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RESEARCH ARTICLE

Pathogenic *Vibrio* Strains Isolated from Human Stool and Water Samples from Western Kenya

Roselida Achieng Owuor[1,2ab], David Miruka Onyango[2a], Eliud Waindi[2b]

[1] *National Commission for Science, Technology and Innovation, Nairobi, Kenya*
[2a] *Department of Zoology, Molecular Microbiology and Biotechnology Laboratory, Maseno University, Maseno, Kenya*
[2b] *Maseno University, Department of Zoology, Maseno University, Maseno, Kenya*

ABSTRACT

Objective: Investigate the type of pathogenic *Vibrio* strains from water and stool samples collected from Migori, Sondu-Miriu, Nyando and Yala regions in Western Kenya.

Methods: A total of 811 samples (596 water and 215 stool samples) were collected during the study periods of May to December 2013 and August to September 2014. Pathogenic *Vibrio* strains were identified through culturing in TCBS Agar, followed by oxidation, string and serological (polyvalent) tests, respectively. The PCR analysis was done using combined primers targeting *Vibrionaceae* 16SrRNA and species specific primers for *V. vulnificus* and *V. cholerae*.

Results: The results showed the presence of *V. vulnificus* and *V. cholerae*. However, *V. parahaemolyticus* was not found in any of the samples. The PCR results for 16SrRNA, Vib 1, and Vib 2 showed polymorphism in the genes, this was an indication of cross combination of genes from more than one strain in one isolate.

Conclusion: The study showed the presence of *V. cholerae* (Ogawa and Inaba) in water and human stool samples. Type B *V. vulnificus* was detected in the water sample collected from River Migori. This information is of essence in controlling and managing cholera in the western part of Kenya. *J Microbiol Infect Dis 2016;6(1): 1-7*

Key words: *V. cholera*e, *V. vulnificus*, *V. parahaemolyticus*, PCR

Batı Kenya'da Su ve İnsan Dışkı Örneklerinde Patojenik *Vibrio* Suşları

ÖZET

Amaç: Batı Kenya bölgelerinden olan Migori, Sondu-Miriu, Nyando ve Yala'dan alınan su ve dışkı örneklerinden patojenik *Vibrio* türlerinin araştırılması

Yöntemler: Mayıs-Aralık 2013 ile Ağustos –Eylül 2014 arasındaki çalışma döneminde toplam 811 (596 su ve 215 feçes) numune toplandı. Örnekler önce TCBS besiyerine ekildi ve üreyen bakterilerden patojenik *Vibrio* suşlarının ayrımı oksidasyon testi, sünme testi ve serolojik (polivalan) testler kullanılarak yapıldı. PCR analizi, Vibronecea 16SrRNA'yı hedefleyen primerlerle spesifik olarak *V. vulnificus* ve *V. cholerae*'yı hedefleyen primerlerinin kombine şekli kullanılarak yapıldı.

Bulgular: Sonuçlar V. vulnifucus ve *V. cholera*e varlığını gösterdi. Ancak, izolatların hiçbirinde *V. parahaemolyticus* bulunmadı. 16SrRNA, Vib 1 ve Vib 2 için yapılan PCR sonuçları, bir izolatta bir suştan çok genlerde çapraz kombinasyonu teyit eden gen polimorfizmi varlığını gösteriyordu.

Sonuç: Bu çalışma gösterdi ki suda ve insan dışkı numunelerinde *V. cholera* (Ogawa ve Inaba) varlığını gösterdi. Migori ırmağından toplanan su numunelerinde Tip B *V. vulnificus* tespit edildi. Bu bilgiler Kenya'nın batı kesiminde koleranın yönetimi ve kontrolü için temel unsurdur.

