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The Brief Case

(For answers to the self-assessment questions and take-home points, see page 1672 in this issue [doi:10.1128/JCM.02661-15].)

Safe To Go Back in the Water? *Vibrio parahaemolyticus* Wound Infection Associated with Brackish Water

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CASE

A previously healthy 12-year-old female presented 1 day after sustaining a laceration of her left lower leg on an unidentified submerged object when she jumped off a dock into a brackish river in Cape Cod, MA. She had initially been evaluated at an urgent care clinic on the day of injury, where the wound was irrigated and sutured. On the following day, she developed worsening pain, erythema, and edema of the leg, as well as fever, and presented to the emergency department, where her vital signs were notable for a temperature of 38.5°C, a heart rate of 112 beats/min, and a blood pressure of 80/32 mm Hg. Her physical examination was remarkable for an intensely painful 1.5-by-2-cm laceration on the left anterior lower leg, which was productive of serosanguinous drainage and was surrounded by a 6-cm region of erythema. Laboratory results were notable for an elevated white blood cell count of $24.7 \times 10^3/\mu l$ with 87% neutrophils. She was administered ceftriaxone, doxycycline, and levofloxacin and admitted to the intensive care unit, where she briefly required hemodynamic support with a dopamine infusion.

On the second hospital day, the patient underwent incision and drainage of the wound. Aerobic, anaerobic, and mycobacterial tissue cultures were sent. A Gram stain of the original specimen showed no polymorphonuclear cells and no organisms. At 24 h, the aerobic culture plates showed growth of oxidase-positive, straight, Gram-negative rods. The organism grew slowly on MacConkey agar, producing small colonies at 48 h, without production of acid. Two slightly different colony morphologies were both identified as *Vibrio parahaemolyticus* by the Vitek 2 automated microbial identification system (bioMérieux, Durham, NC) with percent probabilities of 97% (excellent identification) and 95% (very good identification). These were also identified as *V. parahaemolyticus* by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry with scores of 2.47 and 2.27 (MALDI Biotyper [Bruker Daltonics, Billerica, MA] using the MALDI Biotyper reference library). Susceptibility testing was performed with a microdilution panel (MicroScan Neg MIC 38; Beckman Coulter, Brea, CA) and revealed the organism to be susceptible to all of the antibiotics tested (Table 1).

On the basis of the identification and susceptibility testing of the organism, the patient's antibiotic regimen was changed to ceftazidime and doxycycline. She improved clinically and was discharged on hospital day 9 on oral ciprofloxacin and doxycycline to complete a 14-day course of antibiotics. By 2 weeks after hospital discharge, she had fully recovered and the sutures were removed from her wound, which had healed well.

TABLE 1 *V. parahaemolyticus* antimicrobial susceptibility test results

Drug(s)	MIC(s) (μg/ml)	Interpretation
Amikacin	≤4	S[a]
Cefepime	≤2	S
Ceftazidime	≤1	S
Ciprofloxacin	≤0.5	S
Gentamicin	≤1	S
Imipenem	≤1	S
Meropenem	≤1	S
Piperacillin-tazobactam	≤8	S
Trimethoprim-sulfamethoxazole	≤2/38	S

[a] S, susceptible. The interpretive breakpoints used are those issued by the Clinical and Laboratory Standards Institute (1).

DISCUSSION

Pathogens commonly recovered from soft tissue infections acquired in a setting of salt or brackish water exposure include *Vibrio* species (especially *Vibrio vulnificus* and *V. parahaemolyticus*), *Edwardsiella tarda*, *Aeromonas hydrophila*, *Chromobacterium violaceum*, *Shewanella* species, *Streptococcus iniae*, *Erysipelothrix rhusiopathiae*, and *Mycobacterium marinum* (2). In addition to skin and soft tissue infections, many of these organisms can also cause gastroenteritis, bacteremia, and sepsis; systemic manifestations are more commonly encountered in immunocompromised individuals. With the exception of *S. iniae* (a Gram-positive coccus), *E. rhusiopathiae* (a Gram-positive rod), and *M. marinum* (an acid-fast bacillus), these pathogens are Gram-negative rods. Many *Vibrio* species have a curved or comma-shaped appearance on Gram staining.

