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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 ctbB 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 (5), 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, Chhatak, and Matlabaria and isolated V. cholerae strains (n = 500 [clinical/environmental]; Dhaka [n = 110/94], Matlabaria [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<sup>ET</sup>, hlyA<sup>ET</sup>, and rtxC. Mismatch amplification mutation assay—PCR (9) demonstrated ctxB<sup>CL</sup> allele in 492 V. cholerae O1 ET strains (altered ET), whereas ctxB<sup>ET</sup> 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<sup>ET</sup> 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<sup>ET</sup> had the ET biotype–specific RS1 element gene rstC and repressor gene rstR<sup>ET</sup>, suggesting prototype ET attributes (7).

Three V. cholerae strains carrying ctxB<sup>ET</sup> 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<sup>ET</sup> was isolated from cholera patients in Mathbaria and Chhatak (n = 1 each). Also, 3 O139 strains carrying ctxB<sup>ET</sup> were isolated from surface water in Dhaka. The confirmed V. cholerae O1 and O139 Bengal strains carrying ctxB<sup>ET</sup> were of particular interest because altered ET strains carrying ctxB<sup>CL</sup> have been deemed the cause of endemic cholera in Bangladesh since 2001 (4) and globally (10).

V. cholerae strains carrying ctxB<sup>ET</sup> were closely related to the pre-2001 V. cholerae strains carrying ctxB<sup>ET</sup>, as were the O139 Bengal strains carrying ctxB<sup>ET</sup>. 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 NorI-digested genomic DNA showed identical banding patterns for the 4 V. cholerae O1 strains carrying ctxB<sup>ET</sup> 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<sup>ET</sup>, 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<sup>ET</sup> in surface water of Bangladesh in 2011 and in association the following year with...
cholera may be yet another turning point, considering that the global pattern of cholera is changing rapidly.

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