A Cytotoxin-Producing Strain of *Vibrio cholerae* Non-O1, Non-O139 as a Cause of Cholera and Bacteremia after Consumption of Raw Clams

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Received 23 December 1999/Returned for modification 8 March 2000/Accepted 12 June 2000

We report a case of a cholera-like gastroenteritis subsequent with bacteremia in a healthy man following consumption of raw clams. Although we failed to recover the organism from the patient's stool culture, his blood culture was positive for a non-cholera toxin-producing yet cytotoxin-producing non-O1 and non-O139 *Vibrio cholerae*.

CASE REPORT

A healthy 49-year-old man vacationing with his wife in Ocean City, Md., in mid-July 1998, consumed raw clams at a local restaurant. About 18 h later he developed severe profuse watery diarrhea, nausea, and vomiting. Over the next 24 h he was unable to eat or drink and suffered from constant episodes of nonbloody, watery diarrhea and severe abdominal cramping and pain. While his wife, who did not eat the raw clams, remained well, he appeared extremely ill, febrile, and with shaking chills and was admitted to the hospital due to dehydration and gastrointestinal pain. On initial examination, his vital signs included a temperature of 103.3°F, a blood pressure of 140/80 mm Hg, a pulse of 78 beats/min, and a respiratory rate of 22 breaths/min. His white blood cell count was 13,200/mm³ with 82% neutrophils. His low electrolytes included sodium and potassium levels of 137 mmol/liter and 3.2 mmol/liter, respectively. Blood and stool specimens were submitted for culture prior to his treatment with intravenous hydration, suppository acetaminophen, intramuscular prochlorperazine, and ciprofloxacin. The patient recovered completely and was released from the hospital after 48 h.

**Microbiological investigations.** Cholera-toxin producing vibrios, including *Vibrio cholerae* O1 and O139 serogroups, which mainly spread through contaminated drinking or recreational water, cause noninvasive epidemic cholera in developing countries, but non-O1 *V. cholerae* may be invasive, transmitted to humans by ingestion of raw seafood, and cause episodes of systemic bacteremia and septicemia in patients with underlying medical predisposing conditions, including malignancies, cirrhosis, and immunodeficiency (6, 11, 12). Because a history of raw seafood consumption by the patient was not indicated on the laboratory request slip, the stool specimen received from the patient was processed for common enteric pathogens, including *Salmonella*, *Shigella*, and *Campylobacter* species, by inoculation to blood agar, MacConkey agar, Hektoen enteric agar, and Campy-BAP (Becton Dickinson Microbiology Systems, Sparks, Md.). Initially, thiosulfate-citrate-bile-succrose (TCBS) agar was not inoculated. As a consequence, *V. cholerae* was not isolated from his stool specimen. Of two sets of blood cultures processed by the BACTEC 9240 system (Becton Dickinson Microbiology Systems), one was positive for a curved, highly motile gram-negative rod after 48 h. The isolate was β-hemolytic on sheep blood agar, oxidase positive, fermentative, and grew on TCBS agar as large raised shiny yellow colonies. It was identified as *V. cholerae* biotype 6001010 by using a MicroScan gram-negative Combo panel (Dade International, Inc., Sacramento, Calif.) and was susceptible to trimethoprim-sulfamethoxazole, ampicillin, cefazolin, gentamicin, aztreonam, and ciprofloxacin. The isolate was encapsulated, and the capsular material appeared as faint blue halos around dark purple cells stained by the Hiss method (2). The identification of the isolate was later confirmed by the Pennsylvania Department of Health and Center for Disease Control and Prevention as a non-cholera toxin-producing, non-O1, non-O139 *V. cholerae*. The isolate did not bear the cholera toxin gene (*ctxA*), which was confirmed by a PCR reported by the enteric bacteriology section at the Centers for Disease Control and Prevention.

