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El Tor cholera with severe disease: a new threat to Asia and beyond

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SUMMARY

During epidemics of cholera in two rural sites (Bakerganj and Mathbaria), a much higher proportion of patients came for treatment with severe dehydration than was seen in previous years. *V. cholerae* O1 isolated from these patients was found to be El Tor in its phenotype, but its cholera toxin (CT) was determined to be that of classical biotype. Whether the observed higher proportion of severe dehydration produced by the El Tor biotype was due to a shift from El Tor to classical CT or due to other factors is not clear. However, if cholera due to strains with increased severity spread to other areas where treatment facilities are limited, there are likely to be many more cholera deaths.

Key words: Cholera, El Tor toxin, epidemic, severe dehydration, *Vibrio cholerae*.

INTRODUCTION

Nearly two centuries have elapsed since the first pandemic of cholera spread from its home in the Indian subcontinent. The current seventh pandemic caused by *V. cholerae* O1 El Tor, has not only affected much of the developing world, but has remained as an epidemic and endemic disease in many countries of Asia and Africa [1–3]. The El Tor biotype spread from Indonesia beginning in 1961 and gradually replaced the previous classical biotype of the sixth pandemic [4]. The El Tor and the classical biotypes belong to the same serogroup, *V. cholerae* O1 [5] and they each may exist as an Inaba or an Ogawa serotype. In 1992, a new serotype, O139 emerged in India and Bangladesh as another serotype capable of causing epidemic cholera, but so far has not spread to other continents [6–8]. Although it continues to be recovered sporadically in South Asia, it has remained as a relatively minor contributor to the overall cholera burden. After a period during the 1970s and 1980s when both classical and El Tor strains co-existed, classical strains were last detected in southern Bangladesh in 1990 [7]. Since then the classical biotype of *V. cholerae* O1

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has not been isolated from patients anywhere in Bangladesh either during epidemic investigations or routine surveillance.

Since the El Tor biotype first appeared, many clinical and epidemiological observations suggested that *V. cholerae* of the El Tor biotype, although better adapted to the environment, may be less virulent clinically. This is suggested by the higher case–infection ratio, e.g. more patients infected with strains of El Tor have milder or asymptomatic infections [9, 10]. Clearly, infections with either biotype can be acute, severe and life-threatening, but in general, fewer patients infected with El Tor strains present with severe dehydration [10].

As part of a project on the epidemiology and ecology of cholera, systematic surveillance was undertaken at rural health centres in Bangladesh. The surveillance system collected information on all diarrhoea cases presenting for treatment during a 3-day period every 15 days [11]. Within the context of this surveillance system, we observed an apparent increase in the severity of cholera cases. Because of this clinical impression of increased severity, a review of the clinical presentations of these patients was conducted. The strains of *V. cholerae* collected during surveillance were also assessed to determine the type of cholera toxin (CT) they produced.

MATERIAL AND METHODS

Clinical surveillance

From 1998 to 2001 clinical surveillance for cholera was carried out at four government subdistrict-level hospitals, and from 2004 to the present, surveillance continued at two such hospitals. In the present study, the clinical data from two hospitals in southern Bangladesh are used: Bakerganj (1998–2001 and 2004–2006) and Mathbaria (2004–2006). Bakerganj is located on the upper edge of the estuarine area of southern coastal region of Bangladesh and Mathbaria is located in the southern-most region of the country having an estuarine ecosystem being close to the mangrove swamps of the sunderbans. Both are 31-bed rural hospitals with outpatient and in-patient treatment facilities providing services for ~200 000–250 000 people. Methods for this surveillance system have previously been described [11]. Briefly, physicians from the ICDDR,B visit each hospital and collect information and rectal specimens from all enrolled patients with watery diarrhoea for a 3-day period every 15 days. Dehydration status of patients was determined objectively using WHO criteria and were categorized as no detectable dehydration, some dehydration or severe dehydration [12]. Patients presenting with severe dehydration have an estimated fluid deficit of 10% and are in immediate danger of death unless they are rehydrated immediately with large volumes of intravenous fluids. The specific objectives of this protocol was confined only to study 'epidemiology and ecology of *V. cholerae*' in rural Bangladesh. For statistical analysis, χ^2 tests for difference were used in this study.

