Severe Diarrhea Caused by Cholera Toxin–Producing *Vibrio cholerae* Serogroup O75 Infections Acquired in the Southeastern United States

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**Background.** From 2003 through 2007, *Vibrio cholerae* serogroup O75 strains possessing the cholera toxin gene were isolated from 6 patients with severe diarrhea, including 3 in Georgia, 2 in Alabama, and 1 in South Carolina. These reports represent the first identification of *V. cholerae* O75 as a cause of illness in the United States. *V. cholerae O75* was isolated from a water sample collected from a pond in Louisiana in 2004. Subsequently, 3 *V. cholerae* isolates from Louisiana (2 from patients with diarrhea in 2000 and 1 from a water sample collected in 1978) that had been previously reported as serogroup O141 were also discovered to be serogroup O75.

**Results.** All 8 patients who were infected with *V. cholerae* O75 were adults who became ill after consuming seafood; 2 had eaten raw oysters traced back to the Gulf Coast of the United States. All 10 isolates possessed the cholera toxin gene and were susceptible to 10 antimicrobials. One clinical isolate and 1 environmental (water) isolate had the same pulsed-field gel electrophoresis pattern; 4 clinical isolates shared a common pulsed-field gel electrophoresis pattern.

**Conclusions.** The occurrence of these cases over many years and the concurrent identification of *V. cholerae* O75 in water from a Gulf Coast state suggest that these strains may survive for long periods in this environment. The patients' exposure histories suggest that infection can be acquired from consumption of raw oysters from the Gulf Coast. Clinicians and public health authorities should be vigilant for the occurrence of new toxigenic serogroups of *V. cholerae* that are capable of causing severe diarrhea.

*Vibrio cholerae* serogroup O75 is 1 of ~200 serogroups of *V. cholerae* [1, 2]. Among these, only serogroup O1 and O139 strains, which possess the cholera toxin gene, are known to cause epidemic severe watery diarrhea and are considered by the World Health Organization and by the US Centers for Disease Control and Prevention (CDC) to cause the reportable disease cholera [3, 4]. Many serogroups of *V. cholerae* other than O1 and O139 can cause diarrhea, and certain strains of a few serogroups, including *V. cholerae* O141, can possess the cholera toxin gene [5]. Toxigenic strains of *V. cholerae* serogroup O1 and O141 have been identified in environmental samples from the US Gulf Coast and in diarrheal stool samples from patients who consumed shellfish from the Gulf Coast before becoming ill [5–10].

In 2000, 2 infections (of a series of 11 infections dating back to 1984) that were attributed to cholera toxin–producing *V. cholerae* O141 occurred among Louisiana residents after the consumption of shrimp and crawfish from the Gulf Coast [6]. In 2005, after discrepant serogroup typing results led to the retesting of previously identified *V. cholerae* O141 isolates with a different preparation of antisera, 2 isolates were identified to be strains of cholera toxin–producing *V. cholerae* O75 (H.I., unpublished observation). Retesting of a toxigenic *V. cholerae* O141 isolate from pond water collected in Louisiana in 1978 identified it as toxigenic.
V. cholerae O75 [11]. From November 2003 through October 2007, 6 culture-confirmed cases of toxigenic V. cholerae O75 infection were detected in residents of Georgia [3], Alabama [2], and South Carolina [1]. The organism was also detected in a water sample collected from a Louisiana pond in September 2004. To our knowledge, these represent the only isolates of V. cholerae serogroup O75 reported in the United States; we describe their clinical, epidemiologic, and microbiologic characteristics.

METHODS

Case finding and case investigation. V. cholerae infections are reportable to all US state health departments. Laboratories, hospitals, and clinicians report suspected or confirmed cases to public health authorities and submit isolates to state public health laboratories for additional characterization. Since 1999, in addition to participating in passive surveillance for cases, Georgia has also participated in active surveillance of Vibrio species cases as part of the CDC’s Emerging Infections Program. Public health staff contact clinical laboratories at least once per month to ensure complete reporting of infection due to Vibrio species, and they attempt to interview all patients with use of a standard CDC form. If a patient reports seafood consumption in the week before illness onset, public health staff request a traceback from the department of agriculture in the state(s) where the exposure occurred and/or the seafood was handled.

Environmental isolates. As part of routine surface water quality monitoring, 4 water samples taken from freshwater ponds in 3 inland Louisiana parishes in September 2004 were cultured for Vibrio species. Isolates were forwarded to the Louisiana Office of Public Health Laboratory for confirmation and additional testing and then to the CDC for additional testing.

