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SHORT COMMUNICATION

Novel *Vibrio vulnificus* sequence type 540 infection in a hepatitis patient

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ABSTRACT

Vibrio vulnificus infection is associated with high morbidity and mortality in high-risk patients. Poor prognoses could lead to >50% mortality rate. The present report describes a case of *V. vulnificus* bacteremia in a cirrhotic patient with underlying hepatitis C. He presented with generalised abdominal pain associated with distention and could not ambulate for one week. He also complained of fever for six days and pruritus for 10 days. Tea-coloured urine was noted in continuous bag drainage. The abdomen was distended but soft, with mild tenderness palpated over the left lumbar and iliac region. Blood investigation indicated ongoing infection and inflammation. The aerobic blood culture was identified using the matrix-assisted laser desorption/ionisation-time of flight mass spectrometry and confirmed via 16S rDNA sequencing as *V. vulnificus*. Multilocus sequence typing of the isolated *V. vulnificus* revealed a novel sequence type, ST540. The patient responded well to the intravenous cefoperazone and was then discharged with a four day-course of oral ciprofloxacin, 500 mg twice daily after completing the intravenous cefoperazone for 10 days. Clinical history and physical examination are important for early antibiotic therapy initiation and appropriate surgical intervention. Furthermore, bacterial strain typing is also essential for epidemiological surveillance and potentially anticipating the pathogen's virulence traits, which are vital in controlling and preventing the spread of infection.

Keywords: Infectious disease; Malaysia; MALDI-TOF; multilocus sequence typing; 16S rDNA.

INTRODUCTION

Vibrio vulnificus is a motile, halophilic, gram-negative bacterium belonging to the *Vibrionaceae* family. Other members of the family include; *Vibrio cholerae* and *Vibrio parahaemolyticus*, and they are common causes of acute gastrointestinal symptoms (Jones & Oliver, 2009). However, unlike other species in this family, *V. vulnificus* can cause severe to life-threatening infections in high-risk individuals characterized by three distinct syndromes; acute gastroenteritis, skin and soft tissue infections that may progress to necrotising fasciitis and death (McLaughlin *et al*., 2005; Bross *et al*., 2007). Poor prognosis with a mortality rate of more than 50% has been reported in primary sepsis. Patients usually acquire the infection through the consumption of contaminated or undercooked seafood (e.g. shellfish such as oysters) or, the exposure of open wounds or skin breaks to contaminated sea or brackish water (Klontz *et al*., 1988; Dechet *et al*., 2008; Jones & Oliver, 2009). The infection commonly occurs in immunocompromised patients with underlying chronic illnesses, particularly liver disease or haemochromatosis (Bross *et al*., 2007). The present study describes a case of *V. vulnificus* bacteremia in a cirrhotic patient with underlying hepatitis C infection.

CASE REPORT

A 46-year-old gentleman, who was a chronic alcoholic and claimed to have no known medical illness previously, presented with generalised abdominal pain associated with distention and could not ambulate for one week. He also complained of fever for six days (temperature documented at home) and pruritus for 10 days. Besides, he also experienced loose stool two times per day that lasted for three days prior to admission. Otherwise, there was no bleeding tendencies/ vomiting/shortness of breath or palpitations, urinary tract infection or upper respiratory tract infection symptoms. He denied any history of consumption of contaminated or undercooked seafood or recent travelling. Besides, he claimed no history of swimming or contact with contaminated sea or brackish water. On examination, the patient was mildly febrile (37.6°C), jaundiced, and pale-looking but had adequate hydration. Vital signs showed he was tachycardic (112 bpm), normotensive (112/79 mmHg) and pulse oximetry was 100% under room air. Tea-coloured urine was noted in continuous bag drainage, but no spider naevi or palmar erythema was noted. The abdomen was distended but soft, with mild tenderness palpated over the left lumbar and iliac region. Pedal oedema was felt up to mid-shin. No bleeding or mass was noted rectally.

