Case Report

Fatal non-O1, non-O139 Vibrio cholerae septicaemia in a patient with chronic liver disease

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A 49-year-old male with underlying liver disease presented with fever and signs of sepsis. Non-
O1/non-O139 Vibrio cholerae was isolated from his blood culture, which was positive for the hlyA and toxR genes. We report this fatal case of non-O1/non-O139 V. cholerae sepsis and review the literature on non-O1/non-O139 V. cholerae sepsis in patients with chronic liver disease.

Introduction

Vibrio cholerae are Gram-negative bacteria that are generally considered non-invasive, enterotoxigenic organisms involved in gastroenteritis of varying severity. V. cholerae strains agglutinating with O1 and O139 antisera cause a toxin-mediated acute diarrhoea, cholera. Septicaemia due to this bacterium is on the rise (Coovadia et al., 1983; Jamil et al., 1992; Le Penc et al., 1988; Ling, 1932; Rao & Stockwell, 1980). Strains other than O1 and O139 (called non-O1/non-O139) cannot be biochemically distinguished from V. cholerae O1, and cause sporadic cases of diarrhoeal disease and invasive extra-intestinal illnesses (Restrepo et al., 2006; Sharma et al., 1998) mediated by toxins different from the cholera toxin (Petsaris et al., 2010). Extra-intestinal infections due to non-O1/non-O139 are more common, although their pathogenicity is less-well understood. V. cholerae non-O1/non-O139 bacteraemia is seen in individuals with underlying disorders like cirrhosis, haematological malignancies, diabetes mellitus, gastrectomy or immunocompromised states (El-Hiday et al., 2006; Ko et al., 1998; Toeg et al., 1990). Most of the studies have reported a predominance in cirrhotic patients and the various reasons suggested are decreased serum bactericidal activity, cirrhotic liver with impaired filtration function and increased serum iron levels (Andersen, 1975). We report this fatal case of non-O1/O139 V. cholerae sepsis and review the literature on non-O1/non-O139 V. cholerae sepsis in patients with chronic liver disease.

Case report

A 49-year-old male, previously diagnosed with alcohol-induced chronic liver disease (Child–Pugh class C cirrhosis), presented to the emergency services with a 4-day history of fever, breathlessness and abdominal distension. There was no history of diarrhoea or vomiting. On examination, the patient was drowsy and dehydrated, and showed icterus and bilateral pedal oedema. Examination of the respiratory system revealed basal crepitations in the left lung. The abdomen was distended and free fluid was detected clinically. The patient’s temperature was 38°C, his heart rate was 80 beats min⁻¹ and he had a blood pressure of 90/60 mmHg. A complete blood analysis showed a haemoglobin level of 13.2 g dl⁻¹, C reactive protein of 98 mg dl⁻¹ and a leukocyte count of 6.94 × 10³ μl⁻¹. Liver enzymes were elevated and clotting parameters were abnormal. After blood was drawn for culture, antibiotic therapy was initiated with parenteral meropenem [1 g every 6 h intravenously (IV)] and metronidazole (500 mg every 6 h IV). In view of the abnormal clotting parameters, the patient was infused with 2 units fresh, frozen plasma, after which ascitic fluid tapping was performed and the sample was sent for culture. However, the condition of the patient deteriorated and he died due to cardiorespiratory arrest within 9 h of being hospitalized. Blood cultures were processed and incubated in an automatic culture system (BacT/ALERT; Organon Teknika). Both aerobic and anaerobic blood culture bottles grew curved Gram-negative bacilli at 8 h. On subculture, beta-haemolytic mucoid colonies of curved, oxidase-positive, facultative anaerobic Gram-negative bacilli grew on 5% sheep blood agar plates. The organism fermented glucose, was susceptible to an O/129 vibriostatic compound, grew as yellow colonies on thiosulfate-citrate-bile salts-sucrose agar and was identified as V. cholerae by the automated VITEK 2 compact system using the ID-GN card (bioMérieux) with 95% probability

Abbreviation: IV, intravenously.

The GenBank/EMBL/DDBJ accession number for the partial 16S rRNA gene sequence of the non-O1/non-O139 V. cholerae strain is JX987303.