Anahtar kelimeler: *V. cholerae, V. vulnificus, V. parahaemolyticus*, PCR

Correspondence: Roselida Achieng Owuor,
National Commission for Science, Technology and Innovation, P.O. Box: 30623-00100, Nairobi, Kenya
Email: roselidaowuor@yahoo.com
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INTRODUCTION

The genus *Vibrio* is in the family Vibrionaceae, which also includes the genera Aeromonas, Plesiomonas, and Photobacterium [1]. Chemotaxonomy and genetic analyses have assisted scientists to classify the *Vibrios* based on their respective environmental survival preferences. In this regard, the most pathogenic *Vibrio* strain to human are *V. cholera*e, *V. parahaemolyticus* and *V. vulnificus* and understanding their preferred physicochemical parameters can contribute towards their control. *Vibrio cholerae*, *V. parahaemolyticus* and *V. vulnificus* can co-exist in aquatic environment and in diseased animal [2]. These species also have dynamic genetic makeup. Environmental factors, such as temperature and rainfall which can be broadly termed as "climate", undoubtedly play a decisive role, not only in cholera outbreaks, but in many infectious diseases caused by bacteria pathogens [3]. Cholera epidemics are caused by *V. cholera*e 01 (that include El Tor and classical biotypes) and 0139 serogroups while some strains of serogroups (non-01/non-0139) are associated with sporadic gastroenteritis.

It is important to note that cholera continues to be an important cause of morbidity and mortality in many areas of Asia, Africa, and Latin America [4]. Its control is therefore crucial. Kenya has experienced numerous outbreaks of cholera ever since it was first detected in 1971. The outbreak of cholera that occurred in Kuria West District in August 2010 spread to the neighboring Migori District [5]. After an outbreak subsides in the lake region of Kenya, it has been suggested that *V. cholerae* becomes extinct in that locale but that isolated pockets of disease linger elsewhere in the region, and when the climate becomes favorable, *V. cholerae* re-emerges and spreads from the refuge [6]. During January 2009 to May 2010, cholera was detected in at least 52 districts throughout the country, and 11,769 cases and 274 deaths (case-fatality rate ≈2.3%) were reported to the Kenya Ministry of Public Health and Sanitation. The regularity of these outbreaks indicates that *V. cholerae* might be frequently spread by travelers or that it is endemic to the area.

V. parahaemolyticus is among the leading cause of seafood-borne gastroenteritis in developing and developed countries [6]. In the U.S.A. gastroenteritis due to consumption of *V. parahaemolyticus* contaminated seafood leads to estimated 35,000 cases of infections each year [7]. *V. vulnificus* causes gastroentereritis and wound infections with high fatality rate in immunocompromised patients [8].

Migori, Sondu, Nyando, Yala and Bondo regions in Western Kenya where this study was conducted have in the past experienced cholera outbreaks. The four Kenya rivers (Nyando, Sondu - Miriu, Nzoia and Yala) that drain into Lake Victoria flow from tea and sugar plantations where they collect refuses and contaminants on their way. This makes water related diseases e.g. dysentery, diarrhea and typhoid to be considered important in these sites [9]. In Bondo, change in the water levels and water use by humans and livestock as observed in this study provides conducive environment for the swamps to harbor pathogenic bacteria according to previously reported studies [10]. It has also been reported that the *Vibrio* pathogenic species produce various virulence [11]. This study therefore investigated the type of pathogenic *Vibrio* strains from water and stool samples collected from Migori, Sondu-Miriu, Nyando and Yala regions in Western Kenya. These findings can be used by the government of Kenya to manage and control cholera in these regions.

METHODS

This study was carried out from May to December 2013 and from August to September 2014 in Migori (1° 4´0S, Oo17´48´´S), Nyando (0° 14´19´´N) and Bondo (34° 28´ 0´´E, 34 o 51´ 31´´ E, 34° 16´0´´E) regions in Western Kenya (Fig. 1). It was a cross-sectional study that was conducted according to Collin et al., and Carla et al [10,12]. Patients with diarrhea of unknown or unsuspected origin were recruited in the respective hospital facilities in the study areas irrespective of their age and gender. The water samples were collected from the surface (19 to 55 cm depth) at each sampling site in 50 ml capacity autoclaved universal bottles between June to December 2013; January to February 2014 and August to September 2014 every two weeks at each site. This study also tested water conductivity, salinity and temperature in the selected study regions.

