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Non-O1 *Vibrio cholerae* in Thailand: Homology with Cloned Cholera Toxin Genes

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We examined 281 non-O1 *Vibrio cholerae* isolates from Thailand for homology with genes coding for cholera toxin. Five isolates from environmental sources were homologous with the cholera toxin gene probe and produced both the A and B subunits of cholera toxin.

To determine whether non-O1 *Vibrio cholerae* isolated from patients with diarrhea (4, 5), their close contacts (5), or environmental sources (3, 5) in Thailand contained nucleotide sequences coding for cholera toxin (CT) or *Escherichia coli* heat-labile toxin (LT), we examined isolates with radiolabeled segments of DNA coding for the A and B fragments of CT (7, 8, 10) and for LT (8). Culture supernatants of isolates that hybridized with the CT and LT gene probes were further examined in a GM-1 enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies to the A and B subunits of CT (A. M. Svennerholm et al., in press).

We examined 281 non-O1 *V. cholerae* isolates from human and environmental sources in rural and urban Thailand in 1980, 1981, and 1982 (Table 1). Isolates from humans and animals were recovered from stools or rectal swabs incubated on thiosulfate-citrate-bile salts media (Difco Laboratories, Detroit, Mich.) at 37°C overnight. Non-O1 *V. cholerae* was isolated from water and food in the homes of persons with diarrhea as previously described (5). Isolates from flies were collected during a study conducted to identify enteric pathogens on flies in a village in northeastern Thailand (3). *V. cholerae* was identified by standard techniques (1) and tested by slide agglutination with polyvalent O1 and monospecific Inaba and Ogawa antisera (Difco). All isolates were lyophilized within 1 month of isolation.

The α-^{32}P-labeled gene probe coding for CT was prepared from *E. coli* MS371(pJM17) containing a recombinant plasmid coding for the A and B subunits of CT (10); the DNA probe coding for LT was prepared from plasmid pEWD299 (8). DNA hybridization of isolates (grown on nitrocellulose paper) with the α-^{32}P-labeled LT and CT probes was performed under conditions of high and low stringency as described by Kaper et al. (7), Moseley et al. (8), and Moseley and Falkow (9); cellular DNA of non-O1 *V. cholerae* that hybridized with the CT probe was prepared by the method of Brenner et al. (2). DNA (1 μg) was digested with either *Eco*RI or *Hin*dIII under conditions recommended by the manufacturer (Bethesda Research Laboratories, Gaithersburg, Md.). The DNA fragments were separated by electrophoresis and examined by the Southern technique (12) with the CT probe under high-stringency conditions (9).

Of the 281 non-O1 *V. cholerae* isolates examined, 5 hybridized with the CT probe under both hybridization conditions and with the LT probe under low-stringency conditions (25% formamide); *Eco*RI and *Hin*dIII digests of cellular DNA of these 5 non-O1 *V. cholerae* isolates hybridized with the CT probe, as determined by the Southern technique (Fig. 1). These five isolates were from environmental sources that were not associated with persons who had diarrhea and from whom O1 or non-O1 *V. cholerae* had been isolated (four isolates from water and one isolate from a collection of flies). None of 44 non-O1 *V. cholerae* isolates from patients with diarrhea hybridized with the CT or LT gene probe.

Culture supernatants of six non-O1 *V. cholerae* strains grown in Syncase media (6) with aeration were tested for the presence of the A and B subunits of CT with monoclonal antibodies in a GM-1 ELISA (Svennerholm et al., in press). As shown in Table 2, all five isolates that hybridized with the CT probe produced both the A and B subunits of CT, as determined by the GM-1 ELISA, with regard to immunological reactivity with monoclonal antibodies distinguishing both the A and B subunits of CT.

None of the 276 non-O1 *V. cholerae* isolates from Thailand that did not hybridize with the CT or LT gene probe produced CT or LT, as determined by the GM-1 ELISA (11). Furthermore, none of these isolates contained nucleotide sequences coding for CT or LT.

What role non-O1 *V. cholerae* containing genes coding for CT has in the epidemiology of cholera in Thailand, if any, is uncertain. As the five environmental isolates were not associated with cases of cholera or even with episodes of

TABLE 1. Sources of non-O1 *V. cholerae* isolates from Thailand in 1980, 1981, and 1982

Location	No. of strains from:				
	Patients with diarrhea	Contacts of patients with diarrhea[a]	Water[a]	Food[a]	Animals[a]
Rural areas	11	11	86	10	11[b]
Bangkok	33	25	153	27	0

[a] Some of the specimens were collected from individuals or sources in the homes of patients with diarrhea.
[b] Two of these isolates were from flies.

