Isolation of Nontoxigenic Vibrio cholerae O Group 1 from a Patient with Severe Gastrointestinal Disease

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A nontoxigenic strain of Vibrio cholerae O group 1 was isolated from Florida from the stool of a patient with severe diarrhea. The strain had the same hemolytic and unique phage-sensitivity pattern as all toxigenic isolates from recent cases of cholera in Texas and Louisiana. Identical strains were transiently isolated from sewerage systems in two other Florida communities, suggesting that multiple human infections had occurred. This is the first indication that V. cholerae O1 strains which do not produce cholera toxin may be able to cause gastrointestinal disease in humans. The identification of these strains also raises questions about the relationship between toxigenic and nontoxigenic strains of V. cholerae O1 along the Gulf Coast of the United States.

Cholera is caused by toxigenic strains of Vibrio cholerae O group 1. Over the last several years, environmental studies (stemming in part from the discovery that cholera may be endemic in the United States [2]) have shown that nontoxigenic V. cholerae O1 strains exist in estuarine waters along the Atlantic and Gulf coasts and can be found in sewage along the Gulf coast. Such strains, however, have rarely been associated with human disease and, in particular, have not been associated with diarrheal illnesses (10). In this report, we describe the isolation of a nontoxigenic V. cholerae O1 strain from the stool of a person with severe diarrhea. The strain was of the same phage type as toxigenic strains responsible for outbreaks of cholera in Louisiana and Texas (2–4, 9); during the investigation of this case, nontoxigenic strains of this phage type were also identified in sewage in two Florida communities.

In November 1980, a 46-year-old woman in Pensacola, Fla., developed severe diarrhea and abdominal cramps. The patient reported having ca. 30 stools during the course of her first day of illness; the stool volume was “large”, and stools were greenish in color. When seen at the emergency room of a local hospital, she had a temperature of 102°F, a pulse of 104, and a blood pressure of 100/64 mm Hg. She was treated symptomatically and sent home but continued to have diarrhea. On the third day of illness she consulted her private physician, who collected a stool specimen for culture and treated her with tetracycline. Her diarrhea continued for a total of 5 days.

The patient’s stool specimen was collected in buffered glycerol saline and plated within 1 h. At 18 h there was heavy growth on sheep blood agar of an organism that was subsequently identified as V. cholerae O1 biotype El Tor serotype Inaba. The organism was the predominant organism on the plate; no other known enteric pathogen was isolated. In serum collected 2 and 6 weeks after the onset of illness, the patient did not have detectable agglutinating antibodies or a rise in vibriocidal antibodies to the stool isolate nor could a rise in anti-cholera toxin antibody titers be demonstrated.

In the 5 days before she became ill, the patient had eaten 6 to 12 dozen raw oysters which had been harvested from Appalachicola Bay, Fla. The patient’s husband, who had eaten raw oysters from the same sack, had several “loose stools” on the day after the patient became ill, but did not have a stool specimen collected for culture. The patient’s daughter, who also lived in the household, did not eat any of the oysters and was not ill. The patient was taking anecdotic during the time she was eating oysters; she had no history of gastric surgery.

In a further investigation conducted in Pensacola and surrounding communities (which included the use of thiosulfate citrate bile-salts sucrose agar to culture diarrheal stools), no additional V. cholerae-associated cases of gastroenteritis were identified. During the month after the identification of the case, Moore swabs (1) were placed in the sewerage systems of the major west Florida cities and cities and towns located along Appalachicola Bay. V. cholerae O1 El Tor Inaba was isolated from the initial Moore swabs placed in sewerage systems in Appalachicola and Carrabelle, two towns by Appalachicola Bay.

The patient and sewage isolates were all hemolytic, and when typed by the methods of Lee and Furniss, all had the same unique phage-sensitivity pattern; this combination of hemolysis and phage type was identical to that of strains isolated during the outbreaks of cholera in Texas and Louisiana in 1973, 1978, and 1981 (2–4, 9). Unlike the Louisiana and Texas isolates, these isolates were nontoxigenic in both a Y-1 adrenal cell assay and an enzyme-linked immunosor- bent assay. In a rabbit skin test, they produced only nonspecific bluing factors which were not significantly inhibited by GM1 ganglioside or standard cholera antitoxin. DNA extracts from the isolates were found to lack a gene for cholera toxin production with an Escherichia coli heat-labile genetic probe (5). Neither cells nor culture filtrates cause fluid accumulation in rabbit ileal loops by standard techniques.
In a concurrent environmental survey, several strains of non toxigenic *V. cholerae* O1 were also isolated from Appalachian Bay. The phage-sensitivity patterns of these strains, however, differed greatly from that of the patient and sewage isolates.

The heavy growth of *V. cholerae* O1 on blood agar after direct plating of the patient's stool suggests that she was infected with the organism. The significance of her failure to develop antibodies demonstrable by standard techniques is unclear; most patients with gastroenteritis associated with nontoxigenic non-O1 *V. cholerae* also fail to develop antibodies demonstrable by these techniques (J. Feeley, unpublished data). Although the initial source of the isolate cannot be definitely identified, there is a recognized association between *V. cholerae* infection and eating raw seafood (2, 6). The organism may have been transmitted by the raw oysters eaten by the patient, with her frequent use of antacids increasing her susceptibility to infection. Identification of additional isolates of nontoxigenic *V. cholerae* O1 of the same phage type in sewage suggests that other human infections were occurring at the same time in at least two of the towns along Appalachian Bay.

The pathophysiological mechanism by which this isolate may have caused diarrhea is as yet undetermined. Unlike other disease-associated *V. cholerae* O1 strains, the isolate could not be shown to produce cholera toxin by standard assays and was subsequently found to lack the gene for cholera toxin production. Although initial testing suggested that the isolate did not cause fluid accumulation in rabbit ileal loops by standard techniques, further work has indicated that it does have activity in biological systems such as the RITARD model (8) (N. Pierce, unpublished data). These findings are analogous to those in non-O1 *V. cholerae* gastroenteritis; although disease-associated isolates have been shown to have biological activity in a variety of models (7), less than 10% produce cholera toxin, with lack of toxigenicity associated with absence of a gene for cholera toxin production (5).

The inability to produce cholera toxin is the only identifiable difference between our patient and sewage isolates and the strains of *V. cholerae* O1 associated with the 1973, 1978, and 1981 cases of cholera in Louisiana and Texas; in all instances isolates have been hemolytic, with the same serotype and biotype and the same unique phage-sensitivity pattern (2–4, 9). Not only have no other nontoxigenic *V. cholerae* of this phage type been identified but most nontoxigenic strains have had radically different phage-sensitivity patterns, with resistance to almost all of the phages used for typing (J. V. Lee, unpublished data). Our strains and the toxigenic strains that have caused cholera along the Gulf coast are related in some as yet unknown way; further study of this relationship may lead to a better understanding of the ecology of these organisms and of the persistence of toxigenic strains in the United States.

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**ADDENDUM IN PROOF**

William Spira and associates have recently described a possible extracellular virulence factor (active in a modified rabbit ileal loop assay system) produced by our patient isolate (W. Spira, D. Sack, S. Sanyal, M. Madden, and B. McCardell, Abstr. 19th Joint Conf. Cholera, U.S.-Japan Coop. Med. Sci. Program, Bethesda, Md., 17–19 October 1983). The role of this factor in pathogenicity is still not well defined; further work is in progress.

**LITERATURE CITED**


