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Original Article

Two Cases of Sucrose-Fermenting *Vibrio vulnificus* Infection in Which 16S rDNA Sequencing Was Useful for Diagnosis

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SUMMARY: *Vibrio vulnificus* is a Gram-negative bacterium which is associated with severe infections in humans. We experienced two cases of sucrose-fermenting *V. vulnificus* infection. The causative agents in both cases were unidentifiable by conventional identification systems because of their unique characteristics, and sequencing of 16S rDNA was found to be useful for diagnosis.

INTRODUCTION

Vibrio vulnificus is a Gram-negative bacillus that is associated with gastroenteritis, septicemia and wound infections in humans (1,2). In many cases, patients are infected by eating raw marine products or going sea-bathing (3,4). Persons who are immunocompromised or have chronic liver diseases are especially at risk for infection and unfavorable outcomes, so quick diagnosis and proper treatment are required (5). In clinical practice, culture on thiosulfate citrate bile sucrose agar (TCBS agar), which is an isolation medium for *Vibrio* spp., produces medium-sized, opaque, green colonie, and the organism can usually be identified by an automated identification system using its biochemical characteristics (6). Bacterial identification is usually carried out with conventional test equipment through the additional examination of bacteria's utilization rates of sugars, amino acids, or the like, while it is generally accepted that *V. vulnificus* does not usually utilize sucrose (6). However, the utilization rates of sugars and amino acids are different within a biogroup and, furthermore, vary between strains even in the same group. We report here two cases of *V. vulnificus* infection in which the special nature of the causative bacterium prevented its identification with ordinary test equipment, and in which 16Sr DNA sequencing was useful in making the final diagnosis.

PATIENTS AND LABORATORY INFORMATION

Case 1: A 68-year-old man was admitted to our hospital complaining of symptoms of fever, diarrhea and confusion. He also complained of general fatigue and of swelling and pain in the right knee joint on admission. He had a history of chronic liver cirrhosis and hepatic cancer caused by hepatitis C virus infection. Physical examination revealed no apparent abnormalities except for the swollen right knee. He reported that he had a swim in the sea the day before admission. He was diagnosed with septicemia and hepatic encephalopathy, the most likely focus of which was inflammation of the knee joint. Empirical antibiotic therapy with intravenous cefotiam (1 g) every 12 h began shortly, and relaxation incision of the right knee was performed. However, his general status and laboratory data worsened and, on the third day, we changed the antibiotic to intravenous ceftazidime (1 g) every 12 h. On the same day, the blood culture obtained during the examination grew Gram-negative bacilli. The causative organism was suspected to be *V. vulnificus*; however, VITEK2 (bioMérieux, Marcy l'Etoile, France) identified the organism as *V. vulnificus* with a probability of 24% and *Aeromonas* sp. with a probability of 20%. As the case was suspected to be *V. vulnificus* infection, oral minocycline (100 mg) was given to the patient every 12 h. The fever resolved on the 4th day. Oral minocycline was continued from day 8 to day 13. Although the patient's general condition appeared to be gradually improving without antibiotic therapy, the onset of fever, hypotension and hypoglycemia suddenly occurred on day 20. Eventually, the patient died from suspected exacerbation of the infection, followed by liver kidney syndromes 14 h later. In concurrence with the treatment, the doctor in charge requested our laboratory to collect further microbiological information and to help to make the diagnosis. We processed the bacterium on TCBS agar, and the color of the colonies formed on the agar turned out to be yellow. The results of the biochemical assay we prosecuted are shown in Table 1. The biochemical assays were performed as previously described (7,9). They showed the fermentation of sucrose but not lactose, and, moreover, they showed that there was no ability to utilize D-mannitol or D-sorbitol. Considering the underlying disease of the patient and his clinical features, *V. vulnificus* was suspected as the cause of the infection. Finally we performed a 16S rDNA analysis for the sake of identification in order to complete the diagnosis. The 16S rDNA gene was amplified by PCR with forward primer 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and reverse primer 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) and sequenced. The 16S rDNA analysis of the strain showed sequence homology of 99.8% with the reference strain of *V.*

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Table 1. Profile of the causative strains

	Case 1	Case 2
Motility	+	+
Indole	+	+
L-Arabinose	–	–
Celloboise	+	+
D-Galactosidase	+	+
Lactose	–	–
Maltose	+	+
D-Mannitol	–	–
D-Mannose	+	+
Sucrose	+	+
D-Sorbitol	–	+
D-Xylose	–	–
Growth in 1% peptone		
Without NaCl	–	–
With 1% NaCl	+	+
With 3% NaCl	+	+
With 6% NaCl	–	–

Both strains fermented sucrose and were negative for D-mannitol.
The Case 2 strain was D-sorbitol-positive.

vulnificus. Unfortunately, the patient died from suspected exacerbation of infection, followed by liver kidney syndromes 14 h later on day 21.

