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Vibrio cholerae O139 Bengal: A Descriptive Study

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ABSTRACT

A prospective study was conducted to determine the clinical and laboratory characteristics and the clinical course of cholera due to *Vibrio cholerae* O139 Bengal. The study subjects included 22 adult males with stool culture-proven *V. cholerae* O139. On enrollment, mean ±SD concentrations (mmol/L) of serum sodium, potassium, chloride, and bicarbonate were 134±3, 4±1, 102±4, and 13±4 respectively, and stool sodium, potassium, chloride, and bicarbonate concentrations were 120±24, 18±6, 93±16, and 37±9 respectively. Seventeen patients (7.8%) had faecal leukocytes ranging from 11 to 50 per high-power field. All *V. cholerae* O139 isolates (100%) were susceptible to tetracycline, erythromycin, and ciprofloxacin, 92% to furazolidine, and only 5% to trimethoprim-sulphamethaxazole. The median (interquartile) volume of liquid stool during the first 24 hours was 9 (5-12) litre. The median (interquartile) volume of liquid stool and the amounts of intravenous and oral rehydration fluids required during the entire study period were 16 (9-24) litre, 9 (6-18) litre, and 14 (9-20) litre respectively. The median (interquartile) duration of diarrhoea was 80 (48-104) hours. The median (interquartile) duration of excretion of *V. cholerae* O139 in stool was 5 (3-6) days. Clinical and laboratory features, and case management of cholera due to *V. cholerae* O139 are very similar to conventional cholera due to *V. cholerae* O1.

Key words: *Vibrio cholerae*; Cholera; Prospective studies

INTRODUCTION

Epidemics of severe dehydrating diarrhoea due to a new serotype of *Vibrio cholerae* were first reported from southern India in October 1992 (1), and from the coastal regions of Bangladesh in December of the same year (2). In Dhaka, the capital city of Bangladesh, an untimely outbreak of cholera-like diarrhoea occurred in mid-January 1993. By the end of March of the same year, 107,297 cases of diarrhoea and 1,473 deaths had been reported (3). It was later established that the epidemics in India and Bangladesh were caused by a single, previously unknown serotype of *V. cholerae*, subsequently designated as *V. cholerae* O139 synonym Bengal (4,5). Non-*V. cholerae* O1 have never been repeated to be associated with an epidemic disease; however, they have the potentials to cause outbreaks or sporadic cases of diarrhoea (6). During the epidemic in Bangladesh, adults were predominantly affected, and in its severe form the clinical features of the disease were observed similar to those of cholera due to *V. cholerae* O1. The new serotype of *V. cholerae* O139 has now been well characterized.

In this study, we have described the presenting features in adults with severe dehydrating diarrhoea due to *V. cholerae* O139, along with laboratory profiles, the clinical course of illness in the hospital in terms of liquid stool volume, the duration of diarrhoea, and the duration of faecal excretion of *V. cholerae* O139.

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METHODS AND MATERIALS

The study was conducted at the Dhaka Diarrhoea Treatment Hospital of the ICDDR,B: Centre for Health

and Population Research from September to October 1993. The study protocol was a component of a larger research project on clinical studies of a new cholera epidemic due to hitherto unrecognized pathogens, and was approved by the Ethical Review Committee of the Centre (3). Informed consents for participation in the study were obtained from all patients.

Selection of patients

The patients were selected according to the following criteria: (a) every 4th male patient, aged 18-60 years, who attended the out-patient department of the Dhaka Hospital of the ICDDR,B from 6 a.m. to 9 a.m.; (b) came with a history of watery diarrhoea for less than 12 hours; (c) had signs of severe dehydration; (d) did not take any antimicrobial or antidiarrhoeal medications; and (e) was positive for *V. cholerae* in the initial dark-field microscopic examination of stool. Women were excluded because of difficulties in separating their urine from stool. Patients were excluded if they had systemic illness, such as pneumonia, meningitis, typhoid fever, and suspected septicaemia. The final study subjects included those whose stools were positive for *V. cholerae* O139.