The genus *Vibrio* includes 10 species that are known to cause disease in humans. *Vibrio* species are oxidase and catalase positive and reduce nitrate to nitrite, with the exception of *Vibrio metschnikovii*, which is oxidase and nitrate negative. They are halophilic, requiring NaCl for growth. Most *Vibrio* species can grow

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on standard media, including blood and MacConkey agars. They are usually non-lactose fermenters, with the exception of *V. vulnificus*, which ferments lactose in 85% of cases (3). The use of selective and differential media can enhance the detection of *Vibrio* species and aid in distinguishing among them. Thiosulfate citrate bile salts sucrose medium selects for the growth of *Vibrio* species because of its high salt content and alkaline pH, while the presence of thymol and bromothymol blue allows differentiation between colonies of sucrose-fermenting species such as *V. cholerae*, which appear yellow, and non-sucrose-fermenting species such as *V. parahaemolyticus*, which are green or blue (4). Chromogenic CHROMagar Vibrio agar (CHROMagar Microbiology, Paris, France) is particularly useful for distinguishing *V. parahaemolyticus*, which appears as mauve colonies, from other species of *Vibrio*, which appear as blue or colorless colonies (4). In the case presented here, the organism was successfully identified by a commercial automated system, but automated detection systems can be inaccurate in the identification of *Vibrio* species (3). PCR-based methods can detect *Vibrio* species and are commonly employed in research settings but are not typically used in the clinical laboratory (3, 4). Early evaluations of MALDI-TOF with *Vibrio* species indicate that, with the use of appropriate databases, this method is highly accurate in the identification of these organisms (3).

Of the *Vibrio* species, *V. cholerae* is perhaps the best known because of its propensity to cause epidemics of severe gastroenteritis associated with large-volume diarrhea, dehydration, and high mortality rates, especially in settings of poverty and overcrowding, where it is often spread by the fecal-oral route in contaminated drinking water (3). In contrast, *V. vulnificus* typically causes septicemia or necrotizing skin and soft tissue infections (2). *V. parahaemolyticus* can cause gastroenteritis, as well as skin and soft tissue infections and septicemia. Patients who have underlying liver disease are at particular risk of severe systemic infections and death (5).

V. parahaemolyticus thrives in warm, saline environments and is frequently recovered from saltwater and brackish water and from shellfish that live in these waters. In contrast to most strains in environmental samples, isolates of *V. parahaemolyticus* that are recovered from patients with clinical infections almost always produce the thermostable direct hemolysin (TDH) toxin. The presence of TDH toxin can be detected by the ability to lyse red blood cells on Wagatsuma agar, known as the Kanagawa reaction (4). In temperate climates, the majority of human infections with *V. parahaemolyticus* occur during warmer months (5). The incidence of human infection caused by *V. parahaemolyticus* has been increasing in the United States over the past 2 decades, and while the causes of this increase are not entirely clear, rising water temperatures are thought to play a role (6).

The mechanism and location of injury of the patient presented in this case initially raised concern for a wound infection with a waterborne pathogen, and the rapid progression of a local infection with development of systemic illness was characteristic of infection with *V. parahaemolyticus* or *V. vulnificus*. The absence of lactose fermentation on MacConkey agar suggested that *V. parahaemolyticus* was the more likely pathogen, as only 1% of such isolates ferment lactose, in contrast to 85% of *V. vulnificus* isolates (3). The organism's identity was confirmed by automated biochemical assays and mass spectrometry.

Data regarding optimal antimicrobial therapy regimens for *V. parahaemolyticus* are limited, but there is some literature on the treatment of *V. vulnificus*. Most cases of gastroenteritis require only supportive therapy, while mild wound infections are generally treated with an oral tetracycline or a fluoroquinolone antibiotic. Severe wound infections and septicemia are indications for aggressive antimicrobial therapy in combination with supportive care and, in the case of wound infections, surgical intervention. Although many *Vibrio* isolates are susceptible *in vitro* to a wide range of antimicrobials, as was the case with the patient described here, it appears that for more severe infections, the combination of an expanded-spectrum cephalosporin with either a tetracycline or a fluoroquinolone may be more effective than monotherapy with a cephalosporin (7).

SELF-ASSESSMENT QUESTIONS

1. Which of the following biochemical patterns is characteristic of *V. parahaemolyticus*?
   (a) Non-lactose fermenting, sucrose fermenting, oxidase positive.
   (b) Non-lactose fermenting, non-sucrose fermenting, oxidase positive.
   (c) Lactose fermenting, non-sucrose fermenting, oxidase positive.
   (d) Lactose fermenting, sucrose fermenting, oxidase negative.
2. A wound sustained in which of the following settings would be most likely to become infected with *V. parahaemolyticus*?
   (a) A freshwater pond in summer.
   (b) A brackish stream in late autumn.
   (c) A hot tub in winter.
   (d) An ocean beach in late spring.
3. Which of the following antibiotics is frequently a component of antimicrobial therapy for *Vibrio* infections?
   (a) Erythromycin.
   (b) Ciprofloxacin.
   (c) Penicillin.
   (d) Vancomycin.

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