Available online at www.aexpbio.com

ISSN : 2348-1935

RESEARCH ARTICLE

Annals of Experimental Biology
2015, 3 (3):8-13

Isolation and Characterization of *Vibrio Cholerae* from Water Sources in Zaria, Nigeria

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ABSTRACT

Water is life and has a profound effect on human health. Vibrio cholerae is autochthonous in aquatic environment and is an important human waterborne pathogen causing cholera. This study was designed to determine the presence of Vibrio cholerae in some water sources in Zaria, Nigeria. A total of 207 water samples were collected from various water sources located in Sabon Gari and Zaria Local Government Area of Kaduna State and cultured on thiosulphate citrate bile saltsucrose agar with prior enrichment on alkaline peptone water. Presumptive isolates were identified and characterized using both conventional biochemical method and identification kit. Isolates were serotyped using Vibrio cholerae antisera kit and confirmed by PCR to detect presence of rfbO1, rfbO139 and ompW genes. Overall, V. choleraewas predominant in river (33.3%) followed by well (4.9%) and least predominant in street vended water (2.7%). None was isolated from borehole and pipe borne water. Water samples from SabonGari were most contaminated with an occurrence of 3.2% followed by Samaru (2.6%) while the least contaminated water samples were from Zaria city (1.5%). V. cholerae of the non O1/ non O139 serogroup were isolated from well, river and street vended water with a prevalence of 2.4% in Zaria. Results of this study suggest that the use of PCR targeting ompW species specific gene of V. cholerae in combination with the conventional method would be an important tool in proper identification of V. cholerae.

Key words: Water, *Vibrio cholerae*, *omp*W gene

INTRODUCTION

Water is a necessity for life and an adequate, safe and accessible supply is of paramount importance [1].The impact of water on health is associated with unwholesome sources contaminated with faeces (from humans or animals) containing pathogenic microorganisms [2-4].The quality of drinking water is closely associated with human health and providing safe drinking water is a major public health priority [5].Traditionally, water has been considered to be the most important vehicle for cholera transmission [6].

Vibrio cholerae is a gram-negative bacterium of the family Vibrionaceae and is known worldwide as the etiological agent of cholera [7]. Over 200 serogroups of *V. cholerae* are known of which only O1 and O139 serogroups have been associated with the diarrhoeal disease generally known as cholera [8]. All other serogroups termed non-O1/non O139 are reported as causative agents of sporadic and localized outbreaks of a cholera-like disease [9].*V. cholerae*is found mostly in aquatic environment; water therefore plays an important role in the transmission and epidemiology of cholera [10]. Monitoring this bacterium in water sources is therefore important [11].

Conventional cultural methods currently used for diagnosis of *V. cholera* remains the gold standard [12]. It involves selective pre-enrichment of samples in alkaline peptone water (APW), plating onto thiosulphate citrate bile salt sucrose (TCBS) agar and followed by morphological, biochemical and serological characterization [13], but the process however, is laborious and time consuming [14]. Furthermore, effective characterization and differentiation of V. *cholerae* from certain members of *Vibrio* species such as *V. mimicus* or *Aeromonas species* with respect to their biochemical properties is quite difficult [14]. This study was therefore aimed at using a combination of biochemical and PCR specie-specific primers targeting *ompW* genes to detect *V. cholerae* from water samples in Zaria.

MATERIALS AND METHODS

Study Area: The study was conducted in SabonGari and Zaria Local Government areas of Kaduna State. Three different locations were randomly selected which are Samaru, SabonGari and Zaria city. A total of 207 water source were identified and selected comprising of62 different boreholes, 61 hand dug wells, 44 different pipeborne points, 37 Street vended water hawkers irrespective of source of collection and3 open river flowing through the localities.

Sample collection: One liter of water was collected from each of the 207 water sources aseptically into sterile containers, sealed, labeled appropriately and transported immediately to Laboratory of the Department of Microbiology, Faculty of Science, Ahmadu Bello University Zaria, for further analysis.

Sample Processing: One liter each of the water sample was filtered through 0.45µm membrane filter (Millipore) and the membranes were subsequently transferred into 100ml sterile alkaline peptone water (Oxoid) and incubated for 8h at 37°C. The membranes were then placed aseptically on sterile thiosulphate citrate bile salt sucrose (TCBS) agar (Oxoid) and incubated for 24h at 37°C. Yellow smooth and slightly flattened colonies with opaque centers and translucent peripheries appearing after 24h of incubation were presumably considered as *Vibrio* species and were sub-cultured and for each sample, a representative pure colony was selected and stored on sterile nutrient agar (Oxoid) slant [6].