The collected stool and water samples were processed within 4 to 9 h post collection for strains identification and characterization by standard biochemical procedures according to Sakazaki [13]. The isolates developed purple colour within 10 minutes on a filter paper saturated with oxidase reagent (1% N, N, N, N'-tetramethyl p-phenylenediamine.2HCl). Bacteria that showed or did not show positive results were subjected to serological agglutination test by using polyvalent antisera (Ramel Europe Ltd. Clipper Boulevard West crossways, Dartford Kent, DA2 6PT UK) for Ogawa and Inaba. Amplification of positive *Vibrio* strains was done according to Nandi et al

[14]. Briefly, Twenty microliters of PCR master Mix + 5 microlitre DNA template were aliquoted into 0.2 ml eppendorf tubes, thoroughly vortexed and then centrifuged at 10,000 rpm for 6 sec (Eppendorf centrifuge 5415D, Germany) followed with amplification using MJ Gradient Thermocycler (PTC -225, Peltier Thermocycler, BioEnymes, Germany) PCR with the following conditions: building up of Hot start temperature at 95°C for 1min, denaturization temperature at 95°C for 30 seconds, annealing temperature of 55°C for 1minute, and extension temperature of 68°C for 1minute and final extension at 68°C for 10minutes. The amplified amplicons were loaded on to a casted 1.5% agarose gel (2 grams agarose powder + 100ml 1 x TBE Buffer+0.2 microlitre of ethidium bromide) with a gene marker of 100 bp, a negative control and positive control. This was then let to run for 45 minutes at 135 V after which the UV pictures were taken using the UV photo transmitter (Gel Logic 100 Imaging System, Kodak).