Case 2: A 55-year-old man with a history of liver cirrhosis and hepatic cancer saw a local internist complaining of diarrhea and pains in the left foot. He reported that he had eaten an abalone the day before. On the following day, he became hypotensive, went into shock, and was transferred to a regional emergency hospital. When admitted to the hospital, he had purpura on the anterior surfaces of the distal portion of the left leg and the right ankle. An empiric therapy with intravenous injections of meropenem trihydrate (1 g) every 12 h and aggressive resuscitation were started immediately after he was diagnosed with septicemia, disseminated intravascular coagulation and necrotizing fasciitis. Intravenous meropenem injections were discontinued on day 8, while cefepime dihydrochloride injections (2 g) were stated and administered every 12 h until they were finally discontinued on day 15. Debridement of the lower legs was carried out six times in the ensuing months until the patient finally received a skin graft. Infective lesions of the lower legs were sterilized and treated with gentamicin sulfate to prevent secondary infection. Culture of the fluid obtained from a bullous edema obtained in the first debridement grew Gram-negative bacilli. Considering the clinical course of the disease and the result of the Gram stain, the organism was suspected to be *V. vulnificus*, but we could not confirm the diagnosis by VITEK 2. As the doctor in charge asked our laboratory to make the diagnosis, we made a further microbiological evaluation. The bacterium processed on TCBS agar formed yellow colonies. The results of biochemical assay we perfomed are shown in Table 1. The bacteria metabolized sucrose and had no ability to utilize lactose, D-mannitol or D-sorbitol. Because the characteristics were not typical of *V. vulnificus*, sequence analysis of 16Sr DNA was performed by the same method used in Case 1. The result showed that 98.8% of the sequence was identical with the reference strain for *V. vulnificus*, and based on this we finally diagnosed the case as *V. vulnificus* infection.

DISCUSSION

V. vulnificus is an extremely invasive and destructive natural marine Gram-negative bacillus that was previously described as "lactose-positive *Vibrio*," as it metabolizes lactose. This characteristic distinguishes *V. vulnificus* from other vibrios (6). The main clinical symptoms of *V. vulnificus* infection are primary septicemia and soft tissue infection (8). The mortality rate of the infection is extremely high, especially in patients with liver disease, even if appropriate antibiotic therapy is provided (5). In clinical practice, this organism can be identified by commercial identification systems using the biochemical characteristics of each species. We could not, however, make a definite diagnosis of the strains we encountered with a conventional identification examination in clinical settings. Additionally, we carried out the examination of the biochemical characteristics, and, ultimately, identified the strains by 16S rDNA sequencing.

According to the previous report, 3 - 15% of *V. vulnificus* strains are positive for sucrose fermentation (9). Other biochemical test results and properties have been reported to vary among strains (10). Because of such variations, strains with atypical characteristics are difficult to identify or can be misidentified by commercial phenotype-based identification systems. These facts suggest that careful interpretation of the results of automated identification systems is required in clinical practice. The strains we encountered have unique propertie, and such strains may already be disseminating in Japan, while the epidemic may be underestimated because of the insufficient ability of conventional identification systems to identify the strains.

Although these cases involved infections caused by *V. vulnificus* strains that had peculiar characteristics, the patients had very severe clinical courses as in a typical *V. vulnificus* infection. Because the virulence of these cases was as high as that of past cases, early diagnosis was crucial. Clinicians need to be well aware that some cases of *V. vulnificus* infection are difficult to diagnose, as in our cases. In addition to our cases, there may have been cases that had unfortunate consequences due to the lack of a proper diagnosis. Similar cases may arise. Early diagnosis and proper treatment are essential to obtain good clinical outcomes (11); thus, we have to accumulate cases, define their characteristics and improve the system by which early diagnoses are made.

We believe that this is the first report of sucrose-fermenting *V. vulnificus* infections in which 16S r DNA sequencing was essential for making the final diagnosis.

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