To account for his watery diarrhea, studies for a cytotoxin were undertaken. Cytotoxin activity of the culture filtrates of the *V. cholerae* isolate grown in tryptic soy broth and brain heart infusion broth (Becton Dickinson Microbiology Systems) media were tested on HEp-2 cell monolayers (8). The cell-free filtrate of tryptic soy broth but not brain heart infusion broth showed cytotoxicity at a 1:32 dilution on HEp-2 cells. The cytotoxin activity of the filtrate was neutralized by the addition of the patient's serum, derived 6 weeks postinfection, to the HEp-2 cell monolayer prior to its inoculation with *V. cholerae* cell culture extract. However, the hemolytic activity of the strain remained intact when a blank filter paper disk saturated with the patient's serum was placed along the line of inoculation on the sheep blood agar.

*V. cholerae* non-O1 was not detected in the stool specimen of this patient despite processing of the specimen for the recovery of enteric pathogens. TCBS medium, which would have enhanced detection, was not inoculated simply because the history of consumption of raw clams by the patient was not indicated. This case underscores the importance of a history of traveling to the coastal regions and ingestion of raw seafood by patients, which should be effectively communicated between the primary physicians and the microbiologists, when the diarrheal stool specimens are submitted to a microbiology laboratory. The patient’s admission blood culture became positive for a slightly curved gram-negative rod, which was initially suspected to be a *Campylobacter* or a *Vibrio* species. It was subsequently identified as *V. cholerae* by both API 20E and MicroScan gram-negative Combo identification systems. The isolate failed to
grow on MacConkey agar, which also might have contributed to the lack of recovery of the organism from the initial-stool specimen. The strain was a non-cholera toxin-producing, non-O1, non-O139 *V. cholerae* which clinically caused cholera symptoms (1). On the other hand, the isolate was encapsulated, beta-hemolytic, and demonstrated a rather high titer of cytotoxin activity on a HEp-2 cell monolayer. Although our present report cannot fully characterize the hemolytic and cytotoxic activities of the strain, it is noteworthy that only the cytotoxicity was neutralized by the patient’s serum. Non-cholera toxicigenic strains of both O1 and non-O1 serotypes of *V. cholerae* have been associated with cholera-like symptoms in humans (1, 10). These reports hence strengthen the role of the cytotoxin in conjunction with hemolysin, encapsulation, and any other potential virulence factor(s) accounting for entero-pathogenicity and bacteraemia in this patient.

The majority of cases of bacteraemia caused by non-O1 serogroups of *V. cholerae* have been reported in patients with chronic underlying medical problems, including cirrhosis, renal failure, hematological malignancies, and immunodeficiencies (9). In contrast, this patient was an active, healthy 49-year-old man, with no identifiable underlying conditions, who ingested raw shellfish. His bacteraemia most probably resulted from the transmural migration of the organism through the gastrointestinal tract. Although we did not identify the specific serotype of this isolate, it is possible that among the 193 currently recognized serotypes of *V. cholerae* (14), they may individually vary regarding the extent of their virulence and pathogenicity. We suggest that while investigators search for new virulence factors generated by *V. cholerae*, serotyping of the isolates involved in serious human infections may indicate that a particular serotype is associated with invasive diseases.

To date, there are no published guidelines for antibiotic therapy of non-O1 *V. cholerae* infections (4, 9). Nonetheless, this isolate was susceptible to trimethoprim-sulfamethoxazole, ampicillin, cefazolin, gentamicin, aztreonam, and ciprofloxacin in vitro, and the patient appeared completely afebrile after 48 h of therapy with intravenous ciprofloxacin.

Both consumption of raw seafood and exposure of open wounds to salt water are known to be common mechanisms of transmission of non-O1 *V. cholerae* in the United States (3, 7, 13). However, this case augments previous findings that clams consumed in the United States may harbor *V. cholerae*, which can cause life-threatening septicemia in individuals with underlying liver disease (5). Therefore, the consumption of raw and undercooked shellfish should be considered potentially hazardous.

REFERENCES