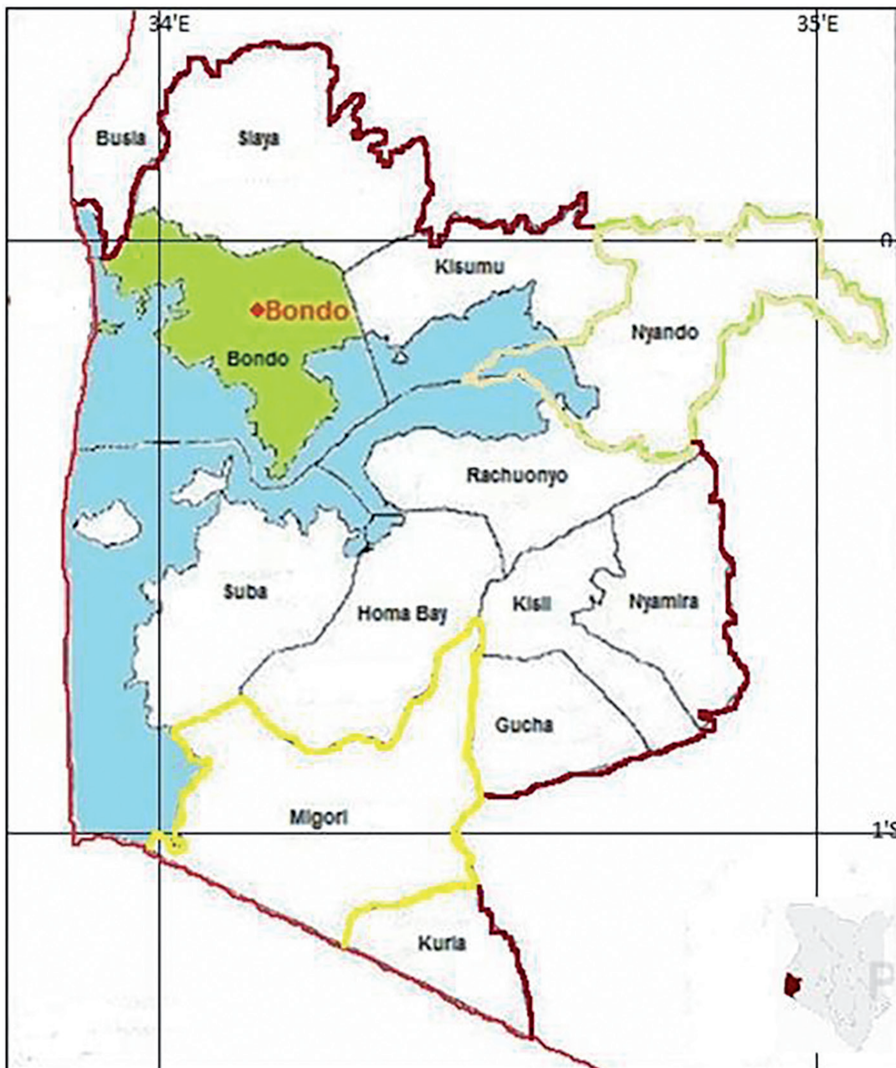

Figure 1: Map showing the locations of the study area (Courtesy of Agriculture Livestock Sector Working Group of the Kenya Food Security Steering Group (KFSSG), 2007

RESULTS

Out of the 811 samples collected, 596 were environmental and 27% were clinical samples. Out of the environmental samples, 11 were found to be of *Vibrio* species based on the conventional microbiological culture characteristic of *Vibrio*. The isolates showed yellow, green or blue color in TCBS media and ranged between 2 to 3 mm in size depending on the specific strain. The isolates further formed strings when a colony was placed on the slide, one drop of sodium deoxycholate solution added onto it and broken using a plastic loop. In addition, of the clinical samples, 4 were found to be of *Vibrio species* based on their growth characteristics in various media.

Significance difference was observed in the physicochemical parameters. This was the case in the depth of the water in Migori River (0.18+ 0.16m) and Sondu Miriu River (0.2+0.16 m). Dissolved oxygen showed very strong significant at 5.3 +1.5 mg/l and 4.09 +0.8 mg/l at R. Migori and R. Sondu Miriu respectively. Water temperature showed strongly significant at 28.9+ 2.6°C in Bondo swamp. Asterisks in Table 2 show the statistical significance differences. Further analysis (Table 3) showed significant change in salinity (158.9µS*cm^{-1}), water temperature (0.20°C), dissolved oxygen (3.1mg/l) and pH (4.43). This was an indication that these parameters depend on seasons and other geomorphometric indices.

This study showed that the waters where *V. cholerae* was isolated had a pH of 7.7 to 8.2 ($P \le 0.01$), temperature of 22 -28°C ($P \le 0.01$), water sanity of 17-161.2 µS•cm^{-1} (0.2 to 2.3% ($P \le 0.01$). These conditions correlate well with the epidemiological distribution of cholera and conditions required for thriving of *V. vulnificus*. [14] It was observed that water temperature directly correlates with cases of cholera.

Table 1. Primer sequences used in the amplification of *Vibrio* strains

Primer	Sequence 5'- 3'	Length	Reference
Bacterial (rrn)16SrRNA-1	AGRGTTYGATYMTGGCTCAG	20	Myoung*et al.,* 2011
Bacterial (rrn) 16SrRNA-2	GGYTACCTTGTTACGACTT	19	Myoung*et al.,* 2011
Vib 1	GTGGTAGTGTTAATAGCACT	20	Myoung*et al.,* 2011
Vib 2	TCTAGCGGAGACGCTGGA	18	Myoung*et al.,* 2011
Vib 3	GCTCACTTTCGCAAGTTGGCC	21	Myoung*et al.,* 2011
PI	GAAATAAAGCAGTCAGGTG	20	Fields *et al.,* 1992
P2	GGTATTCTGCACACAAATCAG	21	Fields *et al.*, 1992

