Genetic similarities and microbial source tracking of *Vibrio cholerae* from Bomadi and Burutu towns in Delta State, Nigeria

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Abstract

This study employed the use of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) molecular technique to determine the genetic relatedness of *Vibrio cholerae* strains from clinical and environmental sources from Bomadi and Burutu towns in Delta State, Nigeria. This was done to trace the source of January to March 2021 cholera epidemics in these localities. A total of 24 fecal samples, 16 freshwater samples, 16 brackish water, and 24 seafood were collected and subjected to standard enrichment, isolation, and biochemical characterization protocols for the identification of *V. cholerae.* Representative isolates from different sources in the two settlements were subjected to PCR probe for 16S rDNA and the products obtained were spliced with *Taq*I restriction endonuclease. The DNA fingerprints obtained were used to determine the genetic similarities and construction of the phylogenetic tree using DICE+UPGMA. Antibiotics susceptibility of strains from samples were investigated to ascertain the best choice of drugs for treatment. The result showed 100% *V. cholerae* distribution in clinical and freshwater samples. The electrophoresis image of PCR product of 16S rDNAs of *V. cholerae* strains showed seven (7) different clonal clusters of strains with strains of clinical and freshwater samples exhibiting 100% clonal similarities. Antibiotics sensitivity of strains showed pefloxacin, ofloxacin, and ciprofloxacin to be the best line of chemotherapy. The genetic homology in strains of clinical and freshwater samples signified that fres water samples could be the primary source of *V. cholerae* infection in these creek communities

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Introduction

The genus *Vibrio* is widely associated with the aquatic environment which consists of water bodies and kinds of seafood (Lipp *et al*., 2003; Huq *et al*., 2005). Some *Vibrio* species have been implicated in outbreaks due to consumption of contaminated waters (Idika *et al*., 2000; Dutta *et al*., 2006) and seafood (Eyisi *et al*., 2003; Elhadi *et al*., 2004). The most important among these potentially pathogenic *Vibrio* spp in terms of public health importance is the *V. cholerae*.
Vibrio cholerae is a free-living bacterium in the family *Vibrionaceae* and when the lysogenic conversion of this bacterium is effected through the incorporation of the genome of filamentous bacteriophage designated as CTX phage (Waldor and Mekalanos, 1996; Faruque *et al*., 1998; Faruque *et al*., 2003), the *V.cholerae* becomes toxigenic and capable of secreting extracellular substance known as cholera enterotoxin (Joblings and Holmes, 2001; Naha *et al*., 2013). The pathophysiological attribute of this cholera toxin is altering the normal physiological changes of the adenylate cyclase enzyme system in the intestinal mucosal cells (Todar, 2005; Igbinosa and Okoh, 2008). This changes result into excessive outflow of fluids and electrolytes from the mucosal cells into the intestinal lumen, which results into diarrhea. *Vibrio cholerae* has a well-documented history of seven (7) distinct global epidemics and it has the ability of emergence of new serogroups as evident in the emergence of *V.cholerae* O139.
Cholera outbreaks continue to occur in many suburban settlements especially localities with questionable hygiene and inadequate infrastructures for the provision of safe drinking water (Aladese and Ariyo, 2017). These settlements are very prominent in many creek communities of the Niger-Delta region of South-south, Nigeria. Many of these creek villages are

characterized by lack of an effective water treatment for consumption, absence of an effective human waste disposal system and the practice of open defecation into water bodies. These among others have been attributed as the possible reasons for many reported and undocumented pockets of cholera outbreaks.

Microbial source tracking is the process that employs diagnostic approach especially molecular biology and DNA fingerprinting techniques to determine clonal similarities between clinical and environmental strains (Zulkifli *et al*., 2009; Whitehouse *et al*., 2010). This study attempted the use of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to ascertain the genetic relatedness and clonal similarities between strains of *V.cholerae* from clinical samples and environmental sources in the recent cholera outbreaks from January-March 2021 in Bomadi and Burutu communities in Delta State, Nigeria. The study is further aimed at tracing the source of the epidemic which in turn will provide appreciable insights on how to curb future re-occurrence of epidemics in many of these creek localities.

Materials and Methods

Study areas

Bomadi (5^0 10’ N; 5^0 56’E) is one of the Ijaw ethnic communities in Delta State in South-south Nigeria (figure 1a). Bomadi town is surrounded by several settlements such as Uwheru, Torugbene, Ogriagbene, Kpakiama, Azebiri and Patani. Bomadi is a prominent locality along the Forcados River. The local inhabitants engage primarily in fishing and farming.

Burutu (5^0 21’ N; 5^0 31’E) is a creek community which lies on the coast of Forcados River (which lies 30km from the Bight of Benin). Burutu is a historically economic location that linked the river transportation with the sea during the activities of the Royal Niger Company in the late 19th century. The town is located west of Warri and Ughelli which are both important political and economic locations in Delta State (figure 1b). The local population is majorly Ijaw speaking ethnic tribe with fishing as their major occupation.