Clinical management

Medical history was obtained either from the patients or from their attendants by a physician, and was recorded on a standardized form. A thorough physical examination was performed, including recording of the pulse, respiration, body temperature, and blood pressure.

On enrollment, the patients were weighed on an electronic scale with a precision of 10 g, and were then placed on "cholera cots" to facilitate the quantitative measurement of stool and urine. Assessment of dehydration was done according to the WHO guidelines (7). The patients were rapidly rehydrated (usually within 3-4 hours) with polyelectrolyte intravenous solution (Dhaka solution). The composition of the solution was as follows: sodium 133 mmol/L, potassium 13 mmol/L, chloride 98 mmol/L, and bicarbonate 48 mmol/L. After the initial rehydration with intravenous solution, hydration was maintained with rice-based oral rehydration solutions (ORS) (8). The patients were offered to take normal hospital diet, such as bread, rice, vegetables, and chicken curry after the initial hydration. Patients, who could not be maintained adequate hydration with ORS because of high purging rates and/ or frequent vomiting, were managed with the same intravenous polyelectrolyte fluid. All information, including the amount of intake of intravenous fluid and ORS, stool and urine output, and quantity of vomit, were recorded for each 8 hourly period of the study since enrollment. Neither an antimicrobial agent nor any other drugs were used for the management of the patients. The patients were discharged from the hospital if they did not have liquid stool during two consecutive 8-hour periods and if the infecting strain of *V. cholerae* O139 could not be isolated from the stool samples on two consecutive days. However, asymptomatic patients requested for discharge from the hospital and excreting *V. cholerae* O139 in their stools were given with a 3-day course of tetracycline (500 mg 6 hourly).

Laboratory data collection

Upon enrollment in the study, venous blood sample and small volume of stool were collected and sent to the Centre's laboratory under liquid paraffin for the determination of electrolytes. Another sample of stool specimen was also collected on admission for microscopic examination and culture. After the initial rehydration, another sample of blood was obtained for the determination of haematocrit and a complete blood count. Samples were collected from each patient. The haematocrit was reassessed 24 hours after rehydration to determine the hydration status of the patients. Stool cultures were done on admission, and at every 24 hours until the time *V. cholerae* O139 could not be isolated from the samples of two consecutive days. Stool samples were directly inoculated, as well as inoculated after 6 hours of enrichment in alkaline peptone water onto tellurite-taurocholate gelatin agar (TTGA) for isolating *V. cholerae*. *V. cholerae* O139 was identified by the slide agglutination method, using specific monoclonal antibodies against *V. cholerae* O139 developed at the Centre (9), which was confirmed by a sensitive and highly specific coagglutination test for the detection of the organism directly from the stool samples (10). Antimicrobial susceptibilities of the *V. cholerae* O139 isolates were determined by the disk-diffusion method.

Statistical methods

All data were entered into a personal computer using the STATA statistical software (Stata Corporation, Texas, USA; Release 5, 1997), and were checked, including verification with the data-collection sheets. The mean values with standard deviations and the median values with interquartile range were calculated, respectively, for all normally and non-normally-distributed continuous variables.

Definitions

Duration of diarrhoea was defined as the time between enrollment in the study and the end of last 8-hour period in which a liquid stool was passed. Liquid stool was defined as the one which can be poured from one container to another container without any adherence. Soft stool was defined as the one which takes the shape of the container, but cannot be easily poured like the liquid stool. A study day was a complete 24-hour period after enrollment.

RESULTS

Clinical presentation

Twenty-five patients with a positive dark-field test for *V. cholerae* were initially enrolled into the study. *V. cholerae* O139 could not be isolated from stool samples of 2 patients collected at the time of admission, and another patient had left the study after 24 hours, leaving 22 evaluable patients. The presenting features of the 22 patients are summarized in Table 1.

Laboratory features

Table 2 presents the results of laboratory investigations of the patients done on admission.