Table 2. Analysis of physicochemical parameters in the study region

Sampled parameters	Study Sites					p
	R. Migori Mean ± SD	R. Sondu Miriu Mean ± SD	R. Nyando Mean ± SD	R. Yala Mean ± SD	Bondo Swamp Mean ± SD	
Water Depth	0.18 ± 0.16	0.21 ± 0.17	0.19 ± 0.17	0.22 ± 0.18	0.20 ± 0.17	<0.01
Water Salinity (µS·cm^{-1})	42.27 ± 1.8	41.44 ± 3.6	161.92 ± 11.9	73.65 ± 4.6	17.01 ± 3.1	<0.05
Dissolved Oxygen (mg/l)	5.33 ± 1.54	4.09 ± 0.83	3.81 ± 0.73	3.47 ± 0.26	3.48 ± 0.43	<0.05
Water pH	7.90 ± 0.74	7.91 ± 0.32	8.26 ± 0.40	7.78 ± 0.38	8.09 ± 0.42	<0.05
Air Temperature	23.88±2.43	20.09±0.97	23.48±1.02	23.2±1.99	28.9±1.43	<0.05
Water temperature	23.47±1.30	19.27±0.36	22.45±0.69	21.22±0.74	28.97±2.69	<0.05

Table 3. Analysis of physicochemical parameters in the study region

Variable	No. of Samples	Median value	Minimum value	Maximum value	Change (Δ=)
Water temp (°C)	204	21.9	18.7	35.0	16.3
Air temp (°C)	85	22.9	18.4	30.8	12.4
Water depth (m)	81	0.19	0.18	0.48	0.20
pH	122	8.07	6.87	11.3	4.43
Dissolved O_2 (mg/liter)	144	3.6	2.8	5.9	3.1
Fecal coliforms (log_{10} CFU/ml)	215	4.7	1.28	8.7	7.42
Salinity (µS•cm–1)	191	42.2	10.3	169.2	158.9

The PCR results showed polymorphism with 16SrRNA, Vib 1, Vib 2 (Figure 1). This was an indication that there were cross combination of genes from more than one strain in one isolate. Three water isolates with 16SrRNA had a band between 454 to 473 base pairs (Figure 2) which although does

not discriminate the *V. vulnificus* from other bacteria can be used to avoid detection of type B 16SrRNA of this bacteria in PCR. These isolates were analyzed for vv gene that is responsible for hemolysin gene in *V. vulnificus* using Vib 1 Vib 2 and Vib 3. The bands of 273bp and 825bp showed an indication of Type B *V. vulnificus* from water samples.

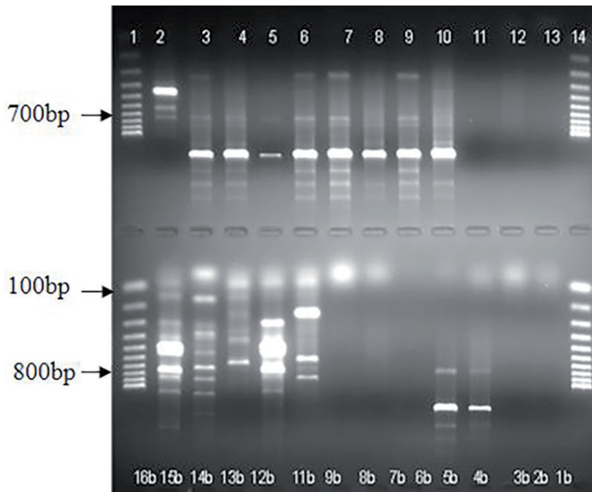

Figure 2. PCR gel bands showing the presence of rrn gene (Bacterial 16S rRNA) used for identification of *Vibrio* species. Lanes 1 and 14 = L= Molecular DNA marker 100bp, lanes 2-9 = isolated *Vibrio* strains, lane 10 = positive controls of fresh *Vibrio* ATCC cultured in HIA slants, lane 12-13 = 3b and 2b are negative control (PCR water).

