

## VIBRIO CHOLERAE O1 INFECTIONS IN JOS, NIGERIA

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A study to determine the prevalence of *Vibrio cholerae* O1 in stool sample submitted for routine examination of enteric pathogens, as well as identify the serotypes and antibiogram of the isolates to commonly used antibiotics was undertaken. The survey involved the examination of 774 (763 stool and 11 rectal swabs) specimens obtained from different patients seen at the Jos University Teaching Hospital (JUTH). Of the total number examined, 34 (4.39%) yielded *Vibrio cholerae* O1. All of them were Inaba serotype of El-Tor biotype. The age group 20-29 years had the highest rate, 21 (6.95%). Rectal swabs yielded a higher number of isolates, 9 (81.82%) from 11 specimens compared to 25 (3.28%) from 763 stool specimens. The organism is most prevalent during the mid-rainy season (June/July) since most of the isolates 29 (85.29%) of the 34 isolates were isolated during this period ( $P < 0.05$ ). Isolates were very sensitive to ofloxacin, erythromycin and tetracycline but resistant to chloramphenicol, ampicillin, cloxacillin and penicillin G. This study demonstrates that *Vibrio cholerae* O1 is endemic in our environment.

### INTRODUCTION

Cholera (Greek, chole, bile) is caused by the Gram-negative *Vibrio cholerae* bacterium of the family Vibrionaceae. Although, there are many serogroups, only O1 and O139 have exhibited the ability to cause epidemics (1). *Vibrio cholerae* O1 is divided into 2 serotypes, Inaba and Ogawa, and 2 biotypes, Classic and El-Tor. Throughout recorded history, Cholera has caused seven pandemics in various areas of the world especially in Asia, the Middle East, and Africa. The first reported case in Nigeria occurred in 1970 (2). Since then, the disease has remained endemic with occasional outbreaks in some states of the country primarily due to lack of good water supply and poor personal and environmental hygiene.

An average of 3,000 stool specimens is processed annually at the medical microbiology department of Jos University Teaching Hospital (JUTH). From the results obtained from these specimens over the past

one decade, it was observed that the rate of isolation of enteric pathogens has been very low. Due to the limited range of facilities in JUTH, only a selected few of the enteric bacterial pathogens are sought for routinely; *Salmonella*, *Shigellae*, and occasionally enteropathogenic *Escherichia coli*. Others such as *Vibrio cholerae*, *Helicobacter* and *Campylobacter* are never sought for routinely. This has led to the always-recurring result "no pathogen isolated".

This study was therefore undertaken to examine the prevalence of *Vibrio cholerae* O1 in stool samples routinely processed in JUTH and identify the prevailing serotype and antibiogram of recovered isolates in the light of reported cases of clinical cholera.

### MATERIALS AND METHODS

#### Samples

The samples analysed in this study included a total of 774 (763 stools and 11 rectal swabs) of patients attending the Jos University Teaching Hospital. These were

brought in clean, transparent, wide mouthed bottles. In suspected cholera cases, rectal swabs were collected by nurses in the wards and inoculated into bottles of sterile alkaline peptone water. Both out patients and nurses were instructed on the mode of collection.

#### **Processing of specimens**

The specimens were processed according to the guidelines on laboratory methods for the diagnosis of *Vibrio cholerae* by the Centres for Disease Control, National Centre for Infectious Diseases and Prevention (CDC/NIVD), Atlanta Georgia, United States of America and as described by Collee and Miles (3). These include macroscopy, microscopy, motility testing, Gram stain, culture and biochemical testing. Others are serology and antimicrobial susceptibility testing. Specimens were inoculated into thiosulphate citrate sucrose bile salt (TCBS) agar (Antec Diagnostics, UK) and alkaline peptone water and incubated at 37°C. After 6 hours of incubation, subcultures were made from the surface growth on alkaline peptone water onto TCBS and incubated overnight. Colonies from TCBS agar were inoculated onto Brain Heart Infusion (BHI) agar for biochemical and serological identification.

#### **Biochemical testing**

Suspected organisms were identified as *Vibrio cholerae* O1 from growth on BHI agar using the following standard tests; oxidase test, stringing test, citrate utilization test, lysine decarboxylase test, direct haemagglutination test, nitrosol-indole test and immobilization by distilled water. All tests were as described by Collee and Miles (3) and Porter and Duguid (4).

