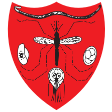
Tropical Biomedicine 38(1): 183-186 (2021)
https://doi.org/10.47665/tb.38.1.012



RESEARCH ARTICLE

A rare and unusual cause of *Vibrio cholerae* non-O1, non-O139 causing spontaneous peritonitis in a patient with cirrhosis

Engku Nur Syafirah, E.A.R.[1], Che Azmi, N.A.[1], Nik Hashim, N.H.H.[1,3], Muhd Besari, A.[2,3], Mustaffa, N.[2,3], Harun, A.[1,3] and Chan, Y.Y.[1,3]*

[1]Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia
[2]Department of Medicine, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia
[3]Hospital Universiti Sains Malaysia, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia
*Corresponding author: yeancyn@yahoo.com

ARTICLE HISTORY

Received: 1 September 2020
Revised: 7 November 2020
Accepted: 11 November 2020
Published: 25 March 2021

ABSTRACT

Spontaneous bacterial peritonitis caused by *Vibrio cholerae* non-O1/ non-O139 is a rare phenomenon. *V. cholerae* is known as a common aetiology of epidemic diarrheal disease and rarely causes extra-gastrointestinal infections. In this report, a 52-year-old man presented to our hospital with a clinical scenario for chronic liver cirrhosis with low grade fever and loose stools. *V. cholerae* was isolated from peritoneal fluid culture, which was further confirmed as non-O1/ non-O139 strain by multiplex polymerase chain reaction. The patient was successfully treated with antimicrobial therapy and peritoneal drainage. This case represents the first isolation of *V. cholerae* non-O1/ non-O139 strain from peritoneal fluid.

Keywords: *Vibrio cholerae*; non-O1/ non-O139; spontaneous bacterial peritonitis; cirrhosis.

INTRODUCTION

Despite significant achievements in public health, cholera remains a major public health challenge worldwide. The global burden of cholera is largely unknown due to under-reporting from endemic and non-endemic countries. The latest estimate of global cholera burden is 1.4 – 4.0 million cases per year, with 21 000 – 143 000 of deaths in cholera-endemic countries (Ali *et al.*, 2015). Malaysia is categorized as non-endemic to cholera but several sporadic outbreaks have occurred in the past. Cholera is still a major health problem in northern Borneo, including the Malaysian state of Sabah (Zaw *et al.*, 2019). To date, cholera outbreaks in Malaysia have been caused by *V. cholerae* serogroup O1, El Tor strain, whilst O139 serogroup and non-O1/non-O139 serogroup cases have occurred sporadically and have not been implicated in any major outbreak (Ang *et al.*, 2010; Teh *et al.*, 2012; Jikal *et al.*, 2019). Although non-O1/non-O139 serogroups only cause mild gastroenteritis, there are several case-reports available regarding them causing bacteremia (Deris *et al.*, 2009), splenic abscess (Cavuoti *et al.*, 2002), otitis externa (Díaz-Menéndez *et al.*, 2018), and empyema (Lai *et al.*, 2012). We believe that this is the first case of non-O1/ non-O139 *V. cholerae* causing spontaneous bacterial peritonitis (SBP) from Malaysia.

CASE REPORT

A 52-year-old Malay man with underlying liver cirrhosis secondary to chronic hepatitis C infection was initially electively admitted to our hospital for Digital Subtraction Angiography (DSA) for a left parasagittal Arteriovenous Malformation (AVM). However, in ward, he developed low-grade fever. Upon further questioning, he claimed that he had been having low-grade fever and was passing loose stool for three days. It was associated with intermittent dull aching abdominal pain. He denied history of having outside meals and consumption of raw meat or seafood prior to the diarrhea episodes. Upon examination, he was jaundiced and had signs of liver failure with portal hypertension which included asterixis, spider naevi, palmar erythema and gynecomastia. His blood pressure and pulse rate were 130/ 80 mmHg and 82 beats per minute, respectively. Temperature on admission was 37.3°C. His abdomen was tense with an everted umbilicus and gross ascites. He had bibasal lung crepitations and bilateral lower limb edema up to the knees.

A provisional diagnosis of spontaneous bacterial peritonitis (SBP) was made, and intravenous ceftriaxone was empirically started. Large volume abdominal paracentesis was performed on day 1 and 3 of admission. The peritoneal fluid analysis showed 25 g/L serum-ascites albumin gradient

Published by Malaysian Society of Parasitology and Tropical Medicine.
All rights reserved.

