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Immunochromatographic detection of *Vibrio cholerae* O1 and O139 antigens among patients with diarrhoea in hospitals in Jos, Plateau State, Nigeria

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Abstract
Two serogroups of Vibrio cholerae, O1 and O139 are recognized for explosive outbreaks of severe diarhoea and have an inclination to causing pandemics with 60% of all cases occurring in sub-Saharan Africa. Screening for cholera is seldomly carried out in cases of acute watery diarrhoea in hospitals.

Objective: The aim of this study is to determine the prevalence of Vibrio cholerae O1 and O139 and the associated risk factors among patients presenting with diarrhoea in some hospitals in Jos, Nigeria.

Methods: Immunochromatographic assay was used to detect these antigens among patients presenting with diarrheoa in selected hospitals.

Results: Two cases of Vibrio cholerae O1 infection (2.0%) and no case of Vibrio cholerae O139 was recorded from this study. The cases of Vibrio cholerae O1 were observed among males and the age range for predisposition to infection with Vibrio cholerae O1 was non-specific to any age group. All the positive subjects were resident in urban slums. Risk factors such as source of water revealed that consumption of well water was associated with Vibrio cholerae O1 infection. There was no relation between type of toilet, hand washing, consumption of home cooked food, and symptoms of the infection. However, significant association existed between duration and frequency of diarrhoea with Vibrio cholerae O1 infection.

Conclusion: Vibrio cholerae O1 is present in the studied population hence basic hygiene habits such as regular hand washing and proper disposal of human faecal waste should be emphasized to the public. Regular screening for these pathogens should be integrated into diarrhoeal disease control programs.

Keywords: diarrhoea, cholera, *Vibrio cholera*, Jos nigeria.

Introduction

The species *Vibrio cholerae* consists of many pathogenic and non-pathogenic strains[1]. However, the only serogroups that cause the infection defined clinically and epidemiologically as cholera are *Vibrio cholerae* O1 and O139[2]. These strains have a tendency to cause wide spread outbreaks and a proclivity to bring about pandemics[3]. The disease is a crucial public health challenge in numerous developing countries despite all control measures [4].

Large scale cases of infection continue to cause substantial illness and death among vulnerable populations, both in crisis and prevalent locales, with a potential for rapid spread in areas where sanitation, water and hygiene is poor[5]. However, screening for cholera among patients presenting with diarrhoea is not integrated into national diseases control programs, thus resulting in under-diagnosis [6, 7].

During epidemics, the presence of bacteria with darting movements under dark-field inspection in a stool sample confirms the diagnosis, but certain confirmation still requires isolation of the organism in culture [8]. Rapid diagnosis of cholera by the use of a highly sensitive and specific immuno-chromatographic test used on fresh stools is the first recommended procedure for detection of *Vibrio cholerae* before culture method [9].

The aim of this study is to determine the prevalence of *Vibrio cholerae* O1 and O139 and its associated risk factors among patients with diarrhoea in hospitals in Jos, Plateau State.

Materials and Methods

A cross-sectional study was carried out to obtain the prevalence of pathogenic *Vibrio cholerae* O1 and O139 infection among patients presenting with diarrhoea in Plateau State Specialist Hospital and Faith Alive Foundation Hospital, Jos, Nigeria.

Ethical approval was obtained from the Health Research Ethics Committee of Plateau State Specialist Hospital and Faith Alive Foundation Hospital, Jos, Nigeria. The study included consenting male and female patients presenting with diarrhoea in the two major hospitals and excluded patients who withheld their consent.

One hundred stool samples collected in sterile sample collection bottles were properly labelled with the subject

identification number for every diarrhoeal patient. Samples were analyzed immediately after collection, observed for macroscopic characteristics such as consistency, presence of blood and tested with CTK biotech onsite cholera Ag rapid test (Mesa Rim road, San Diego, USA) using the procedure in the manufacturer's manual. The lateral flow immunoassay consisted of monoclonal anti-*V. Cholerae* O1 and O139 antibodies conjugated with colloid gold (O1/O139-antibody conjugates). The tests, specimens and buffer were brought to room temperature (15-30°C) and an adequate volume (approximately 80 µL) of a test specimen was applied into the sample well of the test cassette. The specimen migrates by capillary action across the cassette. The *V. cholerae* O1/O139 antigen if present in the specimen bound to the corresponding O1/O139 antibody gold conjugate. This immune-complex is then captured on the membrane by the pre-coated anti-*V. cholerae* O1/O139 antibody, forming a burgundy coloured test line, indicating a O1/O139 positive result. Absence of the test line indicated a negative result.