Figure 1a. Map of Nigeria showing Delta State

Figure 1b. Map of a section of Delta State showing the two studied sites

Sample collection

A total of 24 fecal samples, 16 fresh water, 16 brackish waters and 24 seafoods (crabs, periwinkles and shrimps) were collected from the two communities. Fresh water samples were collected from hand-dug wells, brackish waters were sampled along the coast of Forcados River and seafoods collected from markets within these communities. Clinical and water samples were inoculated into Cary-Blair’s medium for immediate transportation to the laboratory. Seafoods were collected into sterile polythene bags, placed on ice and transported into the laboratory for analysis.

Enrichment and isolation

Clinical and water samples (fresh and brackish) from Cary-Blair medium were inoculated into 1% alkaline peptone water for enrichment purposes. This was followed by plating 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. Seafoods were dissected and the hard external layers were discarded. The internal components of these seafoods 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. Characteristic yellow colonies suspected to be *V.cholerae* were subjected to various biochemical and physiological characterisation.

PCR-RFLP Probe

Polymerase chain reaction probe for 16S rDNA for *Vibrio cholerae* was carried out based on the method described by Whitehouse *et al.* (2010) using the following primers

Vibrio cholerae (F): 5' AGA GTT TGA TYM TGG CTC AG 3'

Vibrio cholerae (R): 5' GAA ATT CTA CCC CCC TCT ACA G 3'

Polymerase chain reaction of 16S rDNA was amplified in a thermocycler with 30 cycles at 59^0C. Restriction fragment analysis 16S rDNA fragments amplified by PCR was digested with *Taq*I restriction endonuclease. The *Taq*I restriction enzyme digestions was carried out at 65^0C incubation for 1-2 hrs. This was followed by electrophoresis on 2% agarose gel medium. The DNA fingerprints bands were observed on U-V trans-illuminator at 312nm for presence or absence of traits. These restriction bands on electrophoretic gel bands were digitally photographed on gel documentation machine.

Construction of Genetic Tree

The DNA fingerprints patterns produced by *Taq*I restriction enzyme were evaluated using the Image master software (Pharmacia Biotechnology, Uppsala, Sweden). Informative DNA fragments with molecular weight greater than 100 bp were documented for their presence or absence. The similarity matrix indices and phylogenetic distance tree were constructed using DICE + UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering methods and the percentage of clonal relatedness was determined.

Antibiotics Susceptibility Test

Antimicrobial susceptibility of the strains was determined using the multi-discs diffusion method based on the method described by Enabulele *et al.* (2006). The commercially available antibiotics discs of antibiotics (Augmentin (25 µg), Amoxicillin (25 µg), Cotrimozazole (25 µg), Tetracycline (50 µg), Pefloxacin (5 µg), Ofloxacin (5 µg) and Ciprofloxacin (10µg)) were inserted on Mueller-Hinton agar containing isolated strains, this was followed by incubation at 35^0C for 18-24 hrs. The zones of inhibitions were measured in millimetres and susceptibility and resistance of isolates were determined through comparisons with the standards instituted by the Clinical Laboratory Standard Institute (CLSI, 2011).

Results

The result of the distribution of *V.cholerae* from different sources showed all clinical and fresh water samples had 100% contamination by the cholera bacterium (table 1). Out of a total of 16 brackish water 14 (87.5%) were positive for the presence of *V.cholerae*. Table 2 shows the biochemical characterization of *V.cholerae* strains recovered from different sources in Bomadi and Burutu. All the strains were Gram negative, oxidase positive, highly motile and ferments sugars without gas production. In addition, strains grow in 0-3% sodium chloride supplemented medium.

The information on the strain types, sources and locations of isolation of forty (40) strains selected for PCR-RFLP is shown on Table 3. These strains were from clinical and environmental sources as well as locations (Bomadi and Burutu) of isolated strains. The product of polymerase chain reaction of 16S rDNA of *V.cholerae* from these communities showed a DNA band product of 663bp (figure 2). The PCR-RFLP product as a result of *Taq*I digestion showed four (4) different types of DNA fingerprints patterns (figure 3). The phylogenetic tree of *Taq*I splicing of PCR product of 16S rDNA of *V.cholerae* showed seven (7) different clonal clustering among stains (figure 4). Strains designate VC 25 (clinical; Bomadi) and VC 27 (fresh water; Bomadi) had 100% genetic homology from *Taq*I digestion. Similarly, clinical strains VC 2 (clinical; Bomadi), VC 4 (clinical; Burutu) and VC 23 (clinical; Bomadi) clustered 100% genetic relatedness with environmental strains from fresh water samples of strains VC 12 (fresh water; Bomadi), VC 13 (fresh water; Bomadi) and VC 24 (fresh water; Burutu). The antibiotics sensitivity patterns of strains showed pefloxacin, ofloxacin and ciprofloxacin had 100% susceptibility among clinical and environmental strains (table 4). Antibiotics like tetracycline, cotrimoxazole and augmentin exhibited varying degree of strains sensitivities.