Biochemical characterization: All presumptive isolates were examined biochemically by screening for oxidase reaction, string test and motility followed by use of standard test kit, Microgen GnA+B-ID system (Microgen Bioproducts Ltd) for species confirmation.

Serological identification: Confirmed *V. cholerae* species were further subjected to slide agglutination test using commercially available polyvalent and monovalent O1 and O139 *V. cholerae* antisera (Denka Seiken).

Detection of the (*ompW*) and somatic antigens (*rfbO1*) and (*rfbO139*) by PCR analysis

Genomic DNA of each of the *Vibrio* species isolated was extracted from 24 h Luria Betani (LB) broth culture of the isolates using DNA extraction kit (Qiagen). The presence of various gene traits in *V. cholerae*was determined by PCR targeting genes for outer membrane protein (*ompW*) and somatic antigens (*rfbO1*) and (*rfbO139*). PCR amplification was performed in a 50µl reaction mixture using a thermal cycler (Applied Biosystems). The reaction mixture contained 25µl PCR Master Mix (2X) (Fermentas), 1µl each of forward and reverse primers, 7µl of template DNA, and 16µl of nuclease free water. The thermal cycling conditions were: initial denaturation at 95°C for 5min, 30 cycles of 30s at 95°C for denaturation, 30s at 55°C for annealing, 1min at 72°C for extension and a final extension at 72°C for 7min.PCR products were then electrophoresed through 1% agarose gel to resolve the amplified products which were then visualized under UV light and documented using the molecular gel doc imaging system (BIO-RAD).

RESULTS

From a total of 207 water samples analyzed, 9 yielded yellow smooth and slightly flattened colonies with opaque centers and translucent peripheries on TCBS which were all motile and positive for oxidase and string test characteristic of *Vibrio* species. Biochemical studies confirmed 2 isolates as *V. cholerae* while 7 isolates consisted of other *Vibrio* species. None of the *V. cholerae* isolates showed agglutination with polyvalent and monovalent *V. cholerae* O1 and O139 antisera and therefore were considered to belong to the non-O1/ non-O139 serogroup. Amplification of the target 208bp gene *omp*W was observed for 5 isolates (Plate 1). However, the *rfb*O1 and

*rfb*O139 genes targeting the specific serogroups were absent confirming the result of the serology test performed that the *V. cholerae* isolated were of the non-O1/ non-O139 serogroup.

The prevalence of *V. cholerae* in water sources from different sites in Zaria is shown in Table 1. The result shows that prevalence of *V. cholerae* in Zaria was 2.4%. *V. cholerae* was isolated from well, river and street vended water with water sample from river having the highest occurrence (33.33%) followed by samples from well water (4.9%) while the least occurrence was obtained in street vended water (2.7%). *V. cholerae* was however not isolated from water samples from boreholes and taps in all the three sampling sites.

With regard to the various sites of sampling locations, Sabongari had the highest prevalence of 3.2%. *V. cholerae* was isolated only from well water from 2 out of 19 samples (10.5%). Samaru had a prevalence of 2.6% with the organism being predominant in street vended water with an occurrence of 6.3% followed by well water with an occurrence of 4.2%. Water samples from river, boreholes and wells in this site had an occurrence of 0.0% as the organism was not isolated from them. On the other hand, Zaria city had the least prevalence of 1.5% with *V. cholerae* isolated only in water sample from the river source with a 100% occurrence.