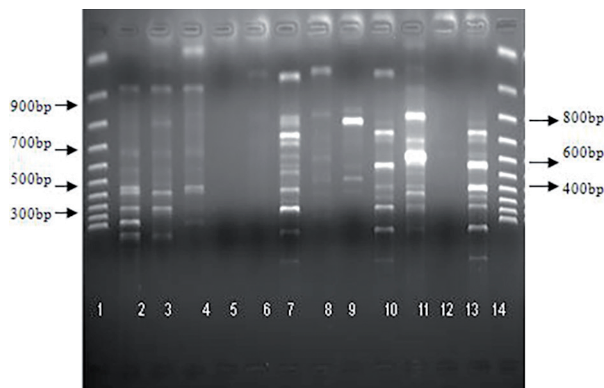

Figure 3. PCR gel band showing presence of rrn gene (16S rRNA) used for identification of *Vibrio vulnificus*. Lanes 1 and 14 = L = Molecular DNA maker 100bp, lanes 2 = positive controls of fresh *Vibrio* ATCC cultured in HIA slants, lanes 5 = negative controls (PCR water), lanes 3, 4, 6 - 13 = isolated *Vibrio* strains.

DISCUSSION

Seasonal factors such as rainfall and hours of sunlight, contribute directly to the physical and chemical characteristics of water that in turn affect plankton populations. The phytoplankton blooms are followed by zooplankton blooms and copepods which have been shown to have a *V. cholera*e flora. [15] Temperatures of ≥ 25°C, pH of ≥7.0 and salinity range of 0.01 to 3.3 are documented to enhance *V. cholera*e counts as a result of phytoplankton blooms (15). Based on the findings from this study, it is alluded that when conditions become favourable *Vibrio* transformed from non-typeable non- pathogenic to pathogenic strains and cause rampant abrupt infections like those recently reported in Migori and Homabay counties where fourteen lives were lost with 637 and 143 confirmed cases respectively [16]. The reported infections in these counties may be attributed to domestic use of contaminated water that would have correlated with the period when bacteria was transforming from non-pathogenic to pathogenic state immediately after the start of the rainy season in the region. However, further studies need to be done to confirm these observations.

It has been reported that factors like water salinity have been found to greatly affect proliferation of *Vibrios*. [1] In this regard, response to fluctuating water salinity in the region between (0.2 and 2.3%) would have contributed immensely in the presence, emergence and re-emergence of the respective *Vibrio* isolates observed in this study. Salinity plays a great role in the growth of *Vibrio* strains. It is documented that *Vibrio* thrive in water salinity of 0.5-17% which when based on this study was attained (0.2 to 2.3%) and thus enhanced their survival [17].

In determining the phenotype of the *Vibrio* strains in the study area, the serological results showed that the type of *V. cholera*e identified in these region were Inaba and Ogawa. This was an important finding since earlier studies according to study done by Kenya Medical Research Institute – CDC in Kisumu in 2007 reported the present of the former (Inaba) and not the later type of V. cholorea. The presence of Ogawa type was also recently confirmed in the recent outbreak of cholera in Migori and Homabay counties by Kenya Medical Research Institute–CDC in Kisumu Western Kenya. This knowledge on the type of *V. cholerae* species in these areas is important in studying the epidemiology and prevention of outbreak of cholera.