Table 1. Distribution of *V.cholerae* from different sources

Samples	Number positive for *V.cholerae*	Total number examined
Clinical	24 (100)	24
Fresh water	16 (100)	16
Brackish water	14 (87.5)	16
Crabs	08 (100)	08
Shrimps	03(37.5)	08
Periwinkles	04(50.0)	08

Table 2. Biochemical characterisation of isolated *Vibrio cholerae* from Bomadi and Burutu, Delta State

Gram's reactions	_
Oxidase reactions	+
Motility	+
String test	+
Lysine decarboxylase	+
Arginine dihydrolase	_
Ornithine decarboxylase	+
5 % chicken erythrocytes	+
Sugars utilisation	
Glucose	Acid production (No gas)
Sucrose	Acid production (No gas)
Lactose	No acid production
Triple sugar iron	Acid/Acid reaction (No gas, No H_2S)
Salt tolerance broth test	
Growth in 0 % NaCl	+
Growth in 1 % NaCl	+
Growth in 3 % NaCl	+
Growth in 6 % NaCl	V

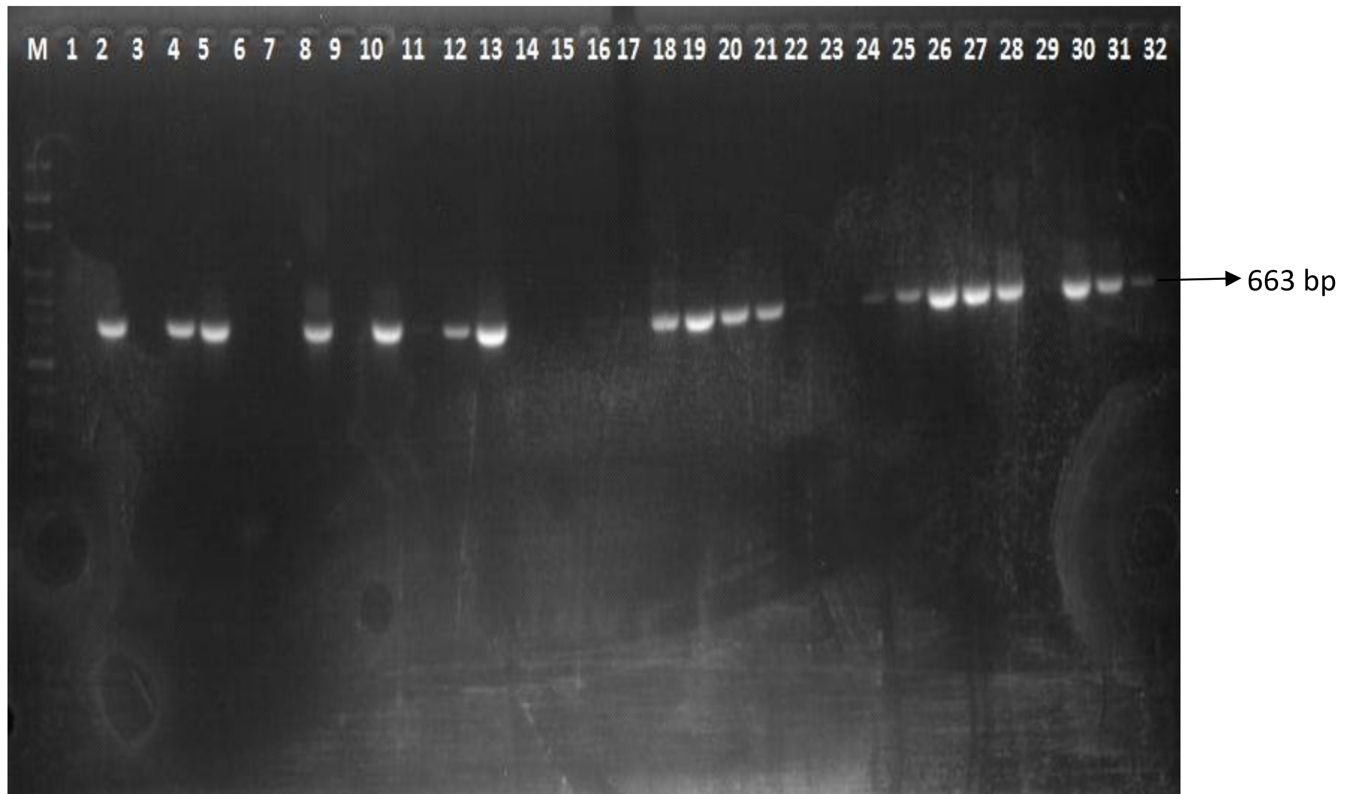
*V positive growth of broth tolerance varies among strains