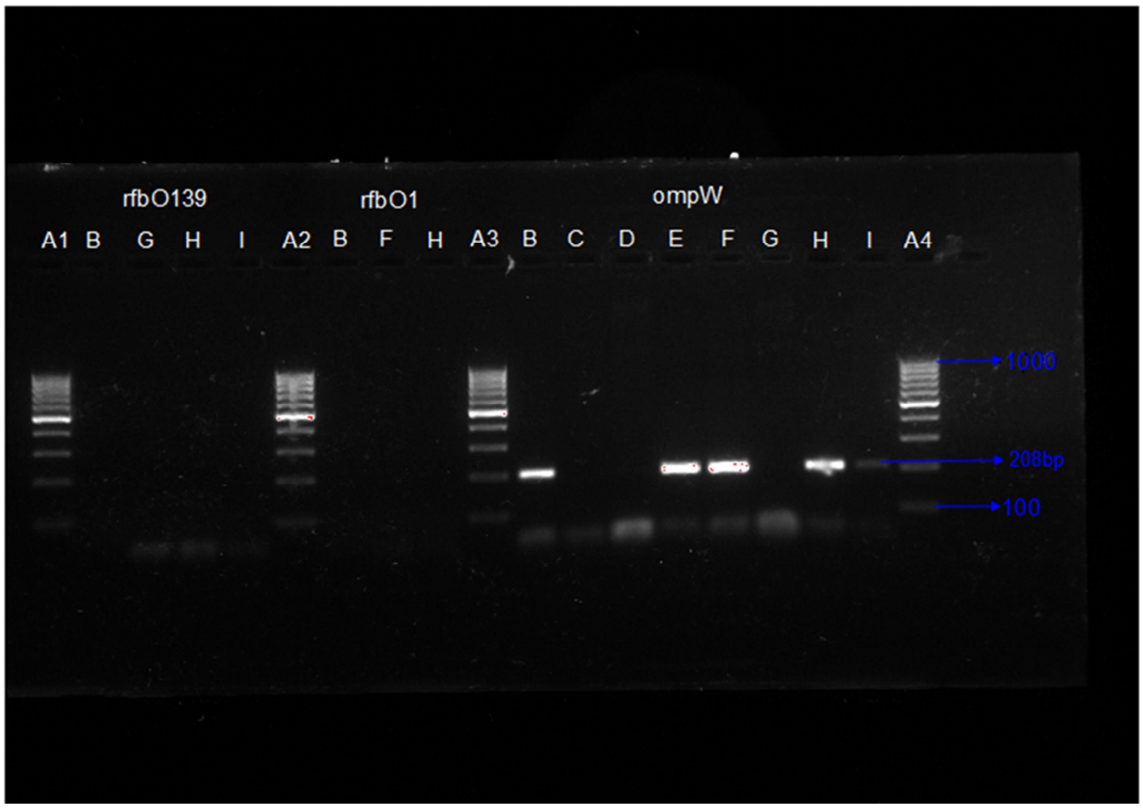

Plate 1: Agarose gel electromatogram of PCR products showing *ompW*, *rfb*O1 and *rfb*O139 genes of *V. cholerae*.

Key:
Lanes A1, A2, A3 and A4 represent 100bp molecular ladder.
Lanes A1 to A2 represents isolates tested for *rfb*O139 gene.
Lanes A2 to A3 represents isolates tested for *rfb*O1 gene.
Lanes A3 to A4 represents isolates tested for ompW gene of which B, E, F, H, and I lanes represent isolates exhibiting positive *V. cholerae* with amplicon size of 208bp *omp*W gene but negative for *rfb*O1 and *rfb*O139 genes.
Statistical analysis showed that there was a significant difference and association between the sources of water and occurrence of *V. cholerae* in Zaria with $P = 0.03$ ($P \leq 0.05$) (Table 2). For Samaru and Sabongari, $P \geq 0.05$ (0.729 and 0.331 respectively) and this indicates that there was no significant difference and association between the source

of water and occurrence of the organism in the two sample sites. However in Zaria city, there was a significant difference and association between the source of water and occurrence of the organism with P = 0.00 (P≤ 0.05).

Table 1: Distribution of *V. cholerae* from different water sources in Zaria

Location	Samaru		Sabongari		Zaria city		Total	
Source	N	p (%)	N	p (%)	N	p (%)	N	p (%)
Borehole	22	0 (0.0)	17	0 (0.0)	23	0 (0.0)	62	0 (0.0)
Well	24	1 (4.2)	19	2 (10.5)	18	0 (0.0)	61	3 (4.9)
Pipeborne	14	0 (0.0)	16	0 (0.0)	14	0 (0.0)	44	0 (0.0)
Street vendor	16	1 (6.3)	9	0 (0.0)	12	0 (0.0)	37	1 (2.7)
River	1	0 (0.0)	1	0 (0.0)	1	1 (100)	3	1 (33.3)
Total	77	2 (2.6)	62	2 (3.2)	68	1 (1.5)	207	5 (2.4)

Key: N = number of samples; P = number of positive samples; (%) = percentage positive

Table 2: Comparative occurrence of *V. cholerae* in water sources in Zaria using Kruskal-Wallis test

Sources	Samaru		Sabongari		Zaria city		Total	
	Nmr		Nmr		Nmr		Nmr	
Borehole	22	40.00	17	32.50	23	35.00	62	106.50
Well	24	38.40	19	29.42	18	35.00	61	101.41
Pipeborne	14	40.00	16	32.50	14	35.00	44	106.50
Street vendor	16	37.59	9	32.50	12	35.00	37	103.70
River	1	40.00	1	32.50	1	1.00	3	72.00
χ^2	772.037		62	4.602	68	67.000	207	16.344
P		0.729		0.331		0.000*		0.003*