Initial laboratory values showed a white blood cell count of 29,000 cells/mm^3 with a differential of 87.3%, 7.6% lymphocytes, and 4% monocytes with the C-reactive protein value of 28.3 mg/L, suggesting the presence of an ongoing infection and inflammation. The liver function test revealed hyperbilirubinemia (total bilirubin of 112.5 µmol/L) with predominantly direct hyperbilirubinemia (107 µmol/L), hypoalbuminaemia (19 g/L), transaminitis with serum aspartate transaminase level of 223 (U/L), and alanine transaminase of 53 (U/L). Serum lactate dehydrogenase was raised with a value of 374. For the coagulation profile, the international normalised ratio (INR) was prolonged (2.97) with a PT/APTT ratio of 34.6/1.35. Screening for other viral infections sent on admission revealed that the patient was positive for hepatitis C, whereas hepatitis B surface antigen (HBsAg) and HIV antigen/antibody combo tests were non-reactive. The patient was started empirically on intravenous cefoperazone (1 g twice daily) and metronidazole (500 mg once daily) for sepsis secondary to spontaneous bacterial peritonitis.

The blood culture sent on admission was positive after 48 hours of incubation, resulting in gram-negative bacilli from the aerobic bottle. Flat, spreading, greyish colonies were observed on blood and chocolate agar, whereas non-lactose fermenting colonies were observed on MacConkey agar. The isolate was susceptible to cefotaxime, ceftazidime and ciprofloxacin via the disk diffusion method, and it was identified as *V. vulnificus* using matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) mass spectrometry (Chua *et al*., 2021) (Score value: 2.39). 16S rDNA sequencing (Loong *et al*., 2020) confirmed the bacteria isolate as *V. vulnificus* (Accession no. OQ815973). Multi-locus sequence typing (MLST) performed on the isolate according to the *V. vulnificus* MLST scheme (Bisharat *et al*., 2007) revealed a novel sequence type 540 (ST540). Novel alleles were found for three loci; *dtdS* (allele 185), *lysA* (allele 179) and *pyrC* (allele 134), in addition to the common alleles for the remaining seven loci; *glp* (allele 16), *gyrB* (allele 1), *mdh* (allele 8), *metG* (allele 25), *purM* (allele 64), *pntA* (allele 44) and *tnaA* (allele 7). The constructed minimum spanning tree based on genetically related *V. vulnificus* MLST profiles suggests that the patient in this study could possibly have acquired the *V. vulnificus* infection from shellfish, fish, eel or the environment (Figure 1).

The blood culture results were informed to the ward's managing team, and the patient responded well to the prescribed antibiotics. Ultrasonography of the hepatobiliary system showed liver cirrhosis with gross ascites, and focal echogenicity in liver segment VIII/V indicated calcification or choledocholithiasis. The diagnosis was revised to sepsis secondary to acute gastroenteritis and liver cirrhosis secondary to underlying hepatitis C and alcoholic liver disease with portal hypertension. However, underlying spontaneous bacterial peritonitis cannot be ruled out due to underlying liver cirrhosis. Thus, peritoneal tapping was performed in which the fluid culture was negative after five days of incubation. The patient was continued on intravenous cefoperazone as he responded well to it, in addition to covering for bacterial peritonitis due to his underlying co-morbidities. He was discharged after completing the intravenous cefoperazone for 10 days, followed by a four day-course of oral ciprofloxacin (500 mg twice daily). The patient's written consent was obtained for the additional laboratory tests and the publication of this case report.

DISCUSSION

V. vulnificus is a free-living bacterium that colonises estuarine such as salt-or fresh-water environments, and is the leading cause of selfish-associated mortality in the United States (Daniels, 2011). Shellfish such as oysters tend to concentrate *V. vulnificus* up to 100 times greater than the surrounding water (Motes *et al*., 1998).

