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NOTES

Identification of *Vibrio hollisae* Associated with Severe Gastroenteritis after Consumption of Raw Oysters

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***Vibrio hollisae* was recovered from the stool culture of a 40-year-old female hospitalized for severe abdominal cramping, vomiting, fever, and watery diarrhea. She had consumed two dozen raw oysters 5 days prior. There was only a single colony on thiosulfate-citrate-bile salts-sucrose-agar, and definitive identification required conventional test media with 1% NaCl.**

Vibrio hollisae (formerly EF-13) is one of several new clinical *Vibrio* species that has been described in the literature in recent years (6, 7). It is not one of the more common vibrios isolated in the clinical laboratory such as *Vibrio parahaemolyticus* or *Vibrio vulnificus*, but when found, *V. hollisae* has generally been isolated from cases of gastroenteritis and even on rare occasions from cases of septicemia (1, 9–11). Perhaps one of the reasons for its infrequent isolation is the fact that it usually does not grow on thiosulfate-citrate-bile-salts-sucrose (TCBS) agar, which many laboratories depend on for detection of clinical vibrios (1, 3, 4). It is also halophilic and relatively biochemically inert, so definitive identification usually warrants conventional tests with NaCl added to a final concentration of 1%.

In this report, we describe a case of severe gastroenteritis in a previously healthy patient that occurred after the consumption of two dozen raw oysters. The definitive identification took several days to complete, and the patient eventually recovered from the infection. This case study is intended to alert both physicians and clinical microbiologists to the possibility of *V. hollisae* as a pathogen and also to offer some guidelines for a timely and accurate identification.

Case report. The patient, a 40-year-old female, was admitted to the hospital complaining of gradually increasing abdominal cramping over the previous 48 h and the onset of significant vomiting and watery diarrhea during the last 6 h. She had purchased and eaten two dozen raw oysters obtained from the lower Chesapeake Bay 5 days prior to admission, but other dietary and recent travel history was unremarkable. Her initial vital signs were normal, except for a blood pressure of 99/43. Her leukocyte count was 12,400, with 79% segmented neutrophils, 19% lymphocytes, and 2% monocytes. She was treated initially with intravenous fluids and antiemetic medication. By the third day of hospitalization, the patient had not improved and was having profuse watery stools as often as every 30 min. She developed a fever on that day to 100.6°F (38.1°C) and a small right pleural effusion. She was started empirically on ciprofloxacin and ampicillin. Blood cultures showed no growth after 5 days, and stool examinations for ova and parasites as well as a *Giardia*-specific antigen test were all negative. Tests for detection of possible viral pathogens were not ordered. Her stool culture subsequently grew *V. hollisae*, and the organism was susceptible to all antimicrobial agents tested. The patient had no recurrence of fever and experienced a slow gradual improvement of all symptoms. She was discharged on the seventh day of hospitalization feeling remarkably better but still having one to two loose stools per day and occasional light abdominal cramps. She took ciprofloxacin for a total of 10 days and last had a brief recurrence of diarrhea 19 days after the initial onset of her symptoms. No follow-up cultures were ordered. Interestingly, the patient had a friend who consumed only half a dozen oysters with her and developed a similar but milder illness and did not seek medical treatment.

Laboratory identification. After receipt in the laboratory, the stool specimen was plated onto a variety of selective and enteric media, including Hektoen-Enteric agar, MacConkey agar, MacConkey agar with sorbitol, Campy-BAP agar, cefsulodin-irgasin-novobiocin, TCBS, Trypticase soy agar (TSA) with 5% sheep blood, and GN broth (all from Becton Dickinson Microbiology Systems, Cockeysville, Md.). The GN broth was subcultured to another Hektoen-Enteric agar plate after 4 h of incubation at 35°C. The Campy-BAP plate was incubated at 42°C in a Campy Pouch (Becton Dickinson Microbiology Systems), the TSA with 5% blood was incubated at 35°C under 5% CO_2, and all other plates were incubated aerobically at 35°C. There were no suspicious colonies after 24 h of incubation, but after 48 h of incubation a single clear green colony was visible on the TCBS plate, and this was subcultured to TSA with 5% sheep blood. It was this laboratory's protocol to flood the original TSA with 5% sheep blood at 48 h with cytochrome oxidase reagent (tetramethylphenylenediamine; Becton Dickinson Microbiology Systems) for the detection of *Aeromonas*, *Vibrio*, or *Plesiomonas* species. This procedure detected a moderate growth of an opaque, oxidase-positive organism that was slightly beta-hemolytic directly beneath the colony. This was subcultured and found to be morphologically similar to the single colony detected on the original TCBS agar plate. Each colony was set up for identification in an API-20E strip (Biomerieux Vitek, Inc., Hazelwood, Mo.). However, the profile number generated in both cases was 0044006, indicating positive reactions only for glucose fermentation, indole production, and arabinose fermentation and a positive oxidase reaction. This profile did not match any organism in the