(SAAG) which was suggestive of significant portal hypertension (transudative ascites). Peritoneal fluid microscopy showed presence of pus cells, 10–25 cells/mm³. Culture of the fluid grew hemolytic colonies on blood agar (Thermo Fisher Scientific, Massachusetss, USA) and non-lactose fermenting colonies on Mac Conkey agar (Thermo Fisher Scientific, Massachusetss, USA). Gram stain revealed Gram negative curve-shaped bacilli and culture on Thiosulfate-Citrate-Bile Salts-Sucrose agar (TCBS agar) (Thermo Fisher Scientific, Massachusetss, USA) showed yellow (sucrose fermenting) colonies. Identification using TM systems (bioMérieux SA, Marcy-l'Étoile, France) revealed *V. cholerae* with a high identification score. However, *V. cholerae* polyvalent 01 serotyping was negative. Antimicrobial susceptibility test using disk diffusion method showed susceptibility of the isolate to ciprofloxacin, tetracycline, and ampicillin.

Further confirmation of the suspected *V. cholerae* isolate was performed using in-house multiplex polymerase chain reaction (PCR) (Lalitha *et al.*, 2008; Deris *et al.*, 2009; Ang *et al.*, 2010). The PCR reaction contains eight sets of target primers for the identification of *V. cholerae* serogroups (O1, O139 or non-O1/non-O139), biotypes (Classical or El Tor) and toxigenicity by the detection of virulence genes (*ace*, *zot* and *ctx*). An internal amplification control was also included in the PCR assay to detect false negative results due to presence of PCR inhibitors.

In short, the sample preparation for PCR was performed by using the boiling method. One colony of *V. cholerae* was placed into a tube containing 200 µl of PCR water. The bacterial suspension was vortexed vigorously for several seconds and boiled at 100°C for 10 minutes. Two microliters of boiled bacterial DNA template was added into 18 µl of PCR reaction mixture containing 1× PCR amplification buffer (Thermo Fischer Scientific, USA), 3.75 mM of $MgCl_2$, 0.25 mM of dNTPs mix, 6.3 µl of octaplex primer mix, 4 U of *Taq* DNA polymerase (Thermo Fischer Scientific, USA) and 35 pg/ µl of internal control plasmid. The PCR assay was performed in a thermal cycler (PTC-200, MJ Research, USA) under the following conditions: 1 cycle at 95°C for 5 mins; 30 cycles, each consisting of 30s at 95°C, 30s at 60°C, and 30s at 72°C; and a final round extension for 30s at 60°C and 5 mins at 72°C.

The PCR amplicons were subjected to electrophoresis on 2.0% (w/v) of agarose gel and visualized by UV illumination as shown in Figure 1. The suspected *V. cholerae* isolate (Lane S1) showed two single bands specific for *IoIB* gene (519 bp) of *V. cholerae* and a 150 bp of internal control band. Thus, this electrophoretic profile was identified as a non-O1/ non-O139 strain of *V. cholerae* by PCR.

The diagnosis of SBP caused by *V. cholerae* was finally confirmed. Repeated peritoneal fluid culture on day 3 of admission did not show any bacterial growth after 48 hours of incubation. He was given intravenous ceftriaxone for seven days and responded well to this; he was later discharged with oral ciprofloxacin for another five days. His DSA procedure was postponed to a later date. Case notification was performed to the District Public Health Department for further action on their part.

DISCUSSION

V. cholerae is a facultative anaerobe, comma-shaped Gram-negative bacilli that is oxidase-positive, highly motile and has a unipolar flagellum. Catalase positive and sucrose fermentation are the properties that differentiate *V. cholerae* from other *Vibrio* species. *V. cholerae* is classified into more than 200 serogroups based on the difference in the composition of the O-specific polysaccharide (OSP) chains of lipopolysaccharide (LPS) molecules (Johnson *et al.*, 2012). In most cases, *V. cholerae* infections present with intestinal