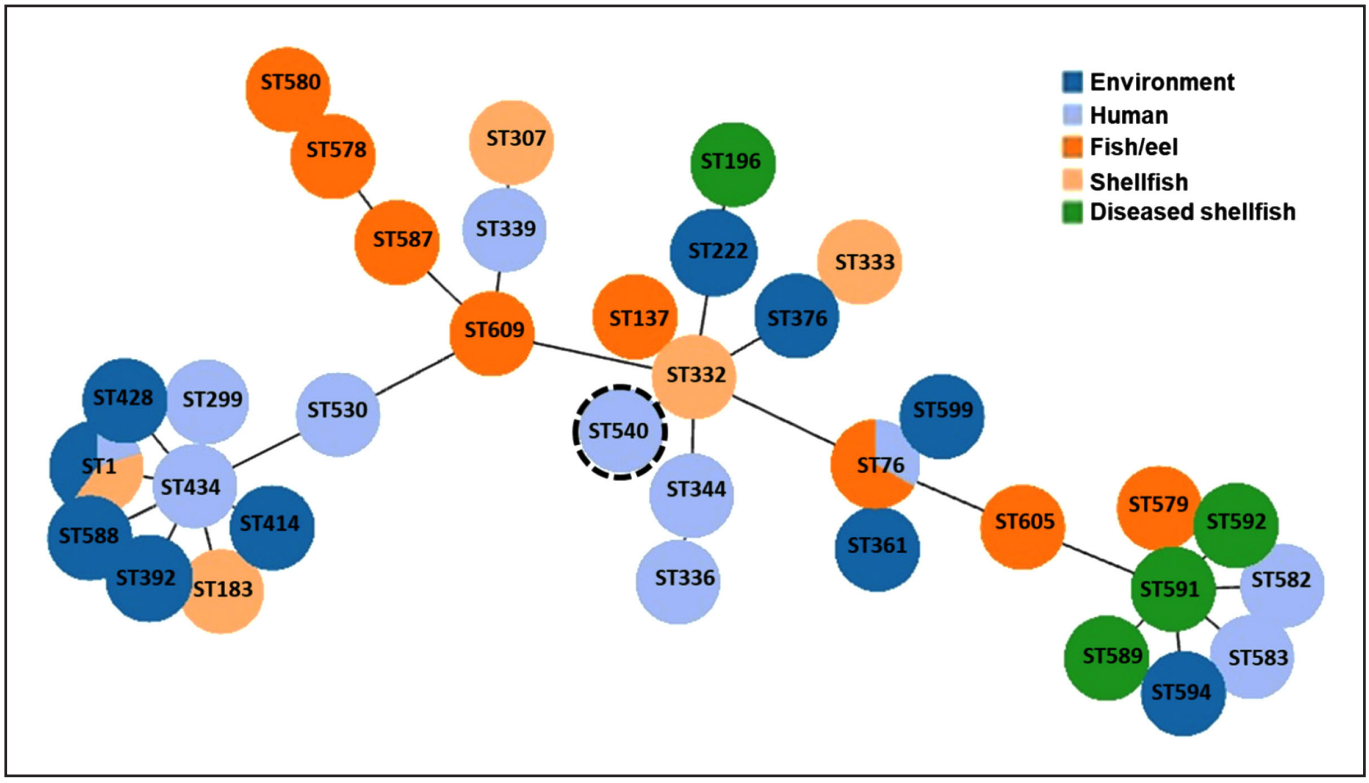

Figure 1. Minimum spanning tree showing the source of infection and the genetic closeness between *V. vulnificus* ST540 and other *V. vulnificus* MLST profiles. The minimum spanning tree was plotted as far as quintuple locus variants from ST540. Each node represents a distinct ST and each branch represents a single locus variant. Branch lengths are not proportional to genetic distance. The legend on the figure shows the different sources of *V. vulnificus* infection, represented by different colors. *V. vulnificus* ST540 is represented by a black circle with dotted lines. *V. vulnificus* MLST profiles were extracted from https://pubmlst.org/organisms/vibrio-vulnificus and the minimum spanning tree was constructed using Phyloviz Online, https://online.phyloviz.net/index.