Clinical course

The main clinical features observed during the hospital stay for the first 120 hours are shown in Table 3. All the patients became alert at the end of their initial rehydration. No change in stool consistency was observed during the first 24 hours, when stool was liquid, similar to that of 'rice water.' The volume of liquid stool was reduced by over a half (55%) during the second 24 hours of the study, and by 96 hours, stools of 77% (17/22) of the patients became soft. Four (18%) patients continued to have liquid stools up to 120 hours, but none had liquid stool beyond 136 hours. Vomiting was present in 64% (14/22) and 36% (8/22) of the patients during the first 24 and 48 hours respectively. Only 9% of the patients persisted vomiting up to 120 hours. Quantity of vomitus and other fluid loss are shown in Table 3. The

Table 1. Clinical characteristics of patients (n=22) with *V. cholerae* O139

Characteristics	
Age (years)	38±10
Body weight (kg)	41±5
Duration of diarrhoea (hour)	8±2.5
Vomiting, n (%)	22 (100)
Duration of vomiting (hour)	9±2
Anorexia, n (%)	5 (23)
Abdominal pain, n (%)	2 (9)
ORS taken at home, n (%)	19 (86)
Temperature £37 °C, n (%)	22 (100)
Severe dehydration*, n (%)	22 (100)

Values are mean±SD, unless otherwise indicated
* According to the WHO guidelines

Table 2. Laboratory characteristics of patients (n=22) with *V. cholerae* O139

Blood	
Pre-hydration haematocrit (%)	56±3
Specific gravity	1.04±0.003
Peripheral blood white cells (after rehydration)	
Total white cells/c mm	18,000±5,000
Neutrophils (%)	81±9
Lymphocytes (%)	16±8
Serum electrolytes (on admission) mmol/L	
Sodium	134±3
Potassium	4±1
Chloride	102±4
Total carbon dioxide (T Co_2)	13±4
Stool	
*Erythrocytes/HPF**	
None, n (%)	8 (36)
1-10, n (%)	11 (50)
11-20, n (%)	3 (14)
*Leukocytes/HPF**	
<10, n (%)	5 (22)
11-20, n (%)	5 (22)
21-50, n (%)	7 (34)
>50, n (%)	5 (22)
*Machrophages/HPF**	
None, n (%)	8 (36)
1-10, n (%)	14 (64)
Electrolytes (mmol/L)	
Sodium	120±24
Potassium	18±6
Chloride	93±16
Total carbon dioxide (T Co_2)	37±9

Values are mean ±SD, unless otherwise indicated.
*HPF=High-power field

Table 3. Hospital course of patients (n=22) with cholera due to *V. cholerae* O139

Outcome	0-24 hours	25-48 hours	49-72 hours	73-96 hours	97-120 hours	Enrollment to cessation of diarrhoea
Liquid stool (L)	9 (5.0-12.0)	4 (3.0-6 .0)	3 (1-6)	0.5 (0-1.5)	0 (0–0.6)	16 (9–24)
No. of patients with vomiting, n (%)	14 (64)	8 (36)	8 (36)	2 (9)	2 (9)	0
Vomitus (L)	0.9 (0-1.5)	0 (0-1.4)	0 (0-3)	0 (0-0)	0 (0-0)	1.5 (1-4)
Urine (L)	2 (1-5)	4 (3-6)	5 (3-6)	5 (2.5-6)	4 (3-6)	10 (6-16)
Intake (L)						
I.V. fluid	7 (6-13)	0 (0–3)	0 (0-0.5)	0	0	9 (6-18)
ORS	6 (5-7)	4 (3-5)	3 (1-5)	1 (0-3)	0 (0-0)	14 (9-20)
Cumulative no. of patients with soft stool (%)	0	2 (9)	8 (36)	16 (73)	20 (91)	

Values are median (interquartile range), unless otherwise indicated

median (IQ) duration of diarrhoea was 80 (range 48-104) hours.

Requirement of rehydration fluids

Requirement of intravenous and oral rehydration fluids at various times during the study are shown in Table 3. Intravenous fluid was required for the initial rehydration of all the study patients (100%). The mean SD of intravenous fluid required for the initial hydration was 6 (1) litre or 15 (3) mL per kg of admission body-weight. Half of the patients could maintain hydration using rice-based ORS after their initial rehydration, and did not require any further intravenous fluid during their hospitalization.