A supplementary table is available with the online version of this paper.
(biotype profile number 04277011515072221) and a ‘very good identification’ confidence level. The organism failed to agglutinate V. cholerae O1 and O139 antisera. Cultures of ascitic fluid were sterile. Antibiotic susceptibility testing was performed by using the automated VITEK 2 compact system using the AST-N909 card and by using the standard disc diffusion method on Mueller–Hinton agar. The susceptibility results were interpreted using the Clinical Laboratory Standards Institute interpretive criteria for infrequently isolated or fastidious bacteria (CLSI, 2010; Rao & Stockwell, 1980). The isolate was susceptible to ampicillin, piperacillin, ticarcillin, cefazolin, cefuroxime, cefotaxime, ceftazidime, gentamicin, tobramycin, amikacin, ciprofloxacin, ofloxacin, tetracycline, chloramphenicol and trimethoprim–sulfamethoxazole.

The identity of the organism was further confirmed by PCR amplification of V. cholerae species-specific conserved intergenic spacer region between 16S and 23S rRNA (Rivera et al., 2001). A 1538 bp 16S rRNA gene sequence of the strain was studied using bacterial universal primers (Rivera et al., 2001). A single colony of the bacterial isolate was grown in Luria–Bertani medium for 16 h at 30 °C and genomic DNA was extracted using the acid guanidinium thiocyanate/phenol/chloroform extraction method. Analysis of the partial sequence was performed with an automatic DNA sequencer using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin Elmer, Applied Biosystems). The nucleotide sequence was compared to sequence data available in GenBank (using the BLAST program at http://www.ncbi.nlm.nih.gov/BLAST), which revealed 99% similarity to V. cholerae. The nucleotide sequence was submitted to the GenBank database (accession no. JX987303). The serogrouping of the isolate, carried out by a reference laboratory (National Institute of Cholera and Enteric Diseases, Kolkata, India), showed agglutination with both O2 and O9 antisera. As repeated tests showed the same cross-reaction, we could not determine the serotype of the organism. The organism was characterized for its virulence factors and toxin production by PCR for the following genes: ctxA, tcpA, ctxB, hlyA, rtxA, zot, ace, toxR and ompU (Kumar et al., 2010; Rivera et al., 2001; Singh et al., 2002). The isolate was found to be non-toxigenic, as the PCR failed to amplify the genes encoding the cholera toxin and the toxin co-regulated pilus; it amplified only the genes hlyA (haemolysin) and toxR.

Discussion and overview of available literature

Non-O1/non-O139 V. cholerae septicaemia has frequently been reported in cirrhotic patients (Andersen, 1975). Our patient was a chronic alcoholic with Child–Pugh class C cirrhosis. As cirrhotic patients show decreased serum bactericidal activity and impaired liver filtration, invasion of the bloodstream by an essentially intestinal pathogen can occur, as seen in this case. The source of this organism can vary. Non-O1 V. cholerae are ubiquitous in aquatic environments and are present in coastal and estuarine areas throughout the world (Morris, 1990). Seawater is the main natural reservoir for non-O1/non-O139 V. cholerae due to its requirement for trace amounts of sodium chloride for growth. However, it can also grow in fresh water (Stypulkowska-Misiurewicz et al., 2006). Raw or undercooked shellfish and contaminated water are the epidemiological vehicles commonly implicated in human enteritis due to non-O1 V. cholerae. There are also reports of septicemia due to non-O1 V. cholerae, acquired via consumption of undercooked seafood (Petsaris et al., 2010). Contamination by direct invasion through grazed skin or wounds has also been reported (Anderson et al., 2004). Our patient came from an area where backwaters are a major source of potable water. It is likely that the patient was infected by an environmental strain, which was able to invade the bloodstream due to the patient suffering from achlorhydria, which is frequently associated with hypergastrinaemia and low pepsinogen I serum levels in patients with cirrhosis (Pérez-Ayuso et al., 1989). Diagnosis of V. cholerae sepsis can be a challenge because of its uncommon nature. Most microbiology laboratories in India routinely diagnose V. cholerae O1 infection from suspected cases of cholera. This diagnosis can be confirmed by agglutination with O1 antisera. However, non-O1/non-O139 antisera are usually available only in reference laboratories, making definitive diagnosis at the initial stages extremely difficult. Molecular studies are promising but do not aid the patient immediately. As seen in our case, patients receive empirical antibiotic therapy, and definitive therapy can be initiated after the laboratory reports this unusual organism.

Like most strains of non-O1/non-O139 V. cholerae, the strain isolated from this patient was also sensitive to commonly used antibiotics. However, the delay in presenting to the hospital proved fatal for the patient. Secondly, empirical treatment was started, assuming that the patient had sepsis due to the usual Gram-negative organisms and not V. cholerae, which proved to be inadequate in this case. However, other factors such as underlying co-morbid conditions might also play an important role in treatment failure in these patients.

