JOURNAL OF CLINICAL MICROBIOLOGY, May 1983, p. 918–920
0095-1137/83/050918-03$02.00/0

Vol. 17, No. 5

NOTES

Isolation of Nontoxigenic *Vibrio cholerae* O1 from a Human Wound Infection

JEFFREY M. JOHNSTON,[1]†* LOUISE M. McFARLAND,[2] HENRY B. BRADFORD,[2] AND CHARLES T. CARAWAY[2]

Field Services Division, Epidemiology Program Office, Centers for Disease Control, Atlanta, Georgia 30333,[1] *and Office of Health Sciences and Environmental Quality, Department of Health and Human Resources, New Orleans, Louisiana 70160*[2]

Received 10 November 1982/Accepted 18 February 1983

Vibrio cholerae serotype O1 organisms that do not produce cholera toxin and, in fact, lack the genetic material encoding the enterotoxin have recently been detected in coastal regions of the United States. Although these organisms have been assumed to be nonpathogenic, they have been considered a potential reservoir of toxigenic *V. cholerae*. In 1979, nontoxigenic *V. cholerae* O1 was isolated from a leg wound of an accident victim residing in New Orleans. The only known risk factors of the patient, besides his debilitated condition, were alcoholism and the consumption of raw oysters before recognition of his wound infection. Coincident with the identification of the isolate from the leg wound, an identical nontoxigenic *V. cholerae* O1 isolate was cultured from the sewage system serving the residence of this patient. Nontoxigenic *V. cholerae* O1 seems to be capable of multiplying in human tissue and may produce extraintestinal infection. This indigenous inhabitant of temperate coastal regions may not be avirulent and may be of public health significance.

During the seventh cholera pandemic, hypotoxigenic variants of the pathogenic El Tor biotype of *Vibrio cholerae* O group I were isolated from marine environments and studied as possible vaccine strains (17). Recently, nontoxigenic or hypotoxigenic (4) isolates of *V. cholerae* O1 have been reported from various parts of the world, including Guam (16), England (2), and Russia (22), and from oysters in Florida (12), bayous in Louisiana, and the Chesapeake Bay (7). The isolation of such an organism from humans is exceedingly rare and, in fact, has been documented only once (6). In this paper we describe a wound infection from which nontoxigenic *V. cholerae* O1 was isolated and the detection in a sewage system of an identical organism which we believe came from the patient.

Case report. In June 1978, a 45-year-old male was struck by a car and sustained multiple fractures and lacerations. During his hospitalization, the patient underwent several surgical procedures, including split-thickness skin grafting of bilateral tibial wounds. After each procedure, the left leg was placed in a long leg cast and the right leg was placed in a short leg cast; intermittent heavy drainage was noted beneath both casts. The patient was treated with cephalosporin antibiotics through mid-July, as well as with Betadine soaks throughout his hospital course. A culture obtained from the left tibial wound after cessation of antibiotic treatment grew *Enterococcus* sp. and *Pseudomonas stutzeri*; nonetheless, he was afebrile during his entire 109-day hospital stay.

The past medical history of the patient was remarkable only for gout requiring Indocin. He had no history of peptic disease, gastric or intestinal surgery, or liver disease, although he did drink heavily. He did not take antacids or iron medications.

The patient was in a nursing home from September 1978 until March 1979, when he returned to a Jefferson Parish rehabilitation center where he had lived and worked before his accident. On 27 March 1979, the left leg cast was removed and a dressing was applied. The wound appeared to be well healed, with adequate skin coverage and no drainage. A radiograph taken at that time raised the suspicion of osteomyelitis of the comminuted fragment and proximal tibia beneath the wound. On 10 April 1979, the patient was seen in

† Present address: Department of Medicine, University of Utah Medical Center, Salt Lake City, UT 84132.