#### **Antimicrobial susceptibility testing**

Sensitivity of isolates to antimicrobial agents was determined on BHI agar plates using the disc diffusion method of Scott (5). From a pure culture of the isolate to be tested, a uniform streak was made on the agar plate. The antibiotic discs (Antec Diagnostics, UK) were then placed on the plates and incubated at 37°C overnight. Interpretation of results was done using the zone sizes. Zone diameters of inhibition of  $\geq 18$ mm were considered sensitive, while 13-17 mm intermediate and  $< 13$  mm were considered resistant. All isolates were tested for sensitivity to the following antibiotics; ampicillin (10mcg), chloramphenicol (10mcg), streptomycin (10mcg) tetracycline (10mcg), cotrimoxazole (25mcg), erythromycin (5mcg), ofloxacin (95mg) and penicillin G (10 units).

#### **Statistical analysis**

The data obtained were subjected to the Chi-squared test using a probability of  $P < 0.05$  as the level of significance.

#### **RESULTS**

A total of 774 samples made up of 763 stool and 11 rectal swabs were examined over a period of 8 months (January-August 1996). The age range of the patients was 0- 69 years. Of the total number of specimens examined, 34 (4.39%) were positive for *Vibrio cholerae* O1. The highest numbers of isolates were recovered from the age group 20-29 years, followed by age group 30-39 years with 5 isolates. The age brackets 0-9 years, 10-19 years, and 40-49 years had 2 isolates each. Only one isolate each was recovered from the age groups 50-59 and 60-69 years (Table 1). The difference is not statistically significant ( $p > 0.05$ ).

Macroscopic examination of the specimens showed that 189 were watery, 321 soft formed, 15 blood-stained, 206 hard formed and 32, mucoid. The watery samples yielded the highest number of isolates (21), soft-formed specimens 3 and hard formed 1. The blood stained and mucoid specimens yielded no isolates. The remaining 9 positive were from rectal swabs (Table 2).

Table 3 shows the prevalence of *Vibrio cholerae* 01 in in-patients and out-patients. Of the 580 samples taken from out-patients, 6(1.03%) were positive for *Vibrio cholerae* 01, while 28 (14.43%) of the 194 samples from in-patients yielded *Vibrio cholerae* 01. This difference is statistically significant ( $p < 0.05$ ). The monthly isolation of *Vibrio cholerae* 01 is shown in Table 4. Only one isolate was recovered in January, none in February, March, and April. Two isolates were obtained in May, 20 in June, 9 in July, and 2 in August. Serological screening of all the 34 isolates showed that all were of the Inaba serotype and El-Tor biotype.

Table 5 shows the *in-vitro* susceptibility pattern of the isolates. All the isolates were sensitive to tetracycline, erythromycin and ofloxacin. The only strain showing the widest range of susceptibility was isolated in January. It was sensitive to cotrimoxazole and gentamicin in addition to the other three antibiotics. This difference is statistically significant ( $p < 0.05$ ).

**Table 1: Prevalence of *Vibrio cholerae* 01 isolated in relation to age**

Age groups (Years)	No examined	No positive (%)
0-9	142	2(1.41)
10-19	109	2(1.83)
20-29	302	21(6.95)
30-39	108	5(4.63)
40-49	55	2(3.64)
50-59	37	1(2.70)
60-69	21	1(4.76)
<b>Total</b>	<b>774</b>	<b>34(4.39)</b>

$$X^2 = 8.97 \quad df = 6 \quad p > 0.05$$

**Table 2: Type of samples treated and the number of *Vibrio cholerae* 01 isolated**

Types of stool sample	No examined	No positive (%)
Watery	189	21(11.11)
Soft formed	321	3(0.93)
Blood stained	15	0(0.00)
Hard formed	206	1(0.49)
Mucoid	32	0(0.00)
<b>Total</b>	<b>763</b>	<b>25(3.28)</b>
Rectal swab	11	9(81.82)

**Table 3: Prevalence of *Vibrio cholerae* 01 isolated from the various specimen sources**

Source of specimen	No examined	No positive (%)
Out patients	580	6(1.03)
In-Patients	194	28(14.43)
<b>TOTAL</b>	<b>774</b>	<b>34(4.39)</b>

$$X^2 = 50.81 \quad df = 1 \quad p < 0.05$$

**Table 4: Monthly incidence of *Vibrio cholerae* 01 in JUTH**

Period	No examined	No positive (%)
January	25	1(4.00)
February	29	0(0.00)
March	125	0(0.00)
April	111	0(0.00)
May	103	2(1.85)
June	163	20(12.2)
July	100	9(9.00)
August	68	2(2.95)
<b>Total</b>	<b>774</b>	<b>34(4.39)</b>