Table 3. List of selected *V.cholerae* strains used for PCR-RFLP analysis

Strain designate	Strain type	Source of isolation	Location of isolation
VC1	Clinical	Faecal samples	Burutu
VC2	Clinical	Faecal samples	Bomadi
VC3	Clinical	Faecal samples	Burutu
VC4	Clinical	Faecal samples	Burutu
VC5	Environmental	Brackish Water	Burutu
VC6	Environmental	Seafoods	Bomadi
VC7	Clinical	Faecal samples	Bomadi
VC8	Environmental	Brackish Water	Burutu
VC9	Environmental	Brackish Water	Bomadi
VC10	Environmental	Brackish Water	Burutu
VC11	Environmental	Seafoods	Burutu
VC12	Environmental	Fresh Water	Bomadi
VC13	Environmental	Fresh Water	Bomadi
VC14	Environmental	Seafoods	Bomadi
VC15	Environmental	Seafoods	Bomadi
VC16	Environmental	Brackish Water	Bomadi

VC17	Environmental	Seafoods	Burutu
VC18	Environmental	Seafoods	Burutu
VC19	Environmental	Brackish Water	Burutu
VC20	Environmental	Seafoods	Burutu
VC21	Environmental	Brackish Water	Burutu
VC22	Environmental	Seafoods	Burutu
VC23	Clinical	Faecal samples	Bomadi
VC24	Environmental	Fresh Water	Burutu
VC25	Clinical	Faecal samples	Bomadi
VC26	Environmental	Seafoods	Burutu
VC27	Environmental	Fresh Water	Bomadi
VC28	Environmental	Fresh Water	Bomadi
VC29	Environmental	Fresh Water	Bomadi
VC30	Clinical	Faecal samples	Burutu
VC31	Environmental	Seafoods	Burutu
VC32	Environmental	Fresh Water	Burutu
VC33	Environmental	Seafoods	Bomadi
VC34	Environmental	Seafoods	Bomadi
VC35	Clinical	Faecal samples	Burutu
VC36	Clinical	Faecal samples	Bomadi
VC37	Clinical	Faecal samples	Bomadi
VC38	Environmental	Fresh Water	Burutu
VC39	Clinical	Faecal samples	Bomadi
VC40	Environmental	Brackish Water	Bomadi

(a)



(b)

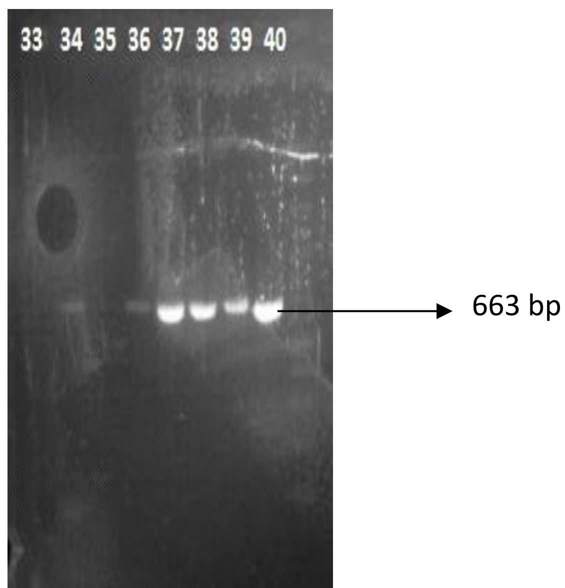
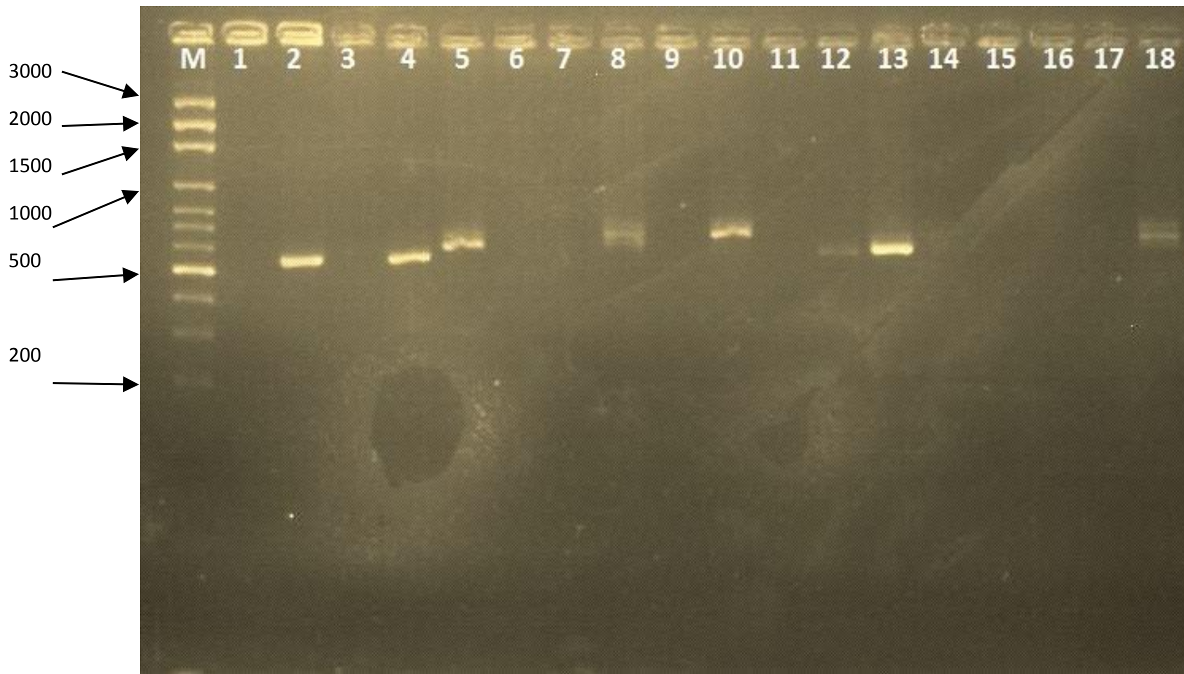