Key: N = number of samples; mr = mean rank; χ^2 = chi square; P = P - value
** = Statistically significant P-values (where P ≤ 0.05).*

DISCUSSION

V. cholerae isolated from the water sources (well, street vendor and river) were of the non-O1/non-O139. Non-O1/non-O139 *V. cholera* sero group in the past has been considered of negligible importance [15]. Presently, this strain can no longer be ignored [16],as several studies have revealed isolation of the organism from various water sources and its association with hospitalized patients presenting with diarrhoea, peritonitis and also in immunocompromised cases in several parts of the world.[9][15-20]In a research conducted by Marin et al[21],*V. cholerae* strains from sequential outbreaks in Nigeria were characterized and it was observed that a lineage of *V. cholerae* non-O1/non-O139 serogroup was circulating with atypical El Tor strains isolated from patients with cholera like cases. Their findings indicated that non-O1/non-O139 serogroups were the important cause of diarrhoeal diseases in Nigeria. The presence of theseserogroup in water is of public health importance and suggests that environmental conditions are suitable for survival of epidemic *V. cholerae* O1 in the water source [19].

The outer membrane protein (*omp*W) gene is one that is highly conserved among *V. cholerae* strains which serves as a good species-specific marker for *V. cholera* [10] and this has led to the development of primers targeting the gene for rapid identification of the microorganism [22]. In some cases biochemical tests may not be able to differentiate *V. cholerae* from other *Vibrio* speciesas a result of sharing serological markers and phenotypic characters among *Vibrio* species [23]. PCR on the other hand able to identify and characterize organisms based on genotypic traits hence, more sensitive. A similar study was conducted by Baron et al[24], and result of their work showed that PCR can be used for checking doubtful biochemical identification of *V. cholerae* using the *ompW* species-specific primer. In Nigeria, majority of the rural populace do not have access to potable water therefore, depend on water from borehole, well, stream or river for domestic use [25]. Zaria is known to be one of the oldest towns in Northern Nigeria which is very large with a growing population from different parts of the world. However, the town has been known to have unending water problems despite being blessed with an abundant water resource [26].Most of the populace depends on water from wells and boreholes for domestic use. Also street vendors are seen at all times hawking water in large gallons pushed in trolleys. Factors such as improperly covered wells, poor drainage systems were located wells, location of wells in close proximity to septic tanks and several unhygienic practices could causes of contamination of the wells with the organism. Ground water sources such as wells and springs are often believed to be of good quality with regard to bacterial pathogens transmitted by faecal-oral route.

Most of the street vendors purchased water from private owners of motorized boreholes that pump the water directly into large storage tanks with hose attached for transfer into containers for the street vendors while a few obtained

water from public wells. Some of these containers or gallons were observed to have cracks which are not properly sealed while some use dirty polythene bags to seal the leaking edges. Interaction with some street vendors revealed that the containers are not washed frequently. While the source of contamination of the street vended water may have varied and could not be determined, evidence suggests that the contamination may have occurred from any of the factors observed during sampling. In 1996 there was an outbreak of severe diarrhoea in Kano. In an attempt to find the possible risk factors, Hutinet al[27] conducted a research and their results showed that street vended water was associated with the illness. Similarly, consumption of street vended water has also been reported to be a significant risk factor associated with cholera epidemic in Sierra Leone [28] and Latin America.[29[30][31] Vended water is common in many parts of the world where scarcity or lack of infrastructure limits access to suitable quantities of drinking water. Although water vending is more common in developing countries, it also occurs in developed countries. Nigeria is also not an exception because municipal water supply is not available for all households, water vendors can be observed at all times in Zaria. The non-detection of the pathogen in water samples from taps and boreholes analyzed in this study may be a reflection of the water treatment process and depth of the boreholes respectively.

CONCLUSION

Findings of this study revealed the presence of *V. cholerae* of the non-O1/non-O139 serogroup in some water sources in Zaria with a prevalence of 2.4%. This indicates that water plays a significant role in transmission of the organism, and drinking water from such contaminated sources could pose a threat to public health. Such information is important for raising awareness regarding the presence of *V. cholerae* of the nonO1/nonO139 serogroup so that precautionary measures are taken on time. The organism was successfully characterized using PCR targeting the species-specific gene, *ompW*. Although traditional identification system based on cultural and biochemical characteristics are important, a combination of both conventional and molecular technique using PCR targeting species-specific gene (*ompW*) should be encouraged for reliable confirmation of *V. cholerae* in our environment.

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