Ingestion of raw or undercooked shellfish especially raw oysters can cause primary *V. vulnificus* septicaemia and can be fatal in high risk groups such as patients with underlying liver disease particularly cirrhosis, alcoholism, or medical conditions like haemochromatosis and renal failure that result in an iron overload state (Kim *et al*., 2007). Primary sepsis usually presents with bacteraemia without any obvious focus of infection. About one-third of patients may develop shock within 12 hours of admission with complications such as disseminated intravascular coagulation and gastrointestinal bleeding (Mead *et al*., 1999). Moreover, *V. vulnificus* may bypass the hepatic reticuloendothelial system and directly enter the portal system in patients with portal hypertension (Haq & Dayal, 2005), similar to the patient in the present study. Additionally, capsular polysaccharides and lipopolysaccharides of *V. vulnificus* may induce potent systemic inflammatory response and prevent complement-mediated lysis in the patient. Other virulence factors associated to *V. vulnificus* infection include Type IV pili for initial attachment and colonisation, production of extracellular toxins such as the elastolytic protease VvpE, the phospholipase A_2PlpA, and the multifunctional autoprocessingrepeats-in-toxins (MARTX) toxin that enhance tissue infiltration, destruction, bacterial dissemination from the intestine and subsequent septicaemia (Chung *et al*, 2010; Baker-Austin & Oliver, 2018; Choi & Choi, 2022). The patient in this case presented with acute gastroenteritis with bacteraemia, evidenced by positive *V. vulnificus* blood culture. Although the patient denied consuming raw shellfish and travelling before his illness, we suspect that he could have obtained the infection through consumption of under-cooked seafood. Since he complained of stomach bloating during admission, we suspect that he could have suffered from acute gastroenteritis caused by *V. vulnificus*. The infection usually is self-limiting, mild to moderate in severity and characterised by abdominal pain or cramps, diarrhoea, nausea, vomiting, chills and fever (Horseman & Surani, 2011). Our suspicion was substantiated by the MLST results, suggesting that ST540 is most closely related to ST332 (obtained from shellfish) (Figure 1). Information on ST332 was extracted from the PubMLST website, https://pubmlst.org/bigsdb?page=info&db=pubmlst_vvulnificus_isolates&id=440.

The diagnosis of *V. vulnificus* infection should be made based on clinical and epidemiological findings and confirmed by microbiological culture testing. Routine blood cultures should be performed to rule out bacteremia in suspected cases, whereas a culture of tissue or swab can be performed for patients with skin lesions such as bullae or abscesses (Hernández-Cabanyero & Amaro, 2020). A stool culture should be sent for patients with gastrointestinal symptoms such as diarrhoea, which would then be inoculated onto thiosulfate-citrate-bile-salt-sucrose (TCBS) agar to isolate the organism (Brayton *et al*., 1983). Culture isolation of *V. vulnificus* has high specificity but requires a longer turnaround time. Molecular methods such as MALDI-TOF mass spectrometry and polymerase chain reaction (PCR) have shortened the time for identifying pathogens for early diagnosis and appropriate antibiotic commencement for the affected patients. Both MALDI-TOF mass spectrometry (Chua *et al*., 2021) and 16S rDNA sequencing (Loong *et al*., 2020) successfully and accurately identified the isolated pathogen in this study as *V. vulnificus*. The subsequent *V. vulnificus* MLST revealed a novel ST540 and the resulting minimum spanning tree suggested that ST540 most likely arose from shellfish (ST332) and its ancestors have been very successful in colonizing different ecological niches from healthy shellfish, fish and eel to diseased shellfish, human patients and the environment (Figure 1) (Hernández-Cabanyero & Amaro, 2020).

Prompt antibiotic administration is important for managing patients with suspected *V. vulnificus* infection. Case fatality rates for *V. vulnificus* infections have been documented to increase with the delay in diagnosis and initiation of antibiotics treatment (Dechet *et al*., 2008). For patients with septicaemia or severe wound infections, it is recommended to start the patient on a combination therapy of third-generation cephalosporin such as cefotaxime (2 g) intravenously for every eight hours or ceftriaxone (1 g) intravenously daily along with a tetracycline (e.g. minocycline (100 mg) orally twice daily and doxycycline (100 mg) orally twice daily) or a fluoroquinolone (e.g. ciprofloxacin (500 mg) orally twice daily) (Kotton *et al*., 2015). Appropriate renal or hepatic-adjusted dosage is required to prevent drug toxicity. Early surgical debridement is also indicated for patients with severe wound infections such as necrotising fasciitis. In this case, the patient responded well to the intravenous cefoperazone antibiotic therapy followed by fluoroquinolone (ciprofloxacin) and he was discharged after completing two weeks of antibiotic therapy. During a follow-up one month post-discharge, he did not have fever or any symptoms suggestive of bacterial peritonitis.

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Conflict of interest

The authors declare that they have no conflict of interest.

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