a clinic complaining of pain at this wound site. An examination revealed a large shallow-cratered ulcer on the distal portion of the left leg which was soaked and redressed. When next seen on 1 May 1979, this ulcer was larger, with necrotic material in its crater and a surrounding edge of erythema and edema. A culture was obtained, but the patient left before the wound could be debrided. On 16 May 1979, a *Vibrio* species isolated from this culture was confirmed by the Louisiana Division of Laboratories as *V. cholerae* O1, serotype Inaba. This isolate was resistant to colistimethate but sensitive to all of nine other antibiotics tested. *Proteus mirabilis, Escherichia coli*, and *Pseudomonas aeruginosa* were also isolated from the wound culture. *V. cholerae* O1 was again isolated from a culture obtained from the wound on 17 May 1979. At that time the ulcer was erythematous and necrotic, with copious foul-smelling exudate. On 20 May 1979, the man moved to another state, where he died in August of unrelated causes. Unfortunately, no medical follow-up is available subsequent to his departure from Louisiana.

Epidemiological investigation. The patient was interviewed by one of us (L.M.M.) on 17 May 1979. He had moved in 1977 from New York to Louisiana, where he worked intermittently at the rehabilitation center. There was no history of diarrheal illness, recreational or occupational exposure to seawater, or travel outside the United States. He had eaten raw oysters during the last week of February 1979, but after that his only seafood consumption had consisted of fried fish. After the development of an ulcer at the wound site in early April, a visiting nurse taught the patient to debride his wound several times a day into a basin. The soiled bandages and necrotic tissue were subsequently flushed down his toilet. During the interview, a culture was taken of the leg ulcer, and stool and blood specimens were collected.

Since the occurrence of a cluster of *V. cholerae* O1 infections in Louisiana in the fall of 1978, routine sewage surveillance for *Vibrio* organisms has been maintained throughout the southern part of the state. On 13 May 1979, *V. cholerae* O1 was identified for the first time in Jefferson Parish from one of the nine sewage treatment plants by using the Moore swab technique (1). This particular plant handled 4.8 million gallons of sewage per day and served a population of 63,400 residents, including the rehabilitation facility of the patient, located 3.5 miles away. Attempts to isolate the organism from the sewage lines between the rehabilitation center and the treatment plant were unsuccessful; however, it was later learned that the patient had moved before these attempts were made.

Laboratory investigation. The two isolates (human and sewage) were cultured by methods previously described (5). Both satisfied the minimal criteria required for the identification of *V. cholerae* proposed by Hugh and Sakazaki (13). Each isolate agglutinated in Smith polyvalent O1 and Inaba monovalent *V. cholerae* antisera (18). They were of the El Tor biotype and were strongly hemolytic by a tube dilution method (11), similar to previous toxigenic strains from Louisiana and Texas (5). Both were resistant to polymixin B, agglutinated chicken erythrocytes, and had positive Voges-Proskauer reactions. Both were sensitive to classical phages III and IV and resistant to all five El Tor phage types as well as eight experimental phages (3). Both isolates were nontoxigenic by the Y-1 adrenal cell assay (9) and the enzyme-linked immunosorbent assay (20) for heat-labile toxin and by the infant mouse assay for heat-stable toxin (8). Using recombinant DNA techniques, Kaper et al. demonstrated that neither isolate possessed genes encoding cholera toxin (14).

The stool culture of the patient did not yield *V. cholerae*. His serum did not have detectable vibriocidal antibodies as measured by the Benenson microtiter technique (10), antitoxic antibodies as measured by a newly developed microtiter enzyme-linked immunosorbent assay (21), or agglutinating antibodies to the isolate from his ulcer.

Discussion. Since the discovery of a possible endemic focus of cholera in the United States in 1978 (5), interest in and investigation of *V. cholerae* O1 in the environment in the United States have increased dramatically and the isolation of nontoxigenic strains of this organism throughout the country has steadily increased. Recent reports indicate that 2% of the isolates of *V. cholerae* cultured from oysters harvested from the eastern and Gulf coasts of the United States are nontoxigenic O1 organisms (19). Since 1978, 12 such nontoxigenic *V. cholerae* O1 strains have been isolated from environmental sources in Louisiana, most commonly in pollution-free waters of low, but detectable, salinity during warm months (7). All have been strongly hemolytic and of the El Tor biotype. There has been one Ogawa serotype; the remainder have been Inaba. DNA homology studies by Kaper et al. have demonstrated that all of 28 nontoxigenic U.S. isolates tested (including several from Louisiana) lack the structural genes for cholera toxin production (14).