Duration of excretion of *V. cholerae* O139 in stool

The rates of isolation of *V. cholerae* O139 from the stool samples of the patients during the first 10 study days are shown in the figure. The median (IQ) duration of excretion of *V. cholerae* O139 in the stool was 5 (3-6) days. Two patients were persistently excreting *V. cholerae* O139 in stool beyond 10 days, although their diarrhoea ceased by 72 hours; one left hospital on 10th day with a 3-day course of tetracycline, and other one excreted *V. cholerae* O139 in stool for 21 days.

Antimicrobial sensitivity patterns

During the study period, all *V. cholerae* O139 isolates (100%) were susceptible, *in vitro*, to tetracycline, erythromycin, and ciprofloxacin. Ninety-two percent of them were susceptible to furazolidine, while only 5% were susceptible to trimethoprim-sulphamethoxazole.

DISCUSSION

The results of our study showed remarkable similarities between cholera caused by *V. cholerae* O1 (classical or El Tor) and *V. cholerae* O139. First, like cholera due to *V. cholerae* O1, vomiting preceded the onset of diarrhoea in all (100%) of our patients (11). The vomiting mechanism in cholera is not precisely known; however, its development even before the onset of diarrhoea (and loss of bicarbonate in faeces) raised questions about the role of metabolic acidosis as the principal cause (12,13). Second, the proportion of patients with anorexia, abdominal discomfort, and vomiting was similar to that observed in patients with cholera caused by *V. cholerae* O1 (11). Third, as happens in *V. cholerae* O1 infection (11,12), the disease, caused by this new bacterium, resulted in profuse watery diarrhoea, thereby developing severe dehydration and hypovolemic shock within a very short time of the onset of the illness. This is expected, because *V. cholerae* O139 produces a similar amount of cholera toxin as produced by *V. cholerae* O1 (14). Fourth, fever was typically absent in our patients as was also observed in patients with cholera due to *V. cholerae* O1 (11). These findings are consistent with the pathophysiologic mechanism of cholera which is not associated with any local or systemic inflammatory response (11).

Microscopic examination of stools, however, revealed the presence of many erythrocytes, leukocytes, and macrophages than reported for patients infected with *V. cholerae* O1 (11), raising the question if the bacterium also possessed invasive capabilities. *V. cholerae* O139-associated bacteraemia has been reported to occur in an elderly, immunocompromised woman (15); however,