Laboratory isolates. State public health laboratories in Georgia, Alabama, and South Carolina confirmed and speciated Vibrio isolates that were received from clinical laboratories and forwarded them to the CDC. At the CDC, V. cholerae isolates were screened with antisera to serogroups O1, O139, and O141; tested using PCR for the cholera toxin gene [12]; tested for susceptibility to ampicillin, chloramphenicol, ciprofloxacin, furazolidone, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim-sulfamethoxazole; and subtyped using PFGE with 2 enzymes (SfiI and NotI) [13, 14]. Disk diffusion results were interpreted by criteria established by the Clinical and Laboratory Standards Institute for Vibrio cholerae to determine susceptibility to ampicillin, chloramphenicol, sulfonamides, tetracycline, and trimethoprim-sulfamethoxazole [13]. Criteria standardized for the Enterobacteriaceae family were used to interpret results of susceptibility tests for ciprofloxacin, kanamycin, and streptomycin [13]. Tentative zone-size criteria have been proposed for the testing of furazolidone and nalidixic acid susceptibility for V. cholerae. Patients from whom non-O1, non-O139 toxigenic V. cholerae isolates are obtained are asked to submit a serum specimen for anti–cholera toxin antibody testing [15]. Toxigenic, non-O1, non-O139 isolates were forwarded to the National Institute of Infectious Diseases (Tokyo, Japan) for serogroup testing. All isolates that were previously identified as V. cholerae O141 were sent to the National Institute of Infectious Diseases for serogroup testing a second time.

RESULTS

Clinical isolates. From November 2003 through October 2007, 3 cases of toxigenic V. cholerae O75 infection were detected in adult residents of Georgia, 2 cases were detected in adult residents of Alabama, and 1 case was detected in an adult resident of South Carolina. Five of these 6 patients had medical charts available for review, and all had clinical illness consistent with “cholera,” with severe diarrhea and evidence of dehydration. The 2 Louisiana patients who were previously described [6] both had fever and diarrhea. All 8 patients reported having consumed seafood (5 had eaten raw oysters) within the 7 days before becoming ill. Demographic, clinical, and epidemiologic information is summarized in table 1. The 5 most recent cases for which detailed clinical and exposure information was available are described.

Case reports. The first case involved a 36-year-old white woman with hypertension and hyperlipidemia who experienced profuse watery diarrhea (>10 evacuations per day), accompanied by fever, vomiting, headache, loss of appetite, and dizziness, on 8 November 2003, a day after consuming raw oysters that were purchased from a seafood market in Georgia. She thought that the oyster tags had identified Apalachicola Bay (located on the gulf coast of Florida) as the source but had not kept them. On examination, she was afebrile with normal pulse and blood pressure and slight abdominal tenderness. Her complete blood count and blood chemistries were within normal limits. She was treated with ciprofloxacin and metronidazole, and the illness resolved. Toxigenic V. cholerae O75 was isolated from her stool specimen that was collected on 13 November 2003. Four months after the onset of her infection, her serum anti–cholera toxin antibody titer was elevated (1:1280).

Case 2 involved a 50-year-old white man with chronic hypertension who developed diarrhea and abdominal cramps that lasted for 7 days, beginning on 28 July 2004. He had consumed 2 dozen raw oysters in a Georgia restaurant on 24 July 2004. The oysters were traced back to Apalachicola Bay. He was hospitalized with nausea, vomiting, watery diarrhea (>10 evacuations per day), dehydration, and cramps. On examination, he was afebrile with normal pulse and blood pressure. He had dry mucous membranes and diffuse abdominal tenderness. He was hyponatremic and had mildly elevated blood urea nitrogen and creatinine levels. He was treated with intravenous fluids and
Table 1. Selected demographic, clinical, epidemiologic, and laboratory characteristics of 8 cases of infection with toxigenic *Vibrio cholerae* O75 in residents of the southeastern United States, 2000–2007.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
<th>Case 6</th>
<th>Case 7</th>
<th>Case 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolate name</td>
<td>LA1</td>
<td>LA2</td>
<td>GA1</td>
<td>GA2</td>
<td>GA3</td>
<td>AL1</td>
<td>SC1</td>
<td>AL2</td>
</tr>
<tr>
<td>Age, years</td>
<td>51</td>
<td>80</td>
<td>36</td>
<td>50</td>
<td>58</td>
<td>63</td>
<td>34</td>
<td>44</td>
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<tr>
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<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>White (non-Hispanic)</td>
<td>…</td>
<td>…</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Severe diarrhea</td>
<td>…</td>
<td>…</td>
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<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Fever</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>Abdominal cramps</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Dehydrationa</td>
<td>…</td>
<td>…</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>Travel to or residence in Gulf Coast stateb</td>
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<td>No</td>
<td>No</td>
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<td>Yes</td>
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<tr>
<td>Ate raw oystersb</td>
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<td>No</td>
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<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
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<td>No</td>
<td>No</td>
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<td>No</td>
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<tr>
<td>Ate other seafoodb</td>
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<td>Yes</td>
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<td>No</td>
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<td>Yes</td>
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<tr>
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<td>No</td>
<td>No</td>
<td>No</td>
<td>Unknown</td>
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<tr>
<td>ctxA PCR result</td>
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<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
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<tr>
<td>Anti-cholera toxin antibody titerd</td>
<td>…</td>
<td>…</td>
<td>1:1280</td>
<td>1:800</td>
<td>1:10,000</td>
<td>1:800</td>
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<td>KZGS12.0074</td>
<td>KZGS12.0073</td>
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</tr>
</tbody>
</table>