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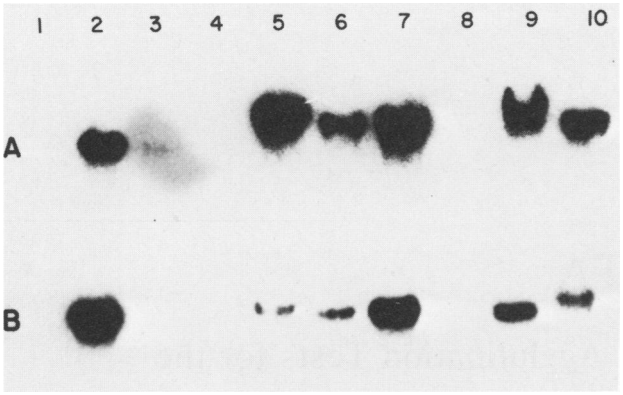

FIG. 1. Whole-cell DNA of non-O1 *V. cholerae* cut with *Eco*RI (A) or *Hin*dIII (B) and examined by Southern blot analysis with a radiolabeled probe for CT genes. Lanes show various *V. cholerae* strains, except as otherwise noted: 1, W-14; 2, WBDH697; 3, WBDH712; 4, W-12; 5, NWBD40/1F1; 6, WBDV101E1; 7, FY-2G; 8, *E. coli* K-12 Xac; 9, 569B (classical); and 10, 2982B (eltor).

TABLE 2. Enterotoxin production by non-O1 *V. cholerae* as tested in a GM-1 ELISA with monoclonal antibodies to the A and B subunits of CT

Strain	Strain concn[a]	Hybridization with the CT gene probe	Absorbance at 450 nm with monoclonal antibody to CT subunit:	
			A	B
Test				
FY-2G	10	+	>2.0	>2.0
	1	+	0.31	0.89
WBDV101E1	10	+	1.11	>2.0
	1	+	0.09	0.79
NWBD40/1F1	10	+	0.94	>2.0
	1	+	0.19	1.20
WBDH697	10	+	0.96	>2.0
	1	+	0.30	1.48
WBDH712	10	+	1.20	>2.0
	1	+	0.08	0.68
W-14	10	−	0.01	NT
	1		NT[b]	NT
Reference	0.5		>2.0	>2.0
	0.1		0.86	>2.0
	0.02		0.36	1.53
	0.004		0.10	0.65

[a] Concentrations of the test strains are given in "fold"; concentrations of the reference strain are given in micrograms per milliliter. The 10-fold concentration was achieved by desiccating the test strains with polyethylene glycol.

[b] NT, Not tested.

diarrhea from which non-O1 *V. cholerae* was isolated, they appeared to be natural inhabitants of the environment. Where the genes were acquired from and what benefit bacteria could gain in retaining genes coding for CT are unknown.

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LITERATURE CITED

1. **Barua, D., and W. Burrows.** 1974. Laboratory diagnosis of cholera, p. 85–126. *In* D. Barua and W. Burrows (ed.), Cholera. Saunders Co., Philadelphia, Pa.
2. **Brenner, D. J., G. R. Fanning, K. E. Johnson, R. V. Citarella, and S. Falkow.** 1969. Polynucleotide sequence relationships among members of *Enterobacteriaceae*. J. Bacteriol. **98:** 637–650.
3. **Echeverria, P., B. Harrison, C. Tirapat, and A. McFarland.** 1983. Flies as a source of enteric pathogens in a rural village in Thailand. Appl. Environ. Microbiol. **46:**32–35.
4. **Echeverria, P., C. Pitarangsi, S. Vibulbandhitkit, B. Eampokalap, P. Boonthai, and B. Rowe.** 1983. A longitudinal study of the prevalence of bacterial enteric pathogens among adults with diarrhea in Bangkok, Thailand. Diagn. Microbiol. Infect. Dis. **1:**193–204.
5. **Echeverria, P., J. Seriwatana, U. Leksomboon, C. Tirapat, W. Chaicumpa, and B. Rowe.** 1984. Identification by DNA hybridization of enterotoxigenic *Escherichia coli* in homes of children with diarrhea. Lancet **i:**64–66.
6. **Finkelstein, R. A., P. Ahasampunna, M. Chulasamaya, and P. Charunmethee.** 1966. Pathogenesis of experimental cholera: biologic activities of purified procholeragen A. J. Immunol. **96:**440–449.
7. **Kaper, J. B., S. L. Moseley, and S. Falkow.** 1981. Molecular characterization of environmental and nontoxigenic strains of *Vibrio cholerae*. Infect. Immun. **32:**661–667.
8. **Moseley, S. L., P. Echeverria, J. Seriwatana, C. Tirapat, W. Chaicumpa, T. Sakuldaipeara, and S. Falkow.** 1982. Identification of enterotoxigenic *Escherichia coli* using three enterotoxin gene probes. J. Infect. Dis. **145:**863–869.
9. **Moseley, S. L., and S. Falkow.** 1980. Nucleotide sequence homology between the heat-labile enterotoxin gene of *Escherichia coli* and *Vibrio cholerae* deoxyribonucleic acid. J. Bacteriol. **144:**444–446.
10. **Pearson, G. D. N., and J. J. Mekalanos.** 1982. Molecular cloning of *Vibrio cholerae* enterotoxin genes in *Escherichia coli* K12. Proc. Natl. Acad. Sci. U.S.A. **79:**2976–2980.
11. **Sack, D. A., S. Huda, P. K. B. Neogi, R. R. Daniel, and W. M. Spira.** 1980. Microtiter ganglioside enzyme-linked immunosorbent assay for vibrio and *Escherichia coli* heat-labile enterotoxins and antitoxin. J. Clin. Microbiol. **11:**35–40.
12. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.