Although he had no exposure to salt water, our patient clearly remembered eating one dozen raw oysters approximately 1 month before his cast was removed. It is conceivable that the organism was introduced into the gastrointestinal tract of the patient via contaminated oysters, multiplied in his bowel, and contaminated his

cast by fecal soilage. Other enteric organisms were also isolated from the wound. *V. cholerae* O1 could then have continued to grow in the necrotic tibial area of a previous trauma; creation of a more aerobic environment after the cast was removed may have enhanced *Vibrio* multiplication. The isolation of nontoxigenic *V. cholerae* O1 of the same phage type from the wound on two occasions 16 days apart and from sewage strongly suggests that the organism multiplied in the wound. Since the organism induced no detectable serological response and other potential wound pathogens were also present, pathogenetic causality cannot be established.

That nontoxigenic *V. cholerae* O1 organisms may be capable of causing human illness is an important issue, given the existence of such organisms in the environment and the potential for human exposure to them. A survey conducted in southern Louisiana in 1975 suggested that 9% of the population regularly eat raw oysters (15). Further epidemiological study of these organisms and their association with humans, as well as laboratory analysis of mechanisms of pathogenicity and molecular relatedness, is necessary. Since the occurrence of cholera in Louisiana in 1978, microbiology laboratories along the Gulf Coast have specifically looked for *Vibrio* species by using special culture techniques; otherwise, isolates such as that described in this report may not be detected. Thus, clinicians should consider *Vibrio* infection in patients with wounds or gastroenteritis and recent exposure to seafood or seawater, and they should alert the microbiology laboratory so that a selective culture medium (thiosulfate-citrate-bile salts-sucrose agar) will be used in addition to routine enteric media. Epidemiologists and other disease control practitioners should recognize the potential significance of these nontoxigenic *V. cholerae* O1 organisms.

We gratefully acknowledge the patient's physician, Ben Ferguson, and the microbiology technician, Anita Pence, of the Veterans Administration Medical Center, New Orleans, La., for initially identifying and informing us of the wound isolate of *V. cholerae*; Timothy Barrett of the Epidemiologic Investigations Laboratory Branch, Centers for Disease Control, for performing serological and toxigenicity tests; and John Lee for phage typing the organisms.