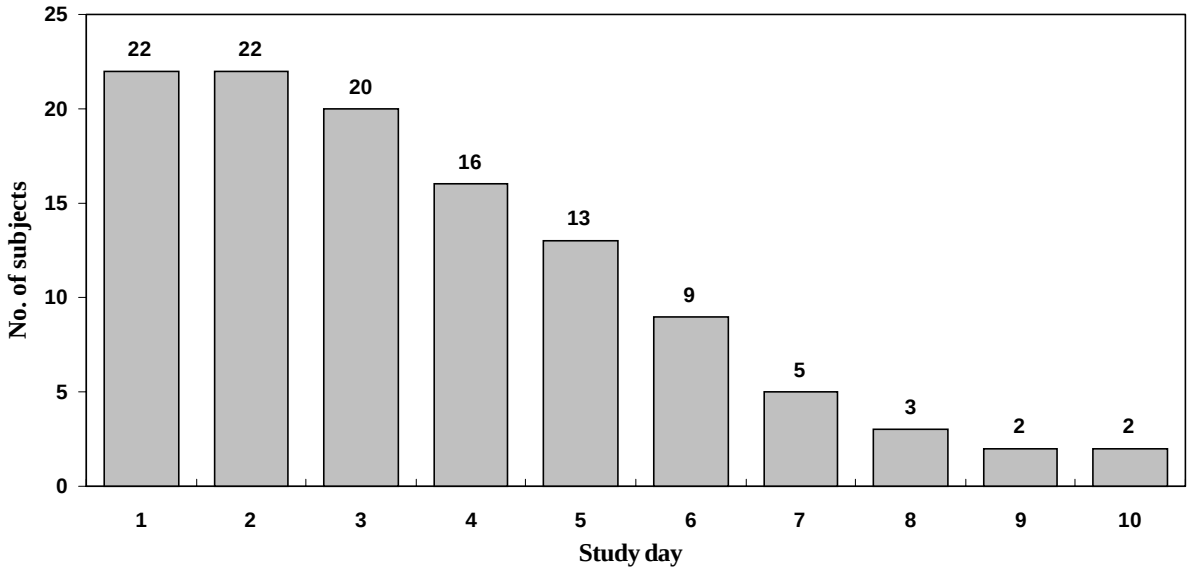

Fig. Number of patients excreting *V. cholerae* O139 in stool by study day

similar cases of *V. cholerae* O1 bacterium have also been reported (16). Unlike *V. cholerae* O1, *V. cholerae* O139 possesses a polysaccharide capsule which is considered to have invasive properties (17), as well as a role in the pathogenesis of the disease. Leukocytosis, as observed in our study patients, may also be a consequence of this property of *V. cholerae* O139. The presence of a large number of pus cells has also been reported to be present in patients with cholera and enterotoxigenic *Escherichia coli*-associated diarrhoea, as well as from apparently healthy individuals in India. Repeated sub-clinical infections by invasive pathogens, superimposed by a new, symptomatic infection, are believed to be the underlying mechanism (18).

Bhattacharya *et al*. reported that 44.3% of their patients with cholera due to this new bacterium had abdominal pain [19]. However, only 9% (2/22) of our study patients had abdominal pain. We do not have a plausible explanation for this difference. Since this is a subjective opinion, there is always a chance of variability.

The serum and faecal electrolyte concentrations of our study patients were similar to that observed in patients with severe cholera due to *V. cholerae* O1 (20), which is conceivable, since the pathophysiologic mechanism is same, and so is the disease severity caused by these two serogroups of *V. cholerae*. In cholera due to *V. cholerae* O1, the volume of stools and vomiting has been observed to decline after 48 hours (11), but in our study, we observed a 55% spontaneous reduction in stool volume, as well as volume of vomitus before 48 hours.

There are several limitations in our study that make general application of the findings difficult. First, we have included only adults in our study. It is possible that the clinical features are different in different age groups. Second, we have studied only those patients who had a more severe disease. Thus, it is beyond the scope of this study to provide information on the whole spectrum of illness as has been described for cholera due to *V. cholerae* O1. Third, only the males were studied in this series. The features and the course of the disease are unlikely to be different in women, although the severity of disease has been reported to be greater in pregnant women with cholera due to *V. cholerae* O1 compared to non-pregnant women (21); the reason for the difference is not clear.

In summary, the results of this study indicate that there are remarkable similarities in the clinical and laboratory features and also in the course of the illness caused by *V. cholerae* O1 and *V. cholerae* O139. The use of same intravenous and oral rehydration fluids has been found to be as useful as in the treatment of cholera caused by *V. cholerae* O1, and none of our patients died or developed any complications. However, treatment with antimicrobials for cholera due to *V. cholerae* O1 has been associated with a significant reduction in the duration of diarrhoea and the volume of watery stools, resulting in reduced requirement of expensive intravenous and oral rehydration fluids (22). Additionally, treatment with appropriate antimicrobials has been useful in shortening the duration of faecal excretion of *V. cholerae* O1 (23), and similar effects of antibiotics in the treatment of cholera due to *V. cholerae* O139 have also been reported (24,25).

Finally, the findings of this study indicate that, in severe diarrhoea due to *V. cholerae* O139, particular attention should be given during the first 24 hours of the disease during which time the patients are most likely to have a significant depletion of fluid and electrolytes, and would need an urgent and efficient replenishment of the losses. Moreover, *Vibrio* excretion can last even up to 21 days without any symptomatic findings, and, therefore, the use of antimicrobial is essential to control the spread of this infection in the community, since we are concerned with patients infected with *V. cholerae* O1.

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