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Vibrio cholerae O1

El Tor and O139

Bengal Strains Carrying ctxBET, Bangladesh

To the Editor: Cholera, caused by Vibrio cholerae, continues to affect millions of persons in disease-endemic areas where safe drinking water is scarce and sanitation is poor. Of 7 cholera pandemics recorded since 1817, V. cholerae serogroup O1 classical (CL) biotype was associated with the sixth, whereas the seventh (ongoing) pandemic was initiated by V. cholerae O1 biotype El Tor (ET), which displaced CL in the early 1960s (1). During 1992–1993, a V. cholerae non-O1 serogroup, designated V. cholerae O139 synonym Bengal, initiated cholera epidemics in India and Bangladesh by transiently displacing V. cholerae O1 ET biotype (2). V. cholerae O139 was less frequently associated with cholera in Bangladesh than V. cholerae ET in 1994 and the years following, until 2005 (3); it has been undetected since then. Meanwhile, V. cholerae ET has shown genetic changes since 2001, and isolates carry the ctxB gene of the CL biotype (ctxBET) in Bangladesh (4). Although the genetic transition from ctxBET to ctxBET was observed during 1998–1999 for V. cholerae O139 biotype, V. cholerae strains carrying ctxBET were considered extinct, i.e., undetected for about a decade.

During June 2010–December 2012, the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B) systematically conducted ongoing epidemiologic ecological surveillance in Dhaka, Chhattak, and Mathbaria and isolated V. cholerae strains (n = 500 [clinical/environmental]; Dhaka [n = 110/94], Mathbaria [n = 90/79], Chhattak [n = 111/16]). Of the 500 V. cholerae isolates, 496 were confirmed as O1 and 4 as O139 Bengal, on the basis of serologic, phenotypic, and genetic properties (3,6–8).
All *V. cholerae* O1 and O139 isolates were positive for *ctxA*, *tlc*, *ace*, and *zot* and possessed ET biotype–specific markers tcpA^ET^, hlyA^ET^, and *rtxC*. Mis-match amplification mutation assay—PCR (9) demonstrated *ctxB^CL^* allele in 492 *V. cholerae* O1 ET strains (altered ET), whereas *ctxB^ET^* was found in 8 isolates (4 *V. cholerae* O1 ET and 4 *V. cholerae* O139).

Nucleotide sequencing of *ctxB* showed that the translated sequences of *V. cholerae* O1 and O139 strains carrying *ctxB^ET^* were identical to those of the ET reference strain N16961 (GenBank accession no. NC_002505), with tyrosine and isoleucine at positions 39 and 68, respectively, as opposed to altered ET, which possesses histidine and threonine at positions 39 and 68, respectively (4). PCR additionally showed that the *V. cholerae* O1 and O139 Bengal strains carrying *ctxB^ET^* had the ET biotype–specific RS1 element gene *rstC* and repressor gene *rstR^ET^*, suggesting prototype ET attributes (7).

Three *V. cholerae* strains carrying *ctxB^ET^* were first isolated in 2011 from surface water: one O1 strain and one O139 strain from Mathbaria and one O1 strain from Chhatak. In 2012, *V. cholerae* O1 carrying *ctxB^ET^* was isolated from cholera patients in Mathbaria and Chhatak (*n* = 1 each). Also, 3 O139 strains carrying *ctxB^ET^* were isolated from surface water in Dhaka. The confirmed *V. cholerae* O1 and O139 Bengal strains carrying *ctxB^ET^* were of particular interest because altered ET strains carrying *ctxB^ET^* have been deemed the cause of endemic cholera in Bangladesh since 2001 (4) and globally (10).

*V. cholerae* strains carrying *ctxB^ET^* were closely related to the pre-2001 *V. cholerae* strains carrying *ctxB^ET^*, as were the O139 Bengal strains carrying *ctxB^ET^*. Two lines of evidence support this close relationship. First, the antimicrobial drug resistance patterns of 3 of the *V. cholerae* O139 strains isolated in Dhaka during 2012 were resistant to trimethoprim/sulfamethoxazole (25 µg), whereas the remaining O139 and 4 O1 strains were susceptible to all drugs tested, including azithromycin (15 µg), ciprofloxacin (5 µg), gentamicin (10 µg), ampicillin (10 µg), tetracycline (30 µg), and erythromycin (15 µg). Second, pulsed-field gel electrophoresis (PFGE) of *NodI*-digested genomic DNA showed identical banding patterns for the 4 *V. cholerae* O1 strains carrying *ctxB^ET^* and the pre-2001 ET strains, including N16961; and the DNA pattern differed from that of the altered ET associated with endemic cholera in Bangladesh (Figure). All 4 *V. cholerae* O139 strains had typical O139 Bengal banding patterns, shown by PFGE, except that 1 strain had an extra band (Figure). Comparison of PFGE patterns with those of previously isolated *V. cholerae* O139 strains (1993–2005) showed that recently isolated strains (2011–2012) belonged to 1 of the ancient clones, suggesting that the strain has been present in Bangladesh since 1993 (Figure).

In conclusion, we provide evidence of the coexistence of *V. cholerae* O1 and O139 strains, which shows that strains carrying *ctxB^ET^*, not isolated for approximately a decade in Bangladesh, have again been isolated (3). Although the epidemiologic importance of the observed genetic change in the *ctxB* is yet to be understood, the finding of *V. cholerae* strains carrying *ctxB^ET^* in surface water of Bangladesh in 2011 and in association the following year with "Figure. DNA fingerprinting patterns of *Vibrio cholerae*. Dendrogram was prepared by Dice similarity coefficient and UPGMA (unweighted pair-group method with arithmetic mean) clustering methods by using pulsed-field gel electrophoresis (PFGE) images of the *NodI*-digested genomic DNA. The scale bar at the top (left) indicates the correlation coefficient (range 90%–100%). *V. cholerae* altered ET (*ctxB^ET^*) strains (pulsotype A) formed a major cluster (cluster I), separated from prototype ET (*ctxB^ET^*) strains (cluster II; pulsotype B) and *V. cholerae* O139 strains (cluster III; pulsotype C), suggesting that they are genetically different. ET, El Tor; Clin, Clinical; Env, environmental."
cholera may be yet another turning point, considering that the global pattern of cholera is changing rapidly.

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