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Isolation of *Vibrio cholerae* Serotype Ogawa from a Florida Estuary

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Vibrio cholerae serotype Ogawa was recently isolated from the estuarine waters of Apalachicola Bay, Fla., in areas that are subject to consistent fecal contamination and in areas that are remote from any apparent source of contamination. The significance of these organisms in the environment has not been determined.

Vibrio cholerae serotype Inaba has recently been found along the Atlantic and Gulf coasts of the United States, in the waters of Chesapeake Bay (3), in salt water marshes and sewers in Louisiana (3), in estuarine waters and oysters from Louisiana (8; A. DePaola, M. W. Presnell, and M. L. Motes, manuscript in preparation), and in Florida waters (U.S. Food and Drug Administration, unpublished data). Oysters isolated from Florida waters have been reported positive for this serotype (7, 8); however, the production of cholera toxin by these isolates has not been demonstrated through conventional assays.

To examine the relationship between human and animal fecal pollution and the incidence of *V. cholerae* in a commercial oyster-growing area, we established 24 sampling stations in Apalachicola Bay, Fla., at sites remote from and near various types of pollution. Seventy-three 4-liter samples of water were collected in sterile Nalgene bottles from selected stations twice in November 1980 and twice in February 1981. All samples were transported to the laboratory on ice and analyzed within 6 h after collection. The microflora in 2-liter portions was concentrated by vacuum filtration through a 142-mm (0.45 μm) Millipore filter, which was placed in a Waring blender containing 100 ml of alkaline peptone water and blended at 14,000 rpm for 1 min. A 10-ml portion of the homogenate was inoculated into tubes containing 10 ml of alkaline peptone water. Five serial 10-fold dilutions were made, and a five-tube most-probable-number test was performed (6). Isolates biochemically similar to *V. cholerae* were serotyped by the slide agglutination test, with group O1 cholera antiserum provided by Harry Smith, Jefferson Medical College, Philadelphia, Pa.

All *V. cholerae* O1 isolates were submitted to the Centers for Disease Control (CDC) and tested for cholera toxin (CT) by the enzyme-linked immunosorbent assay (9) and for stable toxin by the infant mouse assay (4). Pathogenicity testing was conducted in our laboratory by the suckling rabbit test of Dutta and Habbu (5). The CDC confirmed the biochemical patterns and serotypes of all group O1 isolates and found all isolates negative for stable toxin and one weakly positive for CT. Of eight isolates, six were positive by the suckling rabbit pathogenicity test. Pathogenicity in this model was determined by intraintestinal injection of 1 ml (10^8 to 10^9 organisms) of inoculum suspended in phosphate-buffered saline, pH 7.0. Criteria for pathogenicity were gross diarrhea or fluid accumulation in the intestines and cecum within 24 to 26 h after inoculation. Biochemical reactions and toxigenicity and pathogenicity tests on the *V. cholerae* serotype Ogawa isolates are shown in Table 1.

Eight cultures of *V. cholerae*, biotype El Tor, serotype Ogawa, were isolated from four samples of polluted and nonpolluted waters. Previous sanitary surveys by the Food and Drug Administration and the State of Florida have shown that the salinity of the polluted waters of Scipio Creek is low (0 to 5‰) and that these waters are contaminated daily by effluent from the Apalachicola Sewage Treatment Plant. This source of fecal contamination may also be a highly localized source of *V. cholerae* to nearby oyster-growing waters, but not to approved oyster-growing waters across the bay.

In November 1980, we recovered *V. cholerae* serotype Ogawa downstream from the Apalachicola Plant. One month later, during a short-term intensive survey, the CDC isolated *V. cholerae* serotype Inaba from a Moore swab taken at the Apalachicola Plant (Paul Blake, CDC, personal communication). The suckling rabbit test was not performed on the Inaba isolate; however, the Ogawa was found to be positive.

In November 1980 and February 1981, samples from the nonpolluted, approved oyster-growing waters across Apalachicola Bay also

TABLE 1. Biochemistry, toxigenicity, and pathogenicity of *V. cholerae* Ogawa isolates

Isolate No.	Test result																			
	String	Oxidase	Catalase	Citrate (Simmons)[a]	H_2S-TSI	Urea[a]	Gelatin-22c[a]	Indole[a]	Anaerobic glucose	Sucrose	Arabinose	Mannitol	Inositol	Lysine	Arginine	Ornithine	Lactose[a]	Heat-labile toxin[a]	Heat-stable toxin[a]	Pathogenicity
17-17	+	+	+	+	−	−	+	+	+	+	−	+	−	+	−	+	+	±[b]	−	+
40-13	+	+	+	+	−	−	+	+	+	+	−	+	−	+	−	+	−	−	−	−
40-14	+	+	+	+	−	−	+	+	+	+	−	+	−	+	−	+	−	−	−	+
45-20	+	+	+	+	−	−	+	+	+	+	−	+	−	+	−	+	+	−	−	+
45-29	+	+	+	+	−	−	+	+	+	+	−	+	−	+	−	+	+	−	−	−
95-16	+	+	+	+	−	−	+	+	+	+	−	+	−	+	−	+	+	−	−	+
95-17	+	+	+	+	−	−	+	+	+	+	−	+	−	+	−	+	+	−	−	+
95-18	+	+	+	+	−	−	+	+	+	+	−	+	−	+	−	+	+	−	−	+

[a] Performed at the CDC.
[b] Weakly positive.

yielded *V. cholerae* serotype Ogawa isolates. Although these waters (East Hole) are of high salinity (20 to 30%) and are remote from apparent sources of pollution, this area was incriminated as the source of oysters responsible for a symptomatic case of cholera caused by CT-negative *V. cholerae* serotype Inaba in November 1980 (2).

Scipio Creek (polluted) and East Hole (nonpolluted) are two separate and distinct areas about 4.5 miles (7.2 km) apart. Scipio Creek has no effect on the sanitary quality of East Hole, which is remote from any apparent significant source of pollution. The isolation of both serotypes from these areas, which have no known common source of pollution, suggests that the organisms are being introduced by an outside source, as in the polluted waters, or that they are indigenous to the area.

Although the occurrence of a serotype of group O1 *V. cholerae* not previously reported from estuarine waters of the United States is significant, it must be emphasized that unlike the *V. cholerae* group O1 isolates from Asiatic countries, the organisms isolated in our study, except for one weakly positive isolate, were CT negative. Nevertheless, *V. cholerae* organisms of both CT-negative groups O1 and non-O1 have been isolated from patients with cholera-like symptoms (1, 2) and are therefore significant to human health.

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