Figure 2. Electrophoresis gel image PCR products of 16S rDNA of *V. cholerae* strains from Bomadi and Burutu, Delta State.

(a)



(b)

Figure 3. Electrophoresis gel image of PCR-RFLP products of *V. cholerae* strains from Bomadi and Burutu using *Taq*1 restriction digestion of 16S rDNAs.

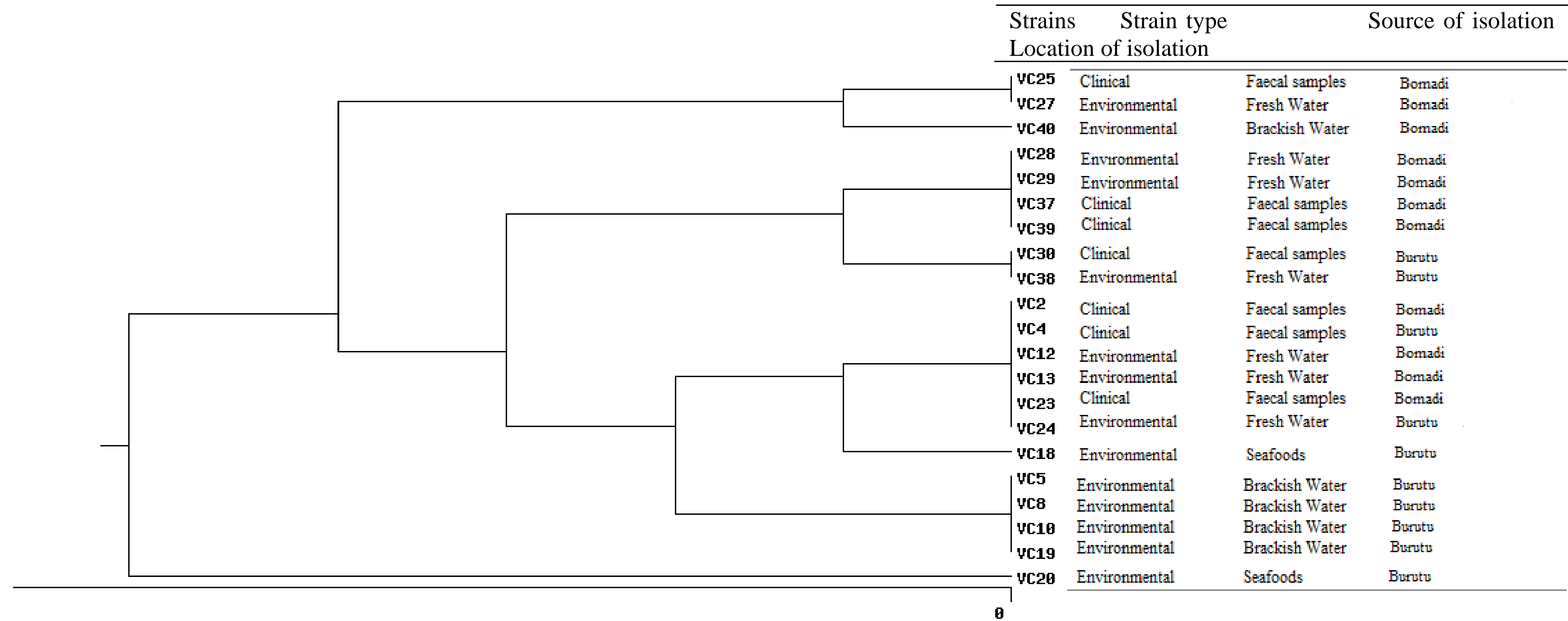


Figure 4. The phylogenetic distance tree showing clonal relatedness of showing from *Taq*I 16S rDNA of *V. cholerae* from Bomadi and Burutu.

