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Vibrio cholerae O1 El Tor and O139 Bengal Strains Carrying *ctxB*^{ET}, 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 (*ctxB*^{CL}) in Bangladesh (*4*). Although the genetic transition from *ctxB*^{ET} to *ctxB*^{CL} was observed during 1998–1999 for *V. cholerae* O139 (*5*), *V. cholerae* strains carrying *ctxB*^{ET} 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 ecologic surveillance in Dhaka, Chhatak, and Mathbaria and isolated *V. cholerae* strains (n = 500 [clinical/environmental]: Dhaka [n = 110/94], Mathbaria [n = 90/79], Chhatak [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*. Mismatch 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*^{CL} 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 *Not*I-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

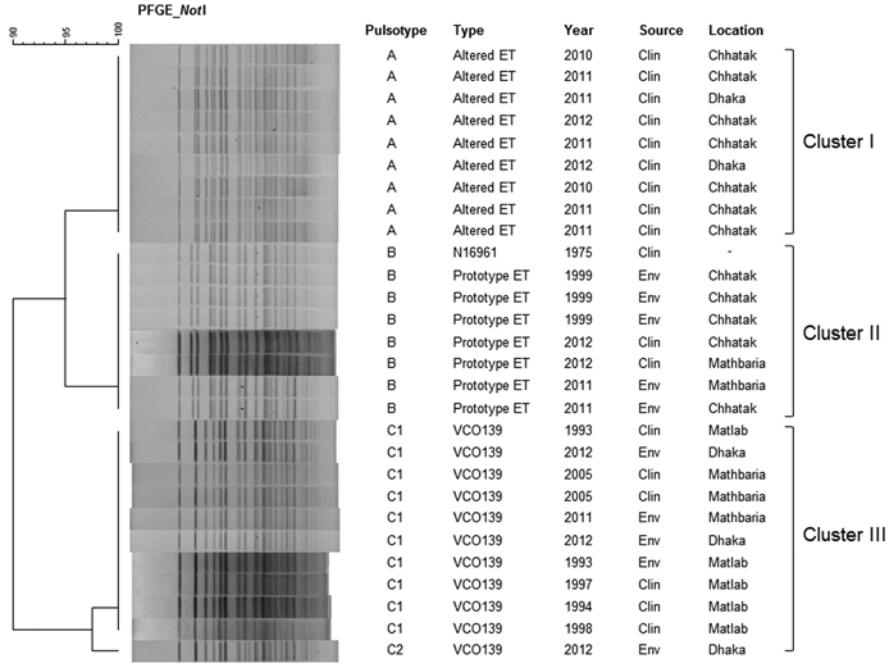

Figure. DNA fingerprinting patterns of *Vibrio cholerae*. Dendrogram was prepared by Dice similarity coefficient and UPGMA (unweighted pair-group method with arithmetic mean) clustering methods by using pulsed-field gel electrophoresis (PFGE) images of the *Not*I-digested genomic DNA. The scale bar at the top (left) indicates the correlation coefficient (range 90%–100%). *V. cholerae* altered ET (*ctxB*^{CL}) 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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