For comparison, this report also includes data collected by the Epidemic Control Preparedness Programme (ECPP) of ICDDR,B from ten rural districts of Bangladesh during epidemic investigations of cholera in the past (1988–1989). Trained physicians of ICDDR,B collected clinical and demographic data in a pre-recorded questionnaire while conducting the epidemic investigations. Details of this method have been described previously [13].

Laboratory methods

Isolation and Identification of V. cholerae

The study physician collected rectal swabs during the clinical surveillance at the Bakerganj and Mathbaria Thana Health Complexes (THCs). The swabs were placed in Cary–Blair medium and were transported to the microbiology laboratory of ICDDR,B in Dhaka where they were cultured using standard bacteriological methods [14]. Rectal swabs were cultured both directly and after a 6-h enrichment in alkaline peptone water on selective media (taurocholate-tellurite-gelatin agar (TTGA) at 37 °C. Suspected colonies resembling *V. cholerae* were tested for agglutination with antisera specific for *V. cholerae* O1 and for *V. cholerae* O139.

Determination of biotype specific B-subunit of CT

The detection of the type of CT was performed using previously described ganglioside GM_1 enzyme-linked immunosorbent assay (ELISA) [15]. *V. cholerae* O1 was cultured in AKI medium at 37 °C overnight [16]. Bacterial supernatants (150 μl) were added to separate wells of GM_1-coated microtitre plates and diluted threefold. Plates were pre-incubated for 1 h at room temperature and analysed for GM_1 bound to CT by using mouse monoclonal antibodies (mAb) specific for the two types of CT produced by the biotypes

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Table 1. *Distribution of acute watery diarrhoea and cholera patients in Bakerganj and Mathbaria, 2004–2006*

	Bakerganj	Mathbaria	
	No. (%)	No. (%)	Total
Total patients	576	649	1225
Patients tested for *V. cholerae*	468	491	959
Patients with *V. cholerae* O1 El Tor	110 (23·5)	89 (18·1)	199 (20·8)

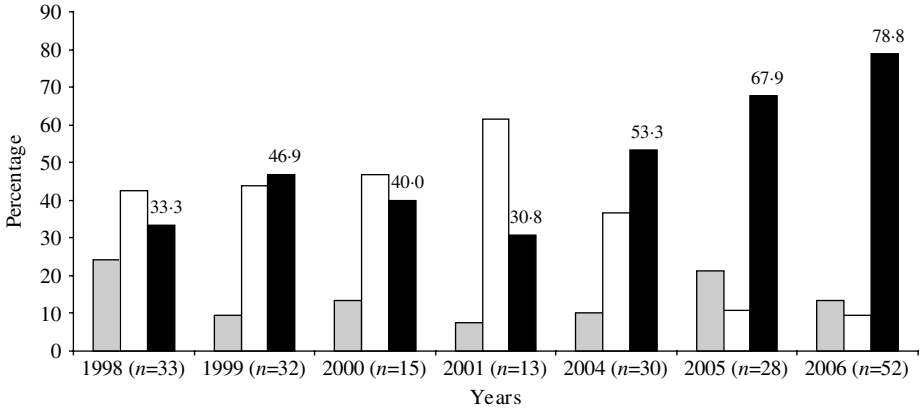

Fig. 1. Dehydration status of *V. cholerae* O1 biotype El Tor-infected patients in Bakerganj, 1998–2001 and 2004–2006. ▨, No dehydration; □, some dehydration; ■, severe dehydration [2006 *vs.* 1998–2001 ($P < 0{\cdot}01$), 2006 *vs.* 2004 ($P < 0{\cdot}05$)].

El Tor (ETC 31:20; mAb raised against CT produced by El Tor strain N16961) or classical (CT21:15; mAb raised against CT produced by classical strain 569B) as well as with the mAb that reacts to the CT subtypes of both biotypes (LT 39:13:1) [17]. After addition of goat anti-mouse immunoglobulin conjugated to horseradish peroxidase (Jackson, USA), the enzyme substrates ortho-phenylene diamine and hydrogen peroxide were added and the optical density (OD) was measured at 450 nm. A computer-based program MULTI (DataTree Inc., USA) was used for calculation of the titres and an OD of 0·4 above background was considered as positive.

The Research Review Committee and Ethical Review Committee of ICDDR,B approved the research protocol.