Table 4. Antibiotics susceptibility of *V.cholerae* from different sources in Bomadi-Burutu, Delta 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	13(54.17)	24	10(62.50)	16	10(62.50)	16	13(54.17)	24
Aug	16(66.67)	24	13(81.25)	16	12(75.00)	16	15(62.50)	24
Cot	13(54.17)	24	09(56.25)	16	09(56.25)	16	12(50.00)	24
Tet	22(91.67)	24	14(87.50)	16	14(87.50)	16	22(91.67)	24
Pef	24(100)	24	16(100)	16	16(100)	16	24(100)	24
Ofl	24(100)	24	16(100)	16	16(100)	16	24(100)	24
Cpx	24(100)	24	16(100)	16	16(100)	16	24(100)	24

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

Discussion

The high percentage of contamination of *V.cholerae* in fresh water and brackish water has shown these sources remain a major vehicle of transmission of cholera bacterium. The public health relevance of fresh and brackish waters in transmission of *V.cholerae* and as a major predisposing factor of the occurrence of cholera epidemic is in concordance with past reports (Idika *et al*., 2000; Ayeni, 2014). The distribution of *V.cholerae* was highest in crabs among the seafoods examined. This finding underlines the importance of crabs as a major environmental reservoir that maintains the survival and persistence of this bacterium in the environment. The result obtained in these seafoods especially in crabs was expected because it is agreement with documented studies on seafoods as a major reservoirs for *V.cholerae* and *Vibrio* spp (Elhadi *et al*., 2004; Adebayo-Tayo *et al*., 2011; Aladese and Enabulele, 2014).

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) has been successfully employed in the past to determine the genetic homology of strains from different sources (Bialkowska-Hobrzanska *et al*., 1990; Yoon *et al*., 2003; Aladese and Pondei, 2019). Strain designate VC 25 (Clinical; fecal sample; Bomadi) clustered 100% in genetic relatedness with VC 27 (Environmental; fresh water; Bomadi). Similarly, strains from fresh water samples VC 28, VC 29 clustered 100% in genetic homology with strains designate VC 37 and VC 39 both of which are clinical strains from fecal samples obtained from Bomadi community. The phylogenetic clustering of clinical and fresh water strains of *V.cholerae* was also observed from isolates obtained from Burutu locality as shown in the 100% genetic relatedness of VC 30 (Clinical; fecal sample; Burutu) and VC 38(Environmental; freshwater; Burutu). The 100% clustering genetic relatedness of these clinical strains with fresh water strains shows there be could high possibility that the recent outbreak of cholera in these localities could be due to consumption of various sources of drinking water in these settlements.

Attempts had been made in recent times to use molecular methods to type strains for their genetic similarities and effectively employ this tools to trace the source of epidemics (Zulkifli *et al*., 2009; Hoffman *et al*., 2010). Prior to the deployment of molecular biology techniques in typing and tracing source of outbreaks there have been attempts in the past to use traditional methods to type these strains in an attempt to trace the origin of infection. Idika *et al*. (2000) and Hutin *et al.* (2003) both employed biochemical and serological procedures to type and trace the possible sources of cholera outbreaks in parts of Lagos State (South-west, Nigeria) and Kano State (North-central Nigeria). Their studies implicated fresh water samples through drinking and in preparation of foods by local food vendors as possible sources of the epidemics. Although, their studies were detailed but the use of molecular biology techniques especially very sensitive protocol like PCR-RFLP gives a more accurate and definitive results which types these strains based on their genetic similarities and composition. This study employed *Taq*I restriction enzyme usually derived from *Thermus aquaticus* and splices DNA nucleotide sequences at 5'-----T CGA----- 3' and 3'----AGC T---- 5' as recognition sites. Our findings on the use of this restriction endonuclease on 16S rDNAs of *V.cholerae* strains showed fresh water samples as the source of infection in the two localities. A similar report by Aladese and Pondei (2019) employed *Mbo*I and *Alu*I restriction endonuclease digestion of 16S rDNAs of *V.cholerae* to obtain DNA fingerprints which effectively traced the sources of cholera epidemics from different localities in Rivers and Bayelsa States in Nigeria. Their findings further affirmed that cholera epidemics in many creek communities from these two states could be traced to contamination of fresh water sources with *V.cholerae*. It is noteworthy to mention that Bomadi and Burutu communities share similar characteristic of creek settlements in the Niger-Delta area of South-south Nigeria with common practice of open defecation in the nearest river. In addition, there is no effective water treatment system in these areas which makes the local population to result into hand dug wells as source of drinking water but the dangers with these is that most of these hand dug wells are too close to these open rivers where human wastes are deposited.

The effective treatment of *V.cholerae* infection is predicated on constant epidemiological surveillance of emergence of antibiotics resistant strains. This study showed ciprofloxacin, ofloxacin and pefloxacin as the drug of choice for treatment. This finding is in agreement with similar studies in the past (Ottaviani *et al*., 2001; Adeleye *et* al., 2008). The presence of amoxicillin, augmentin, cotrimoxazole and tetracycline resistant strains in this study further justifies the public health importance of these strains. Amita *et al*. (2003) demonstrated the presence of plasmid-mediated antibiotics resistance traits among *V.cholerae*. Adeleye *et al*. (2008) opined that the emergence of these strains resistant to these antibiotics could be as a result of over-exposure of strains to these traditional drugs. The presence of resistant strains to these antibiotics especially tetracycline is in

concordance with past studies (Roychowdhury *et al*., 2008; Aladese *et al*., 2015; Ukaji *et al*., 2015)
Conclusively, the application of DNA fingerprint techniques to effectively trace the source of infection continues to be an efficient epidemiological tool that will provide better information and improve the epidemiological surveillance of strains associated with cholera and other epidemics. This expectedly will lead to the development of appropriate strategies and the design of frameworks that will lead to the prevention and control of cholera and other epidemics in many of these localities.

