deekae, S.N. (2013). Assessment of the Physico-chemical Parameters and Quality of Water of the New Calabar-Bonny River, Port Harcourt, Nigeria. *Cancer Biology*, 4(1):1 – 9.

Akubuenyi, F.C., Uttah E.C. & Enyi-Idoh, K.H. (2013). Microbiological and physicochemical assessment of major sources of water for domestic uses in Calabar Metropolis Cross River State Nigeria. *Transnational Journal of Science and Technology*, 3(2): 31 – 44.

Faruque M. (2013). Emergence of a new clone of toxigenic *Vibrio cholerae* O1 biotype El Tor displacing *V. cholerae* O139 Bengal in Bangladesh. *Journal of Clinical Microbiology*, 35:624–630.

Khan MU (1994). Presence of vibrios in surface water and their relation with cholera in a community. *Tropical and Geographical Medicine*, 36:335–340.

Lutendo S. Mudau, M., Stanley M. & Paul R. H.(2016) Cholera and household water treatment why communities do not treat water after a cholera outbreak: a case study in Limpopo Province.

Nwachukwu C.I, & Otokunefor TV (2006). Bacteriolgical quality of drinking water supplies in the University of Port Harcourt, Nigeria. Nig. J. Microbiol. 20: 1383-1388.

Seiyaboh, E.I., Inyang, I.R. and Gijo, A.H. (2013b) Environmental Impact of Tombia Bridge Construction across Nun River in Central Niger Delta, Nigeria. *The International Journal of Engineering and Science*, 2(11): 32 – 41.

Tyagi, T.R. & Agarwal, M.H. (2014). Aquatic Plants *Pistia stratiotes* L. and *Eichhorniacrassipes* (Mart.) Solms: An Sustainable Ecofriendly Bioresources - A Review. *International Journal for Pharmaceutical Research Scholars*, 3(1-2): 540 –550.

Uzoigwe, C. I. & Agwa, O.K.(2012). Microbiological quality of water collected from boreholes sited near refuse dumpsites in Port Harcourt, Nigeria. *African Journal of Biotechnology*, 11(13), 3135-3139.

Dhawale, S. & LaMaster, A. (2003). Microbiology Laboratory Manual. The McGraw Companies Incorporation, USA, p.187.

Yusuf, K.A (2007). Evaluation of groundwater quality in Lagos-City. *J Appl. Sci.* 7: 1780-1784

NSDWQ, (2007). National Standard for Drinking Water Quality. Pp 1-22

WHO, (2003). Total dissolved solids in Drinking-water: Background document for development of WHO Guidelines for Drinking-water Quality WHO/SDE/WSH/03.04/16

Azuonwu, O., Azuonwu, T. C. & Nwizug, W. L. (2017). Bacteriological Quality of Surface, Well, Borehole and River Water in Khana Local Government Area of Rivers State, Niger Delta. *Annals of Clinical and Laboratory Research*, 5 (3):183-188.

Nkwachukwu, I. O., Vincent, E. A., Helen, O. N. & Chukwu, H. C. (2013). Bacteriological Assessment of Selected Borehole Water Samples in Umuahia North Local Government Area, Abia State, Nigeria. *Journal of Environmental Treatment Techniques*, 1(2): 117-121

American Public Health Association (APHA), (2005). Standard Methods for the Examination of Water and Wastewater, 21st Edn., APHA, Washington D C.

Adogo, L.Y., Ajiji, M.A., Anyanwu, N.C.J. & Ajide, B. (2016). Bacteriological and physicochemical analysis of borehole water in Auta Balefi Community, Nasarawa State, Nigeria. *British Microbiology Research Journal*. 11(4): 1-7.

Edema, M.O., Omemu, A.M. & Fapetu, O.M. (2001). Microbiological and physicochemical

Analysis of different sources of drinking water. *Nigerian Journal of Microbiology*. 15: 57–61.

Chatterjee, S. N., & Maiti, M. (1984). Vibriophages and Vibriocins: physical, chemical and biological properties. *Adv. Virus Res*., 29: 263-312

Ramamurthy, T ., Garg, R., Sharma, R., Bhattacharya, S.K., Nair, G.B., Shimada, T., Takeda, T., Karasawa, T., Kurazano, H., Pal, A. & Takeda, Y . (1993). Emergence of *Vibrio cholerae* with epidemic potential in Southern and Eastern India. *Lancet*, 314: 703-704.

Feeley, J.C. (1966). Minutes of IAMS subcommittee on taxonomy of Vibrios. *IJSEM*; 162: 135–142.

Lippi, D. and Gotuzzo, E. (2013). The greatest steps towards the discovery of *Vibrio cholera*. *Clinical Microbiology and Infection*; 1-5

Nardi, M.G. (1954). Discovery of Vibrio cholera by Filippo Pacini, of Pistoia, established in the initial phases of microbiological thought and judged after a century. *Minerva Med* 45: 1024–1029