**NOTE.** AL, Alabama; GA, Georgia; LA, Louisiana; SC, South Carolina.

- Dehydration was defined as dry mucus membranes, hypotension, or elevated blood urea nitrogen and creatinine levels responsive to fluids.
- Exposures in the week before illness onset.
- Patient reported having eaten raw oysters >2 weeks before illness onset but could not remember any more recent seafood exposures.
- A titer >1:400 was considered to be a positive result.
trimethoprim-sulfamethoxazole and was discharged from the hospital after 2 days. Toxigenic *V. cholerae* O75 was isolated from his stool specimen. One month after illness onset, his serum anti–cholera toxin antibody titer was elevated (>1:800).

The third case involved a white woman from Georgia with rheumatoid arthritis, osteoporosis, and hypercholesterolemia who was 58 years of age and was taking medications that included lansoprazole, prednisone, and methotrexate. She became ill on 28 July 2004 and experienced explosive watery diarrhea (>10 evacuations per day), nausea, cramps, myalgias, and headaches. She presented to her primary care provider’s office with a syncopal episode, hypotension, and diaphoresis and was referred to the emergency department. The only seafood that she recalled eating were raw oysters that were eaten 19 days before the onset of illness. Because the seafood consumption occurred outside of the typical incubation period following *Vibrio* exposure, no traceback was done. She was afebrile with dry mucosa and sunken eyes, hypokalemia, and normal renal function. She was treated with fluids, potassium, ciprofloxacin, and lomotil, and she recovered. Toxigenic *V. cholerae* O75 was isolated from her stool specimen. Two months after illness, her serum anti–cholera toxin antibody titer was elevated (1:800).

The fourth case involved a 63-year-old white man from Alabama with a history of Churg-Strauss syndrome, gastro-esophageal reflux, hypertension, dyslipidemia, and bladder and prostate cancer who was taking prednisone; he became ill with vomiting, diarrhea (>10 evacuations per day), and abdominal cramps on 18 October 2004. He had visited the Gulf Coast of Florida between 3 and 10 days before the onset of illness and reported having eaten raw oysters 7 and 10 days before illness onset and various types of cooked seafood on other days. The raw oysters were traced back to Louisiana and Apalachicola Bay. He had a temperature of 38.1°C, a pulse of 125 beats/min, dry mucous membranes, and a mildly tender abdomen. His creatinine level, blood urea nitrogen level, and WBC count were elevated. He was treated with ciprofloxacin and fluids and was hospitalized overnight. Toxigenic *V. cholerae* O75 was isolated from his stool specimen. Two months after illness, his serum anti–cholera toxin antibody titer was elevated (1:800).

Case 5 involved a 34-year-old black man from South Carolina with no underlying medical conditions; he became ill on 6 August 2006 with vomiting, diarrhea, nausea, abdominal cramps, myalgias, and headache. He had traveled to Miami, Florida, the week before illness onset and had consumed seafood, including raw oysters, at local restaurants, but the exact dates and location of consumption were unclear. Because of the lack of specific information, no traceback was possible. He presented to his primary care provider’s office on 10 August 2006 with diarrhea and submitted a stool specimen. He did not require hospitalization or additional treatment. Toxigenic *V. cholerae* O75 was isolated from his stool specimen, along with *Vibrio parahaemolyticus* and *Plesiomonas shigelloides*. The patient did not appear for his follow-up appointment, and no serum sample was obtained.