While determining the genotype of the *Vibrio* strains in this study, species specific ctxA genes responsible for cholera toxin that is a preserve of 01/0139 was observed. Of the analyzed samples twelve isolates had bands of 564 bp. This acted as a confirmation indicator that there were *V. cholera*e

isolates in the water and human stool samples from the study area which was enhanced by change in temperature between 22°C and 28°C. The *V. parahaemolyticus* was absence in the samples. This is because the temperature was much lower than the optimal growth temperature (30°C and 35°C) required for its growth [18]. Furthermore, the 16Sr RNA was used to identify the *V. vulnificus* which was then confirmed by species specific primers for vv responsible for expression of haemolysin. This gene is used for the phenotypic identification of *Vibrio* strains [19]. The amplification of these genes is an indication of the presence of *V. vulnificus* in the samples [20]. Unfortunately, the 16S rRNA sequences of different *Vibrios* spp. show minimum differences making species- specific identification difficult [21]. This hypothesizes the possibility of activities of Rec genes used by *Vibrio* to acquire additional characteristics to enable them survive during adverse conditions. This gene is also known to enables the *Vibrio* to attain phage lytic cycle in addition to expressing genes responsible for adverse conditions e.g., outer membrane protein (ompW) a phenomenon only endowed with *V. cholerae* strains making it highly suitable gene maker for the organism. The virulence markers such as cholera toxin, toxin-coregulated pilus (TCP) are known to be associated with the pathogenic strain of *V. cholerae* 10 or 0139 [22]. These characteristics are made evident with the fact that toxR gene found in both *V. cholerae* and *V. parahaemolyticus* fails to display species-specific identification properties between *V. parahaemolyticus* and *V. cholerae*. The gene probe fails to detect *V. cholerae* despite 52% identity in their toxR gene sequence with *V. parahaemolyticus* [22]. In so doing the isolates become ubiquitous and may have spatial distribution leading to sporadic infection like that recently reported in Migori [23]. This is typical of *V. cholerae* that belongs to non-01/non-0139 serogroup which can be isolated in abundance from aquatic or estuarine sources causing sporadic cases or limited outbreaks of diarrhea in human [24]. In addition, although the precise function of the ompW protein in *V. cholerae* is not well documented, it may play a role in the adherence process, which is likely to facilitate the survival of the organism within the host or in the environment or both leading to sporadic attacks as envisaged in this study. It is reported that while the ompW gene is present in the smaller chromosome (there are two chromosomes in *V. cholerae*), the toxR gene is located in larger chromosome of the organism [24]. This serves as a survival mechanism for this organism. Based on these findings, it is evident that the use of 16S rRNA sequence as the only identification method is not very adequate due to polymorphism observed herein in the respective samples. This is affirmed by lack of appreciable differences between the sequence occurring in *V. cholerae* and other members of vibrionaceae family as reported in other studies [21].

CONCLUSION

This study showed that 0.3% and 0.2% *Vibrio* strains positive samples were from water and human stool respectively. The study also identified the two types of pathogenic *Vibrio* strains namely *V. cholerae* and *V. vulnificus* and their favourable conditions. The type of *V. cholerae* isolated in the region were ogawa and inaba 01/0139 serogroup. The environmental conditions therein in the study area could not support the survival of *V. parahemolyticus* hence its absence in the human stool and water samples from the study area. These findings can be used to formulate policies by the government of Kenya on handling and managing any outbreak caused by these *Vibrio* strains.

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REFERENCES

1. Atlas RM. Principles of microbiology: Bacterial diversity, 2nd edn. Boston: Fishback. 1997;17:80.
2. Mahmud ZH Neogi SB, Kassu A, et al. Occurrence, seasonality and genetic diversity of *Vibrio* vulnificus in coastal seaweeds and water along the Kii Channel, Japan. FEMS Microbiol Ecol. 2008;64:209-218.
3. Colwell RR. Global climate and infectious disease: the cholera paradigm. Science. 1996;274:2025-2031.
4. Pascual M, Rodó X, Ellner SP, et al. Cholera dynamics and El nino-Southern Oscillation. Science. 2000;289:1766-1769.