RESULTS

Clinical observations

The total number of patients with acute watery diarrhoea who were observed during the surveillance periods each 15 days, and the number with *V. cholerae* O1 isolated from their rectal swabs in Bakerganj and Mathbaria sites are shown in Table 1. The proportion of diarrhoea patients with *V. cholerae* O1 from Bakerganj and Mathbaria was 23·5% and 18·1%, respectively.

In 2006, an epidemic outbreak of cholera was observed in our two surveillance sites: Bakerganj and Mathbaria. The striking feature of this outbreak was the large proportion of cholera cases with severe dehydration; 78·8% in Bakerganj and 70·2% in Mathbaria. To assess the significance of this observation we analysed the surveillance data collected during the earlier and the ongoing surveillance period.

In Bakerganj, the proportion of cholera patients with severe dehydration in 2006 (78·8%) was significantly higher ($P < 0{\cdot}01$) than any given year during the earlier phase (33·3% in 1998, 46·9% in 1999, 40% in 2000 and 30·8% in 2001). There was also significant increase in the proportion with severe dehydration between the years 2004 and 2006 ($P < 0{\cdot}05$); however, the difference was not significant between 2005 and 2006 (Fig. 1). In Mathbaria, a significantly higher proportion ($P < 0{\cdot}01$) of cholera patients had severe dehydration in 2006 compared to patients in 2004 and 2005 (Table 2).

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Table 2. *Distribution of severe dehydration of El Tor-positive cholera patients in Bakerganj and Mathbaria, 2004–2006*

	V. cholerae O1 El Tor	
Period	Bakerganj No. (%)	Mathbaria No. (%)
2004	16 (53·3)[a] ($n=30$)	9 (34·6)[c] ($n=26$)
2005	19 (67·9) ($n=28$)	4 (25·0)[c] ($n=16$)
2006	41 (78·8)[b] ($n=52$)	33 (70·2)[d] ($n=47$)

[b] *vs.* [a]: $P<0·05$ (95% CI 1·03–2·03).
[d] *vs.* [c]: $P<0·01$ (95% CI 1·16–3·35).

To examine if this increase in the proportion of patients with severe dehydration was unusual, we compared our findings with the data collected from ten rural districts during the cholera epidemic investigations in 1988–1989 (Table 3). We observed that the proportion with severe dehydration in the El Tor-positive cholera patients was much higher in 2006 ($P<0·001$) and 2005 ($P<0·05$) than in the past (1988–1989). No significant difference was found for the period 2004.

Laboratory observations

The strains of *V. cholerae* O1 biotype El Tor isolated from cholera patients in Bakerganj ($n=153$) and Mathbaria ($n=89$) were examined. The biotype-specific CT ELISA revealed that all the strains of *V. cholerae* O1 El Tor from Bakerganj and Mathbaria produced CT of classical biotype during the entire surveillance period (Table 4).

DISCUSSION

The strikingly large proportion of severe dehydration observed in cholera patients in Bakerganj and Mathbaria caused by the El Tor biotype of *V. cholerae* O1 in 2006 has, to our knowledge, never been previously reported. The proportion with severe dehydration was much higher during the more recent period than during earlier years when about 35% presented with severe dehydration. Patients presenting with severe dehydration are at high risk of death unless they are treated immediately with intravenous fluids to restore circulating blood volume. The patients in our surveillance did receive immediate rehydration.

During the last four decades, the strains of *V. cholerae* causing cholera in Bangladesh have undergone several changes. Major changes include the transition from classical strains to the El Tor biotype. In 1992, the new serotype, *V. cholerae* O139 appeared and predominated for a time before it became less commonly isolated. In the mid-1990s, mutant strains of *V. cholerae*, having characteristics of both El Tor and classical types were isolated and were termed Matlab variants [18]. These appeared to be unusual strains at the time, but strains like these are now commonplace in Bangladesh. The strains recently isolated in Mozambique appear to be typical El Tor strains, but the genome has a tandem repeat of the classical prophage located in the small chromosome [19]. The altered El Tor strains which are typical El Tor, have an El Tor CTX prophage but produce CT of the classical biotype [15]. Since 2001, in Dhaka, all strains examined of *V. cholerae* O1 belonged to the altered El Tor type indicating that a cryptic change occurred in the seventh pandemic El Tor biotype strains of *V. cholerae* O1 [15]. Although the change is subtle, it appears that the *V. cholerae* genome is undergoing changes and thus the new strain is likely to become epidemiologically dominant.