References

Adebayo-Tayo, B.C., Okonko, I.O., John, M.O., Odu, N.N., Nwanze, J.C. and Ezediokpu, M.N. (2011). Occurrence of potentially pathogenic *Vibrio* species in seafood obtained from Oron creek. *Advances in Biological Research*, 5(6): 356-365.

Adeleye, A., Enyinnia, V., Nwanze, R., Smith, S, and Omonigbehin, E. (2008). Antimicrobial susceptibility of potentially pathogenic halophilic *Vibrio* species isolated from seafood in Lagos, Nigeria.*African Journal of Biotechnology*, 7(20): 3791-3794.

Aladese M.A., Pondei J.O. and Enabulele O.I. (2015). Prevalence of Potentially Pathogenic *Vibrio* species in Sea-foods obtained from Markets in Lagos. *International Journal of Basic Science and Technology*, 1(1):39 – 42.

Aladese, M.A. and Ariyo, A.B. (2017). Physico-Chemical Properties and Bacteria Qualities of hand-dug wells in Ebukuma, Rivers State Nigeria. *International Journal of Basic Science and Technology*, 3(1):59-72

Aladese, M.A. and Enabulele, O.I. (2014). Prevalence of potentially pathogenic *Vibrio* species from different locations of the Lagos lagoon. Nigerian Journal of Applied Science, 32: 127-136.

Aladese, M.A. and Pondei J.O. (2019). Genetic relatedness and clonal similarities of *Vibrio cholerae* from clinical and environmental sources in Bayelsa and Rivers States using *Mbo*I and *Alu*I digestion of 16s rDNAs. *African Journal of Biology and Medical Research*, 2(2):12-24.

Amita, C.M., Thungapothra, Y., Nair, G.B. and Amit, G. (2003). Class I integron and SXT elements El Tor strains isolated before and after 1992 *V. cholerae* 0139 outbreak in Calcutta, Indian. *Emerging Infectious Diseases*, 9(4): 500-502.

Ayeni, A. O. (2014). Domestic Water Source, Sanitation and High Risk of Bacteriological Diseases in the Urban Slum: Case of Cholera in Makoko, Lagos, Nigeria. *Journal of Environment Pollution and Human Health,* 2:(1) 12-15.

Bialkowska-Hobrzanhsk., A, Jaskot, D. and Hammerberg, O. (1990). Evaluation of restriction endonuclease fingerprinting of chromosomal DNA and plasmid profile analysis for characterization of multi-resistant coagulase-negative staphylococci in bacteraemic neonates. *Journal of Clinical Microbiology*, 28: 269-275.

Clinical and Laboratory Standards Institute (CLSI) (2011). Performance standards for antimicrobial susceptibility test; Sixteen International supplements. CLSI document M100 S16. 26(3): M7-A7, 26(2): M2-A9, 26(1). Clinical and Laboratory Standards Institute, Wayne, PA. USA 183pp.

Dutta, B., Ghosh, R. and Sharma, N.C. (2006). Spread of cholera with newer clones of *Vibrio cholerae* O1 *El Tor* serotype Inaba. *Indian Jornal of Clinical Microbiology*, 44:3391-3393.

Elhadi, N., Radu, S., Chen, C., and Nishibudi, M. (2004). Prevalence of potentially pathogenic *Vibrio* species in the sea-foods marketed in Malaysia. *Journal of Food Protection*, 67(7):1469-1475.

Enabulele, O.I., Yah, S.C., Yusuf, E.O. and Eghafona, N.O. (2006). Emerging quinolones resistant transfer genes among gram-negative bacteria isolated from faeces of HIV/AIDS patients attending some hospitals in the City of Benin, Edo State, Nigeria. *Online Journal of Health and Allied Sciences*, 5(3):1-9.

Eyisi, O.A.L., Nwodo, U.U. and Iroegbu, C.U. (2013). Distribution of *Vibrio* species in shellfish and water samples collected from the atlantic coastline of South-East Nigeria. *Journal of Health Population and Nutrition*, 31(3)314-320.

Faruque, S. M., Asadulghani, A. R., Albert, M. J., Islam, K. M. and Mekalanos, J.J. (1998). Induction of the lysogenic phage encoding cholera toxin in naturally occurring strains of toxigenic *Vibrio cholerae* 01 and 0139. *Infection and Immunity*, 66:3752–3757.

Faruque, S.M., Zhu, J., Asadulghani, A.R., Kamruzzaman, M. and Mekalanos, J.J. (2003). Examination of Diverse Toxin-coregulated pilus-positive *Vibrio cholerae* strains fails to demonstrate evidence for *vibrio* pathogenicity island phage. *Infection and Immunity*, 71(6):2993–2999.

