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Cholera Due to Altered El Tor Strains of *Vibrio cholerae* O1 in Bangladesh▽

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We determined the types of cholera toxin (CT) produced by a collection of 185 *Vibrio cholerae* O1 strains isolated in Bangladesh over the past 45 years. All of the El Tor strains of *V. cholerae* O1 isolated since 2001 produced CT of the classical biotype, while those isolated before 2001 produced CT of the El Tor biotype.

Vibrio cholerae O1 has two biotypes, namely, classical and El Tor, which are believed to have evolved from separate lineages (7, 8), and these biotypes have traditionally been differentiated by a number of phenotypic traits. Comparative genomic analyses have recently revealed a high degree of conservation among diverse strains of *V. cholerae* but have also shown genes that differentiate the classical biotype from the El Tor biotype (3). Apart from these phenotypic and genetic differences, there are also dissimilarities in the infection patterns of disease caused by the two biotypes. These include the occurrence of more asymptomatic than symptomatic carriers of El Tor strains, who outnumber active patients by a ratio of up to 50:1 (14), better survival of El Tor strains in the environment and in the human host, and more efficient host-to-host transmission of El Tor strains than of classical strains (5). There is firm evidence that the fifth and sixth pandemics of cholera were caused by the classical biotype, while the ongoing seventh pandemic is caused by the El Tor biotype, which has now globally replaced the classical biotype.

Cholera toxin (CT), the principal toxin produced by *V. cholerae* O1 and O139, is responsible for most of the manifestations of the disease cholera. Based on the B subunit of CT, two immunologically related but not identical epitypes have been described: CT1 is the prototype elaborated by classical biotype strains and by U.S. Gulf Coast strains, while CT2 is produced by the El Tor biotype and O139 strains (4). Another classification identifies three types of *ctxB* genes based on three nonrandom base changes resulting in changes in the deduced amino acid sequence. Genotype 1 is found in strains of the classical biotype worldwide and in U.S. Gulf Coast strains, genotype 2 is found in El Tor biotype strains from Australia, and genotype 3 is found in El Tor biotype strains from the seventh pandemic and the Latin American epidemic (12). Thus, the *V. cholerae* O1 El Tor biotype of the ongoing seventh pandemic produces CT of the CT2 epitype and genotype 3, while the classical biotype CT belongs to the CT1 epitype and genotype 1. In this study, we examined a collection of clinical *V. cholerae* O1 strains isolated in Bangladesh during the past four and a half decades, using monoclonal antibodies (MAbs) produced to classical and El Tor CTs, and found that *V. cholerae* O1 El Tor strains isolated since 2001 in Bangladesh produce the CT subtype of the classical biotype.

One hundred eighty-five strains of *V. cholerae* O1, consisting of 31 strains of the classical biotype isolated between 1960 and 1990 and 113 strains of the El Tor biotype and 41 hybrid strains of *V. cholerae* O1 (strains that could not be biotyped as El Tor or classical by conventional phenotypic tests) isolated between 1960 and 2005, were included in this study. These strains were selected from different months within a year and from different years from the ICDDR,B culture collection. All strains were isolated from cases of acute watery diarrhea in patients admitted to the cholera hospital in Dhaka, Bangladesh. The identities of the strains were reconfirmed by the slide agglutination test using specific antisera (13). For biotype analysis, we used

TABLE 1. Cholera toxin subtypes produced by different biotypes of *Vibrio cholerae* O1 isolated from 1960 to 2005, based on an ELISA using monoclonal antibodies specific to the El Tor and classical subtypes of CT

Biotype and isolation period (yr)	No. of strains with CT subtype			
	Classical	El Tor	Both classical and El Tor	No toxin
Classical				
1960–1990	31	0	0	0
El Tor				
1960–2000	6	51	0	7
2001–2005	49	0	0	0
Hybrid[a]				
1960–2000	13	15	2	7
2001–2005	4	0	0	0
Total	103	66	2	14

[a] The strains were biotyped based on conventional phenotypic traits, and those strains that could not be biotyped as El Tor or classical were labeled hybrid strains.

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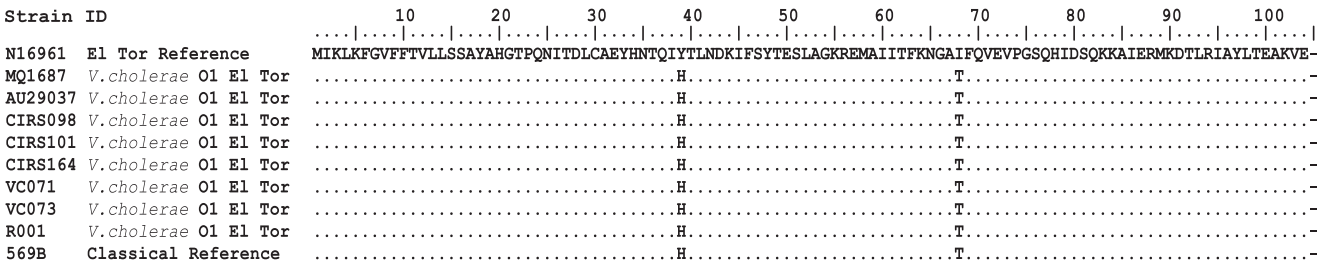