**Environmental isolates.** Toxigenic *V. cholerae* O75 was identified in 1 of 4 surface water samples collected from fresh water sources in Louisiana in 2004. Two other toxigenic isolates were identified as *V. cholerae* O141.

**Antimicrobial susceptibility and PFGE patterns.** All 10 isolates of *V. cholerae* O75 were susceptible to all tested antimicrobials; they represented 2 main PFGE clusters with both enzymes (*Sfi* and *Not*), which differed from the PFGE patterns of *V. cholerae* O1 El Tor and the classical biotype isolates, *V. cholerae* O139 and *V. cholerae* O141 (figure 1). The PFGE patterns observed for the 2004 environmental isolate from Louisiana and the isolate from 1 Georgia patient (GA3) were indistinguishable by use of both enzymes. The PFGE patterns of 3 clinical isolates from 2004 and of the 1 isolate from 2006...
were indistinguishable by use of both enzymes (GA1, GA2, AL1, and SC1). One of the clinical isolates from 2000 and the isolate from 2007 yielded the same PFGE pattern with use of the SfiI enzyme, but they had different patterns with use of the NotI enzyme.

**DISCUSSION**

Classification of *V. cholerae* includes >200 serogroups [1], only a few of which have been shown to possess the cholera toxin gene. Two toxigenic serogroups—*V. cholerae* O1 and *V. cholerae* O139—are capable of causing widespread epidemics of cholera with high case fatality rates. None of the toxigenic *V. cholerae* strains associated with the US Gulf Coast—serogroups O1, O141, and now O75—have caused more than sporadic cases and small outbreaks of severe diarrhea in the United States. This may be attributable to conditions in reservoirs (including Gulf waters) that limit spread, such as the protection of drinking water sources from human waste and environmental contamination. Whatever the reason, infection with toxigenic strains of *V. cholerae* acquired from the Gulf Coast remains rare. Since 1995, only 27 domestically-acquired cases of *V. cholerae* O1 infection [7], 5 cases of *V. cholerae* O141 infection [6], and 8 cases of *V. cholerae* O75 infection have been reported (CDC, unpublished data).

The US Gulf Coast is not the only area where toxigenic non-O1 *V. cholerae* serogroups have been detected in recent years. From 2002 through 2005, Japan reported 3 cases of toxigenic *V. cholerae* O141 infection, 1 case of toxigenic *V. cholerae* O49 infection, and 1 case of toxigenic *V. cholerae* O8 infection [16].

The cases described here demonstrate that toxigenic *V. cholerae* O75 appears to be an emerging infectious disease and that it is capable of causing severe diarrheal illness that results in clinically significant dehydration. Possible predisposing factors present in 2 of the 5 recent case-patients included the use of immunosuppressive medications and acid-blocking medications. Exposure to seafood (specifically, raw oysters) from the Gulf Coast was the salient epidemiologic association for this case cluster. Clinicians should consider toxigenic *Vibrio* infections to be a cause of severe diarrhea in patients with a history of seafood consumption, especially in the southeastern United States. Stool cultures with TCBS media and additional testing available through state health department laboratories can confirm the diagnosis, and empirical antimicrobial therapy can be promptly instituted. Immunocompromised persons, in particular, should avoid raw oysters because of the risk of acquiring *Vibrio vulnificus* infection. The risk of *V. cholerae* O75 infection is an additional reason that raw oysters may pose a danger even to immunocompetent consumers.

The stability of PFGE patterns over time and the similarity of clinical and environmental isolates suggest that these strains persist in the environment, similar to strains of *V. cholerae* O1 and O139. Continued surveillance and submission of *Vibrio* isolates to state public health laboratories, and detailed clinical and epidemiologic information obtained through case interviews are vital to further characterize the epidemiology and prevention measures for this emerging infectious disease. Traceback of potentially contaminated shellfish or finfish should be conducted whenever case histories suggest seafood exposure. Environmental aspects of toxigenic non-O1 and non-O139 Vibrio species, including survival in marine and freshwater environments, requires additional study. The respective roles that horizontal transfer of cholera toxin genes (carried on a bacteriophage, CTX phage) and exchange of O-antigen had in the emergence of toxigenic *V. cholerae* O141, O75, and other non-O1, non-O139 strains remains largely undetermined [11, 17]. Additional research is needed to determine whether the O75 strains are nontoxigenic strains that have acquired the cholera toxin genes from horizontal transfer or whether they represent a lineage of epidemic strains that have undergone genetic exchange in the O-antigen biosynthesis region.

**Acknowledgments**

**Potential conflicts of interest.** All authors: no conflicts.

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