5. Kiiru JN, Saidi SM, Goddeeris BM, et al. Molecular characterisation of *Vibrio cholerae* O1 strains carrying an SXT/R391-like element from cholera outbreaks in Kenya: 1994-2007. BMC Microbiol. 2009;9:275.

6. Nair GB, Ramamurthy T, Bhattacharya SK, et al. Global dissemination of *Vibrio* parahaemolyticus serotype O3:K6 and its serovariants. Clin Microbiol Rev. 2007;20:39-48.

7. Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne Illness Acquired in the United States-major Pathogens. Emerg Infect Dis. 2014;17:7-15.

8. Jones MK, Oliver JD. *Vibrio* vulnificus: disease and pathogenesis. Infect Immun. 2009;77:1723-1733.

9. LVEMP II Consolidated Annual Report 2011-2012 http://lvemp.eac.int/annual-report-july-2011-june-2012.

10. Lutz C, Erken M, Noorian P, et al. Environmental reservoirs and mechanisms of persistence of *Vibrio cholerae*. Front Microbiol 2013;4:375.

11. Zhang XH, Austin B. Haemolysins in *Vibrio* species. J Appl Microbiol 2005;98:1011-1019.

12. Collin B, Rehnstam-Holm AS, Lindmark B, et al. The origin of *Vibrio cholerae* influences uptake and persistence in the blue mussel Mytilusedulis. J Shellfish Res. 2012;31:87-92.

13. Sakazaki R. Bacteriology of *Vibrio* and related organisms In: Barua D, Greenough WB, Cholera, 3rd edn. Planum Medical Books Company, New York 1992:37-55.

14. Nandi B, Ranjan KN, Sarmishtha MP, et al. Rapid method for species-specific identification of *Vibrio cholerae* using primers targeted protein OmpW. J Clin Microbiol 2000; 38:4145-4151.

15. McLaughlin JC. *Vibrio*. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC,Yolken RH, eds. Manual of clinical microbiology, 1st edn. Washington, DC 1995:465-476.

16. Colwell RR, Huq A. Environmental reservoir of *Vibrio cholerae*. The causative agent of cholera. Ann. NY Acad Sci 1994; 740:44-54.

17. International Federation of Red Cross and Red Crescent Societies (2015) Kenya: Cholera Emergency Plan of Action (EPoA) Operation no MDRKE033. reliefweb.int/disaster/ep-2015-000013-ken.2015.

18. Thompson FL, Austin B, Swings J. The biology of *Vibrio*s, 1st edn. ASM Press, Washington, DC 2006.

19. Jay JM, Loessner MJ, Golden DA. Modern Food Microbiology, 7th edn. Dennis Heldman, 2005.

20. Mazel D, Dychinco B, Webb VA, Davies J. A distinctive class of integron in the *Vibrio cholerae* genome. Science. 1998;280:605-608.

21. Nadja B, Silke B, Susanne D, et al. Genotypic Diversity and Virulence Characteristics of Clinical and Environmental *Vibrio vulnificus* isolates from the Baltic Sea Region. Appl Environ Microbiol. 2013;79:3570-3581.

22. Kita-Tsukamoto K, Oyaizu K, Shimidu U. Phylogenitic relationships of marine bacteria, mainly members of the family *Vibrio cholerae*, determined on the basis of 16S rRNA sequence. Int J Syst Bacteriol. 1993;43:8-19.

23. Morris JG. Non- 0 group 1 *Vibrio cholerae*: A look at the epidemiology of an occasional pathogen. Epidemiol Rev. 1990;12:179-191.

24. Oketch A, Kaluoch M. Cholera claims four more as toll goes up. 19 Feb 2015. http://reliefweb.int/report/kenya/cholera-claims-four-more-toll-goes

25. Kim MS, Jeong HD. Development of 16S rRNA targeted PCR methods for the detection and differentiation of *Vibrio vulnificus* in marine environments. Aquaculture. 2001;193:199-211.

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