

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 epitopes 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 epitope and genotype 3, while the classical biotype CT belongs to the CT1 epitope 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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REFERENCES

1. Ansaruzzaman, M., N. A. Bhuiyan, G. B. Nair, D. A. Sack, M. Lucas, J. L. Deen, J. Ampuero, C. L. Chaignat, and the Mozambique Cholera Vaccine Demonstration Project Coordination Group. 2004. Cholera in Mozambique, variant of *Vibrio cholerae*. *Emerg. Infect. Dis.* **10**:2057–2059.
2. Clemens, J. D., D. A. Sack, J. R. Harris, F. van Loon, J. Chakraborty, F. Ahmed, M. R. Rao, M. R. Khan, M. Yunus, N. Huda, B. F. Stanton, B. A. Kay, R. Eeckels, J. D. Clemens, M. R. Rao, B. A. Kay, D. A. Sack, J. R. Harris, B. F. Stanton, S. Walter, R. Eeckels, A.-M. Svennerholm, and J. Holmgren. 1990. Field trial of oral cholera vaccines in Bangladesh: results from a three-year follow-up. *Lancet* **335**:270–273.
3. Dziejman, M., E. Balon, D. Boyd, C. M. Fraser, J. F. Heidelberg, and J. J. Mekalanos. 2002. Comparative genomic analysis of *Vibrio cholerae*: genes that correlate with cholera endemic and pandemic disease. *Proc. Natl. Acad. Sci. USA* **99**:1556–1561.
4. Finkelstein, R. A., F. Burks, A. Zupan, W. S. Dallas, C. O. Jacob, and D. S. Ludwig. 1987. Epitopes of the cholera family of enterotoxins. *Rev. Infect. Dis.* **9**:544–561.
5. Finkelstein, R. A. 25 February 2006, posting date. Cholera, *Vibrio cholerae* O1 and O139, and other pathogenic vibrios. [Online.] <http://gsbs.utmb.edu/microbook/ch024.htm>.
6. Iwanaga, M., K. Yamamoto, N. Higa, Y. Ichinose, N. Nakasone, and M. Tanabe. 1986. Culture conditions for stimulating cholera toxin production by *Vibrio cholerae* O1 El Tor. *Microbiol. Immunol.* **30**:1075–1083.
7. Kaper, J. B., H. B. Bradford, N. C. Roberts, and S. Falkow. 1982. Molecular epidemiology of *Vibrio cholerae* in the U.S. Gulf Coast. *J. Clin. Microbiol.* **16**:129–134.
8. Karaolis, D. K., R. Lan, and P. R. Reeves. 1995. The sixth and seventh cholera pandemics are due to independent clones separately derived from environmental, nontoxigenic, non-O1 *Vibrio cholerae*. *J. Bacteriol.* **177**:3191–3198.
9. Marchlewicz, B. A., and R. A. Finkelstein. 1983. Immunological differences among the cholera/coli family of enterotoxins. *Diagn. Microbiol. Infect. Dis.* **1**:129–138.
10. Mitra, R. K., R. K. Nandy, T. Ramamurthy, S. K. Bhattacharya, S. Yamasaki, T. Shimada, S. Toshio, Y. Takeda, and G. B. Nair. 2001. Molecular characterization of rough variants of *Vibrio cholerae* isolated from hospitalised patients with diarrhoea. *J. Med. Microbiol.* **50**:268–276.
11. Nair, G. B., S. M. Faruque, N. A. Bhuiyan, M. Kamruzzaman, A. K. Siddique, and D. A. Sack. 2002. New variants of *Vibrio cholerae* O1 biotype El Tor with attributes of the classical biotype from hospitalized patients with acute diarrhea in Bangladesh. *J. Clin. Microbiol.* **40**:3296–3299.
12. Olsvik, O., J. Wahlberg, B. Petterson, M. Uhlen, T. Popovic, I. K. Wachsmuth, and P. I. Fields. 1993. Use of automated sequencing of polymerase chain reaction-generated amplicons to identify three types of cholera toxin subunit B in *Vibrio cholerae* O1 strains. *J. Clin. Microbiol.* **31**:22–25.
13. Qadri, F., R. Raqib, F. Ahmed, T. Rahman, C. Wenneras, S. K. Das, N. H. Alam, M. M. Mathan, and A.-M. Svennerholm. 2002. Increased levels of inflammatory mediators in children and adults infected with *Vibrio cholerae* O1 and O139. *Clin. Diagn. Lab. Immunol.* **9**:221–229.
14. Sack, D. A., R. B. Sack, G. B. Nair, and A. K. Siddique. 2005. Cholera. *Lancet* **63**:223–233.
15. Svennerholm, A.-M., and J. Holmgren. 1978. Identification of Escherichia coli heat-labile enterotoxin by means of a ganglioside immunosorbent assay (GM1-ELISA) procedure. *Curr. Microbiol.* **1**:19–23.
16. Svennerholm, A.-M., M. Wikstrom, M. Lindblad, and J. Holmgren. 1986. Monoclonal antibodies to Escherichia coli heat-labile enterotoxins: neutralising activity and differentiation of human and porcine LTs and cholera toxin. *Med. Biol.* **64**:23–30.
17. Tamplin, M. L., R. Jalali, M. K. Ahmed, and R. R. Colwell. 1990. Variation in epitopes of the B subunit of *Vibrio cholerae* non-O1 and *Vibrio mimicus* cholera toxins. *Can. J. Microbiol.* **36**:409–413.