Well-structured questionnaires were used to obtain relevant data. Data collected was checked for completeness and consistency and evaluated using SPSS version 23. Chi-square test was used to determine the association between risk factors and socio-demographic characteristics in relation to the infection. A p-value of less than, or equal to 0.05 was considered significant.

Results

Out of a total of 100 samples collected, two cases (2.0%) of *Vibrio cholerae* O1 serotype was detected. However, no case of O139 serotype of cholera causing *Vibrio cholerae* was detected. The sex-based distribution of prevalence shows that males had a prevalence of 3.8% while there was no occurrence of *Vibrio cholerae* among the female subjects. Though there was a marginal difference in occurrence of the pathogen, there was no significant association (Fisher's exact test =0.496). The age range for predisposition to infection with *Vibrio cholerae* O1 from this cross-sectional study was non-specific to any age group (χ^2= 5.612 and p=0.468), infection was recorded in the 0-10year age group (5.0%) and in the 41-50year age group (10.0%). Occurrence of the pathogen with respect to place of residence of the study subjects revealed that all positive samples [2(5.0%)] were obtained from urban slums (Table 1).

Outcome of the study with respect to other risk factors that predispose to *Vibrio cholerae* O1 infection are presented in Table 2. Consumption of well water (3.9%) was responsible for infection. There was no significant difference in occurrence of infection among individuals who practice frequent (2.0%) and infrequent (2.0%) hand- washing (Fisher's exact test = 1.0). Also infection was detected among individuals who consumed home cooked food (2.4%). However, using pit (1.6%) and open defecation (16.7%) accounted for the cases of *Vibrio cholerae* O1 observed (χ^2 = 7.299 and p =0.063).

Table 3 depicts the symptoms of *Vibrio cholerae* infection as presented by the study subjects. Individuals who tested positive for *Vibrio cholerae* O1 showed mild (2.0%) or no dehydration (2.0%) There was no occurrence of vomiting in both cases of infection. There was significant relationship between the duration (χ^2 =49.942 and $p \leq 0.001$) and frequency (χ^2= 49.634 and $p \leq 0.001$) of diarrhoea with occurrence of *Vibrio cholerae* O1 infection.

Table 1: Prevalence of *Vibrio cholerae* O1 with respect to demographic factors.

Parameter	No. of sample	No. Positive (%)	χ^2	p-value
Sex				
Male	52	2(3.8)		0.496†
Female	48	0(0.0)		
Total	100	2(3.8)		
Age (years)				
0 – 10	20	1(5.0)	5.612	0.468
11 – 20	20	0(0.0)		
21 – 30	14	0(0.0)		
31 – 40	08	0(0.0)		
41 – 50	10	1(10.0)		
51 – 60	21	0(0.0)		
61 – 70	07	0(0.0)		
Residence				
Urban	41	0(0.0)	3.061	0.216
Urban- slums	40	2(5.0)		
Rural	19	0(0.0)		

†= Fisher's Exact Test

* = Significant association exists at $p \leq 0.05$

** = Significant association exists at $p \leq 0.01$

Table 2: Prevalence of *Vibrio cholerae* O1 with respect to risk factors.

Parameter	No. of sample	No. Positive (%)	χ^2	p-value
Source of water				
Pipe-borne	14	0(0.0)	1.961	0.581
Bore-hole	27	0(0.0)		
Well	51	2(3.9)		
Others	8	0(0.0)		
Toilet				
Closet	32	0(0.0)	7.299	0.633
Pit	61	1(1.6)		
Bucket	1	0(0.0)		
Open	6	1(16.7)		
Hand washing				
Frequent	51	1(2.0)		1.000†
Infrequent	49	1(2.0)		
Food handling				
Home	80	2(2.4)		1.000†
Road side	18	0(0.0)		

† = Fisher's Exact Test;

* = Significant association exists at $p \leq 0.05$

** = Significant association exists at $p \leq 0.01$

Table 3: Prevalence of *Vibrio cholerae* O1 with respect to cholera symptoms.