It is known that CT, the principal toxin produced by *V. cholerae* O1 is responsible for the clinical manifestations of cholera. Our laboratory investigations revealed that the El Tor biotype strains of *V. cholerae* O1 isolated from Bakerganj between 1998 and 2001 and between 2004 and 2006 and from Mathbaria in 2006 produced CT of the classical biotype. Therefore, the El Tor strains from Bakerganj and Mathbaria are different from those of the prototype seventh pandemic El Tor biotype strains that produce CT of the El Tor biotype. In contrast to these sites in southern Bangladesh, a retrospective study using the stored *V. cholerae* isolates from Dhaka revealed that all strains of the El Tor biotype isolated from 2001 onwards produced CT of the classical biotype while isolates before 2001 produced CT of the El Tor biotype [20].

Our study suggests that the proportion of severe dehydration in the cholera patients seen in southern Bangladesh in recent years is more than in previous years. The bacterium causing such striking severity was identified as altered El Tor. However, at this stage it is not clear that the change in severity of the disease

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Table 3. *Comparison of severity of dehydration in El Tor-infected cholera patients, between the past (1988–1989) and present (2004, 2005 and 2006) in Bangladesh*

Dehydration status	Ten rural districts (1988–1989)* No. (%)	Bakerganj and Mathbaria (combined) 2004 No. (%)	2005 No. (%)	2006 No. (%)
None	44 (6·1)	4 (7·1)	7 (15·9)	10 (10·1)
Some	420 (58·1)	27 (48·2)	14 (31·8)	15 (15·2)
Severe	259 (35·8)[a]	25 (44·6)[b]	23 (52·3)[c]	74 (74·7)[d]
Total	723 (100·0)	56 (100·0)	44 (100·0)	99 (100·0)

* Part of unpublished data: Siddique *et al.* [13].
[a] *vs.* [b]: $P > 0{\cdot}05$.
[a] *vs.* [c]: $P < 0{\cdot}05$ (95% CI 0·51–0·92).
[a] *vs.* [d]: $P < 0{\cdot}001$ (95% CI 0·41–0·56).

Table 4. *Type of cholera toxin produced by* V. cholerae *O1 biotype isolated from cholera patients at Bakerganj and Mathbaria, Bangladesh: 1998–2001 and 2004–2006*

Biotype isolates by area	Year	No. tested	CT-ELISA El Tor CT	CT-ELISA Classical CT
Bakerganj				
O1 El Tor	1998	13	0	13
O1 El Tor	1999	6	0	6
O1 El Tor	2000	12	0	12
O1 El Tor	2001	12	0	12
O1 El Tor	2004	30	0	30
O1 El Tor	2005	28	0	28
O1 El Tor	2006	52	0	52
Mathbaria				
O1 El Tor	2004	26	0	26
O1 El Tor	2005	16	0	16
O1 El Tor	2006	47	0	47
Classical reference	—	1	0	1
El Tor reference	—	1	1	0

CT-ELISA, Cholera toxin enzyme-linked immunosorbent assay.

is only due to change in the CT. Nor do we have an explanation why the clinical manifestations were not significantly evident until 2006, although the change in CT was detected as early as 1998 in Bakerganj. Further in-depth investigations are needed to explain the possible reasons for change in the severity of the disease.

Interestingly, our study suggests that the new strains which were detected in 1998 in southern Bangladesh did not appear in Dhaka until 2001. Unfortunately, we do not have strains in the southern part of the country prior to 1998 and are therefore not able to determine when they emerged. The implications of cholera produced by the new strain could be profound for many poor countries in Asia and Africa where cholera exists in endemic or epidemic form. In many of these areas, access to good quality health facilities is limited [21]. Inadequate and/or inappropriate treatment [22] greatly increases the risk of death from cholera. The cholera outbreak in 2006 in Angola, one of the poorest countries in Africa, affected over 75% of the provinces and the case-fatality rate in some provinces reached 30% [23]. If these new strains of El Tor were to spread to other countries where treatment

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facilities are limited, their introduction could increase the mortality from cholera even further.

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DECLARATION OF INTEREST

None.

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