LITERATURE CITED

1. **Barrett, T. J., P. A. Blake, G. K. Morris, N. D. Puhr, H. B. Bradford, and J. G. Wells.** 1980. Use of Moore swabs for isolating *Vibrio cholerae* from sewage. J. Clin. Microbiol. **11**:385–388.
2. **Bashford, D. J., T. J. Donovan, A. L. Furniss, and J. V. Lee.** 1979. *Vibrio cholerae* in Kent. Lancet **i**:436–437.
3. **Basu, S., and S. Mukerjee.** 1968. Bacteriophage typing of *Vibrio el tor*. Experientia **25**:299–300.
4. **Blackman, U., S. Basu, and M. J. Pickett.** 1970. *Vibrio cholerae*: production of toxin by a nonpathogenic strain. J. Infect. Dis. **122**:540–543.
5. **Blake, P. A., D. T. Allegra, J. D. Snyder, T. J. Barrett, L. McFarland, C. T. Caraway, J. C. Feeley, J. P. Craig, J. V. Lee, N. D. Puhr, and R. A. Feldman.** 1980. Cholera—a possible endemic focus in the United States. N. Engl. J. Med. **302**:305–309.
6. **Centers for Disease Control.** 1977. *Vibrio cholerae* Alabama. Morbid. Mortal. Weekly Rep. **26**:159–160.
7. **Colwell, R. R., R. J. Seidler, J. Kaper, S. W. Joseph, S. Garges, H. Lockman, D. Maneval, H. Bradford, N. Roberts, E. Remmers, I. Huq, and A. Huq.** 1981. Occurrence of *Vibrio cholerae* serotype O1 in Maryland and Louisiana estuaries. Appl. Environ. Microbiol. **41**:555–558.
8. **Dean, A. G., Y. C. Ching, R. G. Williams, and L. B. Harden.** 1972. Test for *E. coli* enterotoxin using infant mice: application in a study of diarrhea in children in Honolulu. J. Infect. Dis. **125**:407–411.
9. **Donata, S. T., M. King, and K. Sloper.** 1973. Induction of steroidogenesis in tissue culture by cholera enterotoxin. Nature (London) New Biol. **243**:246–247.
10. **Feeley, J. C., and W. E. DeWitt.** 1976. Immune response to *Vibrio cholerae*, p. 289–295. *In* N. R. Rose and H. Friedman (ed.), Manual of clinical immunology. American Society for Microbiology, Washington, D.C.
11. **Feeley, J. C., and M. Pittman.** 1963. Studies on the haemolytic activity of El Tor vibrios. Bull. W.H.O. **28**:347–356.
12. **Hood, M. A., G. E. Ness, and G. E. Rodrick.** 1981. Isolation of *Vibrio cholerae* serotype O1 from the eastern oyster, *Crassostrea virginica*. Appl. Environ. Microbiol. **41**:559–560.
13. **Hugh, R., and R. Sakazaki.** 1972. Minimal number of characters for the identification of *Vibrio* species, *Vibrio cholerae*, and *Vibrio parahaemolyticus*. J. Conf. Public Health Lab. Dir. **30**:133–137.
14. **Kaper, J. B., S. L. Moseley, and S. Falkow.** 1981. Molecular characterization of environmental and nontoxigenic strains of *Vibrio cholerae*. Infect. Immun. **32**:661–667.
15. **Mackowiak, P. A., C. T. Caraway, and B. L. Portnoy.** 1976. Oyster-associated hepatitis: lessons from the Louisiana experience. Am. J. Epidemiol. **103**:181–191.
16. **Merson, M. H., W. T. Martin, J. P. Craig, G. K. Morris, P. A. Blake, G. E. Craun, J. C. Feeley, J. C. Camacho, and E. J. Gangarosa.** 1977. Cholera on Guam, 1974: epidemiologic findings and isolation of non-toxigenic strains. Am. J. Epidemiol. **105**:349–361.
17. **Mukerjee, S.** 1963. Preliminary studies on the development of a live oral vaccine for anti-cholera immunization. Bull. W.H.O. **29**:753–766.
18. **Smith, H. L., and K. Goodner.** 1965. On the classification of vibrios, p. 4–8. *In* Proceedings of Cholera Research Symposium, Honolulu, 1965. Public Health Service publication 1328. Government Printing Office, Washington, D.C.
19. **Twedt, R. M., J. M. Madden, J. M. Hunt, D. W. Francis, J. T. Peeler, A. P. Duran, W. O. Hebert, S. G. McCay, C. N. Roderick, G. T. Spite, and T. J. Wazenski.** 1981. Characterization of *Vibrio cholerae* isolated from oysters. Appl. Environ. Microbiol. **41**:475–478.
20. **Yolken, R. H., H. B. Greenberg, M. H. Merson, R. B. Sack, and A. Z. Kapikian.** 1977. Enzyme-linked immunosorbent assay for detection of *Escherichia coli* heat-labile enterotoxin. J. Clin. Microbiol. **6**:439–444.
21. **Young, C. R., M. M. Levine, J. P. Craig, and R. Robins-Browne.** 1980. Microtiter enzyme-linked immunosorbent assay for immunoglobulin G cholera antitoxin in humans: method and correlation with rabbit skin vascular permeability factor technique. Infect. Immun. **27**:492–496.
22. **Zykin, L. F., V. I. Svyatoy, and R. S. Zotova.** 1978. A study of enteropathogenicity of hemolysing El Tor vibrios. Zh. Mikrobiol. Epidemiol. Immunobiol. **2**:120.