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API-20E data base. The isolate was also tested for susceptibility to a 150-μg disk of O/129 (Vibriostat; Oxoid, Ogdensberg, N.Y.) as well as for its ability to grow in both Trypticase soy broth (TSB) with no additional salt and TSB with 6.5% NaCl. The organism was unable to grow in plain TSB but grew quite readily in the TSB with 6.5% NaCl; this was our first clue that we were possibly dealing with a halophilic *Vibrio* species, since neither *Aeromonas* nor *Plesiomonas* species will grow in this medium. The O/129 Vibriostat result was puzzling because there appeared to be a partial susceptibility to the O/129 disk in that an incomplete or hazy zone of inhibition was seen around the disk; the test was repeated again, with the same results. However, there have been reports of increasing resistance to O/129 among *Vibrio cholerae* isolates from India, Bangladesh, South-East Asia, and Japan (5). An antimicrobial battery for gram-negative bacilli using the Bauer-Kirby disk diffusion method (2) showed the organism to be susceptible to amikacin, ampicillin, aztreonam, carbenicillin, cefazolin, cefotetan, cefoxitin, ceftazidime, ceftriaxone, cefuroxime, cephalothin, ciprofloxacin, gentamicin, imipenem, mezlocillin, piperacillin, tetracycline, ticarcillin, tobramycin, and trimethoprim-sulfamethoxazole.

After comparing the information in the original paper describing *V. hollisae* (3) and the *Manual of Clinical Microbiology* (7) with our profile of glucose fermentation, positive oxidase activity, indole production, arabinose fermentation, incomplete susceptibility to O/129, and salt requirement for growth, we began to suspect that the organism was *V. hollisae*. After receipt of the preliminary report, the physician told us of the recent ingestion of two dozen raw oysters, which confirmed our suspicions. The isolate was shipped to the State of Maryland Department of Health Services Bacteriology Reference Laboratory in Baltimore, Md., and by using conventional media supplemented to contain 1% NaCl it was identified as *V. hollisae*. Their results below were very close to the original description of the organism first proposed by Hickman et al. (3). The state reference laboratory found our isolate to be beta-hemolytic on blood agar. It grew very poorly if at all on MacConkey agar and TCBS and had a triple-sugar–iron agar reaction of alkaline/acid, with a very small amount of gas production. The only positive reactions were for nitrate reduction, arabinose fermentation, and mannose fermentation (galactose was not tested). The organism did not grow in nutrient broth with 0% additional NaCl. It did grow in concentrations of 3 and 6% NaCl but did not grow in concentrations of 8 and 10% NaCl. Negative reactions were reported for the following tests: citrate, urease, methyl red, Voges-Proskauer, esculin hydrolysis, lysine and ornithine decarboxylase, arginine dihydrolase, gelatin, and fermentation of lactose and sucrose. The only discrepancy was that the state reference laboratory recorded a negative reaction for indole production, whereas the Anne Arundel Medical Center recorded a positive reaction for indole production (as originally described in reference 3). A final footnote to this organism's identification is that it was included in a recent clinical trial of the Crystal Enteric/Nonfermenter ID Kit (Becton Dickinson Microbiology Systems) in which 50 clinical *Vibrio*, *Aeromonas*, and *Plesiomonas* strains from the Anne Arundel Medical Center collection were used. With the Crystal system, this *V. hollisae* isolate, as well as another fecal *V. hollisae* isolate from the collection, were both accurately identified after overnight incubation and did not require any additional testing (unpublished data).

Discussion. Gastrointestinal disease associated with *Vibrio* species is generally associated with consumption of seafood, and the majority of the 30 known cases of *V. hollisae* infection were cases of enteric disease that followed the consumption of raw oysters, clams, or shrimp (1, 3, 4, 6, 8–10). This report indicates the importance of clinical microbiologists being aware of the possibility of *V. hollisae*, not only as an enteric pathogen but also as a possible agent of extraintestinal disease, especially in laboratories serving coastal areas. One important practice in this regard is the routine flooding of TSA with 5% sheep blood agar plates for oxidase-positive colonies, even if a TCBS agar plate may be in the original culture setup. The second important point is the realization that several of the clinically significant *Vibrio* species may require additional NaCl for definitive identification. Finally, keeping an open and ongoing discussion with the physician in terms of patient history and recent culinary habits can often be an invaluable clue to the accurate identification of these organisms and thereby result in the prompt and proper treatment of the patient. It may very well be that the actual prevalence of *V. hollisae* among clinical isolates is much higher than previous reports would suggest. This can only be proven by increased awareness among clinical microbiologists and future published case studies and surveillance reports of the isolation, identification, and epidemiology of this presently infrequently isolated aquatic pathogen.

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