Vibrio cholerae (non-O1) Isolated from California Coastal Waters

JANE E. KENYON,* DUNCAN C. GILLIES, DONALD R. PIEXOTO, AND BRETT AUSTIN Santa Cruz County Public Health Laboratory, Santa Cruz, California 95060

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Nineteen strains of Vibrio cholerae non-O1 were isolated from five separate marine sites along the Santa Cruz County coast. This environmental study was initiated after a human case of non-O1 cholera-like diarrhea was acquired endemically.

Studies in recent years have documented the isolation of Vibrio cholerae from aquatic environments in Maryland, Florida, and Louisiana (2, 4-6). Both V. cholerae O1 and non-O1 serotypes have been recovered from waters, sediments, and shellfish of these regions. Cholera disease has been reported in humans ingesting shellfish harboring the organisms and in individuals recreationally exposed to waters from which V. cholerae strains have been isolated (5).

These reports established the prevalence of V. cholerae along the Atlantic seaboard and the Gulf states. We determined that V. cholerae non-O1 is also readily isolated from Pacific coastal waters.

In January 1983, we isolated V. cholerae non-O1 from the liquid stool of a Santa Cruz County resident hospitalized with severe cholera-like disease. Since the patient (a 19-year-old male) had not been outside the county of Santa Cruz, had not eaten shellfish or seafood, and had become ill only after surfing on successive days in coastal waters, we decided to examine waters along the county coast for the presence of V. cholerae.

Marine samples collected in sterile, covered buckets were concentrated by vacuum filtration through 0.45-µm filters (Millipore Corp., Bedford, Mass.). Before filtration, the temperature and salinity of the samples were determined. Isolation of V. cholerae was done and most probable numbers were obtained by the methods of Kaper et al. (5), except that enrichment broths were incubated for 1 week at 4°C after primary plating on thiosulfate-citrate-bile salts agar. Total coliform counts were performed by using a three-tube most-probable-number test with lauryl tryptose and brilliant green-lactosebile broths (Difco Laboratories, Detroit, Mich.) (1).

To date we have sampled seven different sites and isolated 19 strains of V. cholerae from five of these locations. Additionally, Vibrio alginolyticus was recovered from four of the sites, and Aeromonas hydrophilia was recovered from three. Cold enrichment enhanced the recovery of V. alginolyticus but not V. cholerae. V. cholerae strains were tested for agglutination in Difco O1 polyvalent antiserum. None of our isolates was an O1 serotype.

An isolate from the patient and two marine strains were confirmed as V. cholerae non-O1 by the California Department of Health Services and the Centers for Disease Control. Toxin testing of the strain from the patient and of one of our V. cholerae non-O1 isolates was done at the Centers for Disease Control with negative results for both heat-labile and heat-stable toxins. The Centers for Disease Control also reported that these organisms were nonreactive in available antisera. Table 1 summarizes our results.

Although Kaper et al. (5) did not isolate V. cholerae from Chesapeake Bay stations where water salinity was $>17^{\circ}/_{oo}$, our cholera isolates were obtained from a much broader salinity range of <3 to $31.7^{\circ}/_{oo}$. Only strains isolated from the site where the salinity was $<3^{\circ}/_{oo}$ were sorbitol positive. V. cholerae isolates from all other sites did not ferment sorbitol. Another biochemical variable among the strains is the Voges-Proskauer reaction, with the majority of the isolates (58%) having a negative test result. With these exceptions, the characteristics of our marine V. cholerae organisms are similar within the range of tests done.

Kaper et al. reported that isolation of V. cholerae is apparently inversely related to pollution as measured by total coliforms (5). Our data are insufficient to correlate V. cholerae and total coliform most probable numbers (Table 1).

That severe diarrheal disease may be caused by non-O1 V. cholerae was demonstrated in the case of the young surfer. Craig et al. have established that an environmental non-O1 V. cholerae strain produces an enterotoxin identi-

Site (4-liter sample)	Date	Salinity (°/ ₀₀)	Temp (°C)	V. cholerae non-O1 (MPN/liter) ^a	Total coliforms (MPN/100 ml)
Davenport Landing	1/26/83	31.78	16	0.04	ND ^b
Davenport Landing	2/08/83	33.17	14	0	91
San Vincente Beach	2/08/83	28.83	16	0	230
Rio Del Mar River mouth	2/08/83	9.04	16	0.93	2,400
San Lorenzo River mouth	2/24/83	7.43	13.8	4.6	ND
Cowell's Beach	2/24/83	28.83	14.6	0	ND
Santa Cruz yacht harbor	3/16/83	30.39	ND	0.04	ND
Capitola-Soquel Creek breakwater	3/16/83	<3.0	ND	0.43	ND

TABLE 1. V. cholerae (non-O1) isolated from marine waters

^a MPN, Most probable number.

^b ND, Not determined.

cal to, or closely related to, cholera enterotoxin (3, 7). Thus, physicians and public health officials should be aware of the potential for cholera-like diarrhea occurring in persons exposed to environmental non-O1 V. cholerae organisms.

We plan to repeat our V. cholerae isolation attempts during the summer to determine whether there is a seasonal variation in the numbers of organisms present at coastal sites.

Data presented here permit the conclusion that V. cholerae non-O1 is a naturally occurring vibrio in Santa Cruz County coastal waters. The hypothesis that V. cholerae may be found in aquatic environments of other regions of the Pacific Coast has yet to be verified.

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