Hoffman, M., Brown, E.W., Feng, P., C., H., Keys, C., E., Fischer, M. and Monday, S.R. (2010). PCR-based method for targeting 16S-23S rRNA intergenic spaces region among *Vibrio* species. Biomedical Central Microbiology, 10:1-14.

Huq, A., Sack, R. B., Azhar Nizam, I. M., Longini, G., Nair, B, Ali, A., Morris, J. G., Khan, M.N.H.,

Siddique, A. K., Yunus, M., Albert, M.J., Sack, D.A. and Colwell, R. R. (2005). Critical Factors Influencing the Occurrence of *Vibrio cholerae* in the Environment of Bangladesh. *Applied and Environmental Microbiology*, 71 (8): 4645-4654.

Hutin, Y., Luby, S. and Paquet, C. (2003). A large cholera outbreak in Kano City, Nigeria: the importance of hand washing with soap and the danger of street-vended water. *Journal of Water and Health*, 1(1): 45-52.

Idika, I.N., Audu, R.A., Oyedeji, K.S., Iyanda, R. and Egbom, C.A. (2000). Investigation of different water sources as possible cause of cholera outbreak in Lagos, 1997. *Journal of the Nigerian Association of Infection and Control*, 3:111-117.

Igbinosa, E.O. and Okoh, A.I. (2008). Toxigenic *Vibrio cholerae* strains and their associated malaises. *African Journal of Microbiology Research*, 3(5): 200-211.

Joblings, M.G. and Holmes, R.K. (2001). Biological and Biochemical characterisation of variants subunits of cholera toxin: constructed by site-directed mutagenesis. *Journal of Bacteriology*, 183(13):4024-4032.

Lipp, E.K., Rivera, I.N.G., Gil, A.I., Espeland, E.M., Choopun, N., Louis, V.L., Russek-Cohen, E., Huq, A. and Colwell, R.R. (2003). Direct detection of *Vibrio cholerae* and *ctx*A in Peruvian coastal water and plankton by PCR. *Applied and Environmental Microbiology,* 60(6): 3676-3680.

Naha, G., Chowdhury, J., Ghosh-Banerjee, M., Senoh, T., Takahashi, B. Ley, K., Thriemer, J., Deen, L., Seidlein, V., Alief, S. M., Khatib, A., Ramamurthy, E.T., Nandy, R. K., Nair, G. B., Takeda, Y. and Mukhopadhyay, A. K. (2013). Molecular Characterization of High-Level-Cholera-Toxin-Producing *El Tor* Variant. *Journal of Clinical Microbiology*, 51(3):1040-1045.

Ottaviani D, Isidoro B, Laura M, Francesca L, Antonio C, Monica G, Giovoanni S. 2001. Antimicrobial Susceptibility of Potential Pathogenic Halophilic *Vibrio. International Journal of Antimicrobial Agents* 18(2): 135-140.

Roychowdhury, A., Pan, A., Dutta, D., Mukhopadhyay, A.K., Ramamurthy, T., Nandy, R.K., Bhattacharya,S.K. and Bhattacharya, M.K. (2008). Emergence of tetracycline-resistant *Vibrio cholerae* O1 serotype Inaba in Kolkata, India. *Japanese Journal of Infectious Diseases*, 61:128:129.

Todar, K. (2005). The Cholera. University of Wisconsin Madison Publication, United States of America.

Ukaji, D.C., Kemajou, T.S., Ajugwo, A.O., Ezeiruaku, F.C. and Eze, E.M. (2015). Antibiotic susceptibility patterns of *Vibrio cholerae O1 isolated during cholera outbreak in Uzebba (Edo State). Open Science Journal of Bioscience and Bioengineering, **2**(3):33-36.*

Waldor, M. K. and Mekalanos, J. J. (1996). Lysogenic conversion by a filamentous bacteriophage encoding cholera toxin. *Science*, 272:1910–1914.

Whitehouse, C.A., Baldwin, C., Sampath, R., Blyn, B.L., Melton, R., Li, F., Hall, T.A., Harpin, V., Mathews, H., Tediashvili, M., Jaiani, E., Kokashvili, T., Janelidze, N., Grim, C., Colwell, R.R. and Huq, A. (2010). Identification of pathogenic *Vibrio* Species by Multilocus PCR-Electrospray ionization mass spectrometry and its application to aquatic environments of the former Soviet Republic of Georgia. *Applied and Environmental Microbiology*, 76(6):1996-2001.

Yoon,Y.J., Im, K.H., Koh, Y.H., Kim, S.K. and Kim, J.W. (2003). Genotyping of six pathogenic *Vibrio* species based on RFLP of 16S rDNAs for rapid identification. *The Journal of Microbiology*, 41(4): 312-319.

Zulkifli. Y., Alitheen, N.B., Son, R., Raha, A.R., Samuel, L., Yeap, S.K. and Nishibuchi, M. (2009). Random amplified polymorphic DNA-PCR and ERIC PCR analysis on *Vibrio parahaemolyticus* isolated from cockles in Padang, Indonesia. *International Food Research Journal*, 16:141-150.