FIG. 1. Amino acid sequence alignment of CT B subunits of representative El Tor *V. cholerae* isolates from Dhaka, Bangladesh, isolated between 2001 and 2005, that produced the classical subtype of CT, plotted relative to the El Tor reference (identical residues are indicated with dots). The amino acid sequences of the B subunits of *V. cholerae* 569B (classical) and N16961 (El Tor) were obtained from GenBank.

chicken erythrocyte agglutination, sensitivity to polymyxin B, and Mukerjee classical phage IV and Mukerjee El Tor phage 5 tests (11).

The detection of the CT subtype was performed by ganglioside GM1-specific enzyme-linked immunosorbent assays (ELISAs) (15), using mouse MAbs specific for the CT subtype produced by the El Tor (ETC 31:20; MAb raised against the CT produced by El Tor strain N16961) or the classical (CT 21:15; MAb raised against the CT produced by classical strain 569B) biotype as well as a MAb that reacts with the CT subtypes of both biotypes (LT 39:13:1) (16). *V. cholerae* O1 was cultured in AKI medium at 37°C overnight (6). Optical densities of 0.4 or more above the background in the ELISA were considered positive. Nucleotide sequencing of the *ctxB* genes of eight strains of *V. cholerae* O1 El Tor isolated between 2001 and 2005 that produced the classical subtype of CT and multiple sequence alignment was performed as previously described (10). The nucleotide sequences of the reference strains were compared with the corresponding sequences of El Tor strain N16961 (GenBank accession no. NC-002505) and classical strain 569B (GenBank accession no. U25679), which were retrieved from GenBank by BLAST searches.

The CT subtypes of a total of 185 strains of *V. cholerae* O1 isolated over a period of 45 years were examined by the CT subtype-specific ELISA. As shown in Table 1, all 31 classical biotype strains produced CT of the classical subtype. All of the El Tor and hybrid strains of *V. cholerae* O1 isolated between 2001 and 2005 that were included in the study produced CT of the classical subtype. This is in contrast to the El Tor strains isolated from 1971 to 2000, which produced predominantly CT of the El Tor subtype.

Nucleotide sequence analysis of the *ctxB* genes of eight representative El Tor strains of *V. cholerae* O1 isolated from 2001 to 2005 that produced CT of the classical subtype revealed that the strains possess DNA sequences identical to that of the classical type of *ctxB*. The deduced amino acid sequences of all eight representative El Tor O1 strains were aligned with the CtxB sequences of the reference strains N16961 (El Tor) and 569B (classical). The deduced amino acid sequences of all eight representative strains were found to be identical to the deduced amino acid sequence of the CT of the 569B classical reference strain, with a histidine at position 39 and a threonine at position 68 (Fig. 1), thereby confirming the results of the CT subtype-specific ELISA.

The epitype and genotype of the CT of the El Tor strains currently associated with cholera in Bangladesh have shifted from epitype CT2 and genotype 3 to epitype CT1 and genotype 1. Thus, in effect, the present El Tor biotype strains produce CT of the classical biotype. The production of classical CT by the El Tor biotype per se is not novel and has been reported infrequently (1, 11, 17). In fact, U.S. Gulf Coast clones of *V. cholerae* O1 are El Tor strains that possess the classical CT (12). What is novel in the present study is that El Tor strains producing classical CT have completely replaced the prototype seventh pandemic El Tor strains producing the El Tor CT in Bangladesh. Given that there are differences between the classical and El Tor biotypes, the selection of this altered type of strain seems to indicate an evolutionary optimization of the El Tor biotype and could represent a new, more efficient emerging form of the El Tor biotype of *V. cholerae* O1.

These altered El Tor biotype strains cannot be differentiated from other El Tor strains by currently used bacteriological methods, and therefore a revision in methods is needed to track the spread of such strains. The implications of the heterogeneity in the B subunit of the CTs of the altered El Tor strains also need to be assessed from a vaccine and diagnostic perspective. It has been shown that various polyclonal antisera raised against CT1 antigens neutralize CT2 considerably less effectively than they neutralize the homologous toxin (9). Vaccines partly based on the B subunit from classical strains have been shown to protect less efficiently against El Tor strains than against classical strains in field trials (2). How the altered El Tor biotype strains will influence the epidemiology of cholera remains to be seen.

Nucleotide sequence accession numbers. The nucleotide sequences obtained for the *ctxB* genes of strains MQ1687, AU29037, CIRS098, CIRS101, CIRS164, VC071, VC073, and R001 have been deposited in GenBank under accession numbers DQ523204 and DQ523217 to DQ523223.

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