Previous reports of V. cholerae sepsis have documented a higher incidence in Child–Pugh class C patients (Lee et al., 1993; Wiwatworapan & Insiripong, 2008). We reviewed 92 cases via Medline of V. cholerae non-O1/non-O139 bacteraemia in patients with chronic liver disease (Table S1, available in JMM Online). Even though such cases have been reported occasionally from several locations, 70% of the cases are from Taiwan (Table S1). The high seroprevalence of hepatitis B virus (17.5%) and consequent cirrhosis in this region have been postulated as the possible cause (Chen et al., 2007). The case fatality rate in cirrhotic patients with non-O1/non-O139 V. cholerae bacteraemia is high, ranging from 23.8 to 61.5% (El-Hiday et al., 2006; Patel et al., 2009). In a study from
Taiwan, where Ko et al. (1998) reviewed 30 cases of \textit{V. cholerae} non-O1 bacteraemia over a period of 8 years, the case fatality was found to be 47%. It was seen that apart from primary bacteraemia in cirrhotic patients, secondary bacteraemia with invasive soft tissue infection and spontaneous bacterial peritonitis were typical patterns (Ko et al., 1998). Systemic infection with \textit{V. cholerae} non-O1/non-O139 is likely to occur in individuals with poor host defence, as seen in cirrhotic patients. In addition to \textit{V. cholerae} serogroups, many non-cholera \textit{Vibrio} species such as \textit{Vibrio vulnificus} and \textit{Vibrio parahaemolyticus} are serious human pathogens, particularly in cirrhotic patients. Comparatively, the prognosis of non-O1/non-O139 \textit{V. cholerae} gastroenteritis is much better. Reviews of isolated gastroenteritis due to non-O1/non-O139 \textit{V. cholerae} showed a survival rate of 100% (Hlady & Klontz, 1996; Morris & Black, 1985). Although the exact mechanism of pathogenesis of extra-intestinal infection with non-O1/non-O139 \textit{V. cholerae} is not clearly understood, it is postulated that the haemolytic property of the organism may contribute to its virulence in immunocompromised individuals (Feghali & Adib, 2011). Non-O1 strains are known to produce a haemolysin, biologically and antigenically similar to El Tor strains (Yamamoto et al., 1986). This haemolysin is encoded by a gene, hlyA, which was found in the strain isolated from our patient. Haemolysin, along with host factors, might have helped the strain to invade the bloodstream. ToxR, a 32 kDa transmembrane protein, acts as a ‘master switch’ for the control of several virulence genes in \textit{V. cholerae} O1, so that these genes respond similarly and in a co-ordinative fashion to environmental conditions (Miller et al., 1987). Interestingly, our isolate was positive only for ToxR and was not positive for the virulence genes that ToxR regulates. It would be interesting to determine the virulence factors and the mechanism of bloodstream invasion by these bacterial strains.

Even though a number of broad-spectrum antibiotics have been used to treat severe non-O1/non-O139 \textit{V. cholerae} infections, there are no published guidelines (Feghali & Adib, 2011; Namdari et al., 2000). Nevertheless, information regarding dosage and antibiotic selection is limited. A literature review showed that treatment with beta-lactams and beta-lactam derivatives had a poor outcome, with only a 33% recovery rate (Table S1). The drug of choice for treating \textit{V. cholerae} infections is tetracycline, provided that the strain is sensitive to it. In vitro studies have shown a synergistic bactericidal effect when cefotaxime and minocycline were combined (Chuang et al., 1997). Yang et al. (2011) reported that 62.5% of patients treated with a combination of tetracycline and a fluoroquinolone for non-O1/non-O139 \textit{V. cholerae} sepsis survived, therefore, making it reasonable to administer combination therapy for severe infections. Detection of \textit{Vibrio} spp. has important public health implications. Monitoring of non-O1/non-O139 \textit{V. cholerae} strains, which are prevalent in the aquatic milieu, can prevent any outbreak-like situations. Since non-O1/non-O139 \textit{V. cholerae} strains survive on plankton in algal blooms, it is important to monitor their presence, as the contamination of seafood is likely.

Thus, non-O1/non-O139 \textit{V. cholerae} extra-intestinal bacteraemia, though uncommon, results in high mortality. Clinical suspicion of non-O1/non-O139 \textit{V. cholerae} infection should be high in patients with chronic liver disease presenting with features of bacteraemia and peritonitis. It is thus advisable for these patients to avoid contact with seawater or fresh water and to be made aware of the risks of consuming raw or undercooked seafood.

**References**


S. Khan and others