Parameter	No. of sample	No. Positive (%)	χ^2	p-value
Dehydration				
No	49	1(2.0)		0.742†
Mild	51	1(2.0)		

Severe	0	0(0.0)		
Vomiting				
Yes	0	0(0.0)		1.000†
No	100	2(2.0)		
Duration of diarrhoea				
1day	5	0(0.0)	49.942	<0.001**
2 days	21	1(1.9)		
3days	53	0(0.0)		
4days	15	0(0.0)		
5 days	5	0(0.0)		
More	1	1(100)		
Frequency of diarrhoea				
. Once per day	1	1(100)	49.634	< 0.001**
Twice per day	14	0(0.0)		
Three times per day	78	1(1.3)		
Four times per day	6	0(0.0)		
More	1	0(0.0)		

† = Fisher's Exact Test;
* = Significant association exists at $p \leq 0.05$;
** = Significant association exists at $p \leq 0.01$

Discussion

Two cases (2.0%) of *Vibrio cholerae* O1 were observed from this study. This finding is lower than results from another hospital based study conducted in India [10] where a prevalence of (3.93%) was reported. This low figure may be because the dry season in which the study was carried out, limited the survival of the fragile vibrio, thus limiting their explosive potential, however it also indicates that the pathogen is still present within this study area. *Vibrio cholerae* O139 was not detected from this study. This could be because increased awareness, adequate treatment and control measures have been effective in restricting *Vibrio cholerae* O139 to South Asia from where it originates, thus corresponding other studies in Nigeria which indicate that the most common serogroups of *Vibrio cholerae* are *Vibrio cholerae* O1 and non O1 and O139 serogroups [11].

The cases of *Vibrio cholerae* O1 were observed among males (3.8%) and may be as a result of exposure through outdoor activities. This finding does not correspond with reports from UNICEF [12] which reported that women and girls had a higher risk of exposure to a high infectious dose of cholera through their domestic roles. More so, the age range for predisposition to infection from this cross-sectional study was non-specific to any age group. This result does not correspond with studies carried out in Bangladeshi [13] where older age groups had a significant relationship with lower occurrence of *V. cholerae* infection. Also, all the positive subjects were resident in urban slums, possibly because of the high population density and inadequacy of social amenities. This finding does not correspond with studies where rural areas [14] have a higher rate of infection. This study indicates that living conditions of urban slums in this study area should be improved.

Risk factors such as source of water revealed that consumption of well water accounted for the cases of *Vibrio cholerae* O1 recorded. This may be because well water is usually consumed without prior treatment. This is consistent with results from studies conducted in the Philippines, where access to improved water sources such as well was associated with higher cholera due to non-chlorination of well water [15].

Furthermore, occurrence of infection was distributed equally based on frequency of hand washing. No significant relationship was observed and this may be because cholera is endemic in this study area, hence there is increased risk of infection. However, this finding does not correspond to reports from studies by Zohura *et al.* [16] which implicates hand washing as an important contributor to pathogenic *Vibrio cholerae* infection.

With respect to common symptoms of infection, there was significant association between duration and frequency of diarrhoea with occurrence of infection. This could be because frequent diarrhoea especially for an extended period is a common symptom of infection. This is consistent with other studies which indicate that protracted and frequent diarrhoea is a major symptom of *Vibrio cholerae* O1 infection [17]. This finding suggests that tests for *Vibrio cholerae* O1 infection should be carried out, for individuals presenting with frequent and protracted diarrhoea in routine diagnostic tests.

Conclusion

The study indicates that *Vibrio cholerae*, especially the O1 serogroup is prevalent in this study area and also indicate that water, hygiene and sanitation structure of the people within the study area is inadequate, hence they are predisposed to a greater risk of a high infectious dose of *Vibrio cholerae.* In order to limit explosive outbreaks, there should be provision of clean water and proper sanitation for people who live without these basic amenities. More so, the public should be reminded of basic hygienic habits, such as frequent hand-washing with soap as well as appropriate handling, preparation and storage of food. Management centres should be set up in endemic areas and most importantly, regular screening for cholera should be integrated into national diarrhoeal disease control programs in endemic areas.

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