**Table 5: In-vitro antibiotic susceptibility pattern of *Vibrio cholerae* O1**

Antibiotic (mcg)	No examined	No positive (%)
Ofloxacin (5)	34	34(100)
Erythromycin (5)	34	34(100)
Tetracycline (10)	34	34(100)
Cotrimoxazole (25)	34	1(2.94)
Gentamicin (10)	34	1(2.94)
Chloramphenicol(10)	34	0(0.00)
Ampicillin (10)	34	0(0.00)
Cloxacillin (5)	34	0(0.00)
Penicillin (10 unit)	34	0(0.00)

$$\chi^2 = 130.35 \text{ df} = 8 \quad P < 0.05$$

## DISCUSSION

A total of 774 samples were analysed in this study, in which 34 (4.39%) were positive for *Vibrio cholerae* O1. The percentage is low compared to the 18% documented by Shapiro *et al* (6) in rural western Kenya in diarrhoea specimens. Our finding is however significant since the specimens included non-diarrhoea stools. All the 34 isolates were Inaba serotype of the El-Tor biotype. This is contrary to the 1991 outbreak in Jos, in which Ogawa was the predominant serotype. Most strains causing epidemics in Nigeria have been Ogawa serotype (2, 7). Reports of one serotype displacing another have been on the increase. Gomez *et al* (8) reported a case in which Inaba strains, which were dominant in Mexico in 1991, were later sub-planted by Ogawa serotype in 1992. In Calcutta, India, *Vibrio cholerae* O139 displaced El-Tor *Vibrio cholerae* O1 (causative agent of the seventh pandemic), an event that has never happened in recorded history of cholera (1). The toxigenic Inaba serotype of *Vibrio cholerae* O1 biotype El-Tor however reappeared in India in 1998 and 1999, almost a decade after its last dominance in the Calcutta episode (9). Antigenic variations

have also been observed *in-vitro* and that more than one antigenic variant may be isolated from the stool of cholera patients (10).

The highest number of isolates were from the age group 20-29 years with 21 (6.95%), while the least 1 (2.7%) and 1(4.76%) were from the age brackets 50-59 years and 60-69 years respectively. The age group 0-9 years yielded 2 (1.4%) isolates. This finding differs from the report of Samir (11) who stated that in endemic areas, clinical infections are most common among the "unsalted" pre-school children. It however agrees with that of Bhattacharya *et al* (12) who found majority of cases in adults.

Macroscopic examinations of the stool samples showed that watery stools yielded the highest number of isolates 21(11.11%). This agrees with reports in the literature, which showed that cholera is characterized by watery stools often called "rice water stool". Mucoid and blood stained samples yielded no *Vibrio cholerae* O1. This is not unexpected since the organism is non-invasive (1, 13). The percentage isolation from rectal swabs was 81.82%. This agrees with Porter and Duguid (4), who advocate the collection of rectal swabs, especially when screening for carriership.

The percentage isolation from outpatients was low (1.03%) compared to 14.43% in in-patients. This is not surprising since clinical cholera cannot be treated on outpatient basis. The few isolates from the outpatients could be from contacts or carriers. The periodic pattern of *Vibrio cholerae* O1 showed that the highest numbers of isolates were recovered in June. This is probably due to the fact that June

corresponds to the middle of the raining season in Jos during which rivers and streams begin to flood and as such carry along waste from the country side. Also, dams are flooded supplying more water than the treatment facilities can cope with. In addition, because of torrents associated with rains, water pipes become exposed, damaged and contaminated by faecal material.

The isolates were very sensitive to ofloxacin, erythromycin and tetracycline. The only isolate recovered in January showed a wider range of susceptibility to the other antibiotics. It is possible that this strain mutated and later developed multiple drug resistance thereby leading to the epidemics of June and July. Coppo *et al* (14) had reported the introduction of cholera into Somalia by an initial drug susceptible strain. An alternative explanation could be that this strain was supplanted by a new strain brought to the state from neighbouring Kano where an epidemic had been reported since January.

This study has shown that cholera is endemic in our environment. It is therefore recommended that stool specimens should be routinely examined for *Vibrio cholerae*.

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