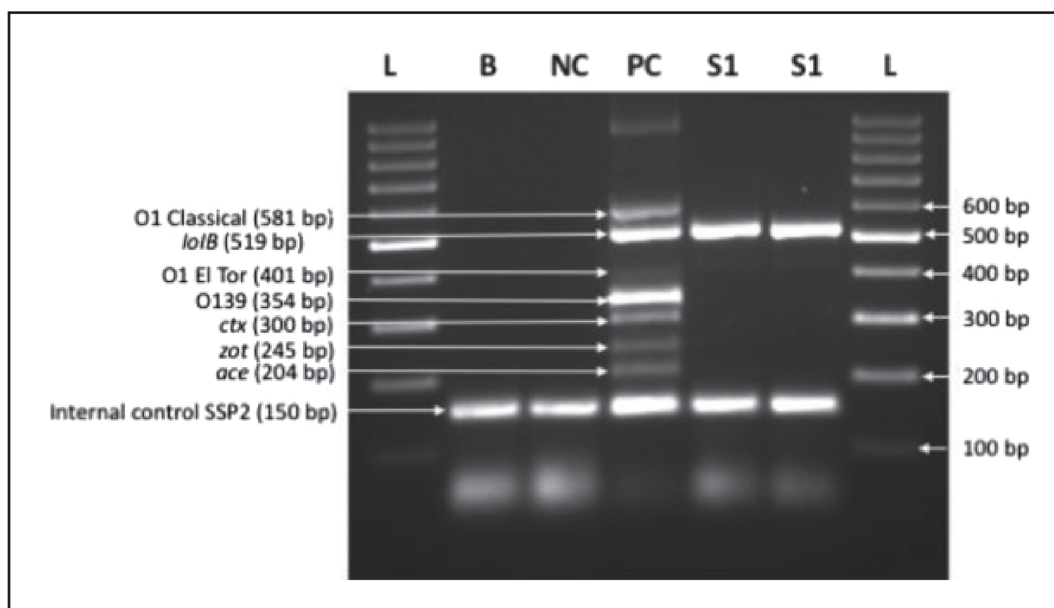


Figure 1. Representative of agarose gel electrophoresis of the suspected *V. cholerae* isolate performed by octaplex PCR assay which targeted serogroups, biotypes, and virulence genes of *V. cholerae*. Lanes L: 100 bp DNA marker; B: Blank; NC: Negative control; PC: Positive control; S1: suspected *V. cholerae* isolate (duplicates). The positive control consists of seven genes linked to *V. cholerae* and one internal control. Seven genes represent all serogroups of *V. cholerae* that are O1, O139 and non-O1/ non-O139, biotypes – El Tor and Classical; all the toxigenic genes (*ctx, zot* and *ace*) and *IoIB* gene that are highly conserved among the species *V. cholerae*. The internal control primers were designed from *Plasmodium falciparum* sporozoite surface protein 2 (SSP2) gene.

symptoms and for serogroup non-O1/non-O139, it causes only mild gastroenteritis. Humans may be infected with *V. cholerae* non-O1/non-O139 serogroups following ingestion of contaminated seafood (e.g., oysters, mussels, prawns) or wound exposure to contaminated water, as this organism has a natural reservoir in sea and coastal waters (Center for Disease Control and Prevention, 2014). However, in our patient, there was no history of consuming raw seafood and no recent exposure to the aquatic environment.

In a study conducted by Chen *et al.*, acute gastroenteritis followed by bile duct infection and primary bacteremia would be the most common clinical manifestations of *V. cholerae* non-O1/non-O139. Peritonitis is rare and accounts for only about 6% of total cases (Chen *et al.*, 2015). The pathogenesis of extra gastrointestinal infections is not yet well-understood. However, several postulates have been described in a number of studies suggesting that these infections may have occurred due to a) prior disruption in mucosal barrier which increases intestinal permeability, b) small intestinal bacterial overgrowth, c) achlorhydria status d) immunosuppression, e) translocation of viable *V. cholerae* through M cells and f) hemolysin production (Jabeen *et al.*, 2010; Lata *et al.*, 2009). Catheters and other devices used for invasive procedures are another potential cause of infection (Lata *et al.*, 2009). SBP is a particularly known complication in patients with more severe liver function damage (Child-Pugh classification C), without a definitive intra-abdominal cause that can be surgically treated and often following bleeding from the upper gastrointestinal tract due to portal hypertension (Alaniz & Regal, 2009). In many cases, the infection is asymptomatic; the typical signs of fevers and abdominal pains may not be very evident (Lata *et al.*, 2009). These are often revealed when there is a worsening of symptoms that follow the course of liver cirrhosis, i.e. increased ascites and failure of diuretic treatment; development of hepatic encephalopathy, hepatopulmonary syndrome, coagulation disorders or even diarrhea (Nusrat *et al.*, 2014). Our patient was in a hypoalbuminemic and immunocompromised state that further predisposed him to develop SBP due to *V. cholerae*. The negative blood culture result in this patient suggested that the infection may only be localized to the peritoneum.

Diagnosis of *V. cholerae* peritonitis can be a challenge due to its unusual nature. SBP is confirmed by detection of polymorphonuclear (PMN) leukocytes in ascites > 250 cells/ mm^3 and positive ascitic fluid culture without other apparent source of infection (Setoyama *et al.*, 2019). However, in our setting, we did not perform differential counts for peritoneal fluid culture. Thus, diagnosis made in this patient was based on clinical suspicion. While high PMN counts and ascitic fluid culture are used as gold standard for SBP diagnosis, ascitic fluid culture is not rapidly accessible, thereby delaying the diagnosis and treatment of infection (Wahab *et al.*, 2018). Many microbiology laboratories in Malaysia routinely diagnose *V. cholerae* O1 infection from suspected cases of cholera by confirming O1 antiserum agglutination. However, non-O1/non-O139 antisera are usually only available in reference laboratories, complicating diagnosis confirmation at the initial testing stage. As seen in our case, empirical antibiotic therapy was initiated early; however, a more definitive therapy could only be initiated after the laboratory confirmation of this organism.

To date, no guidelines have been established for the treatment of non-O1/non-O139 infections. Anderson *et al*. has suggested the use of parenteral third generation cephalosporin as the mainstay of treatment, given for at least one week, followed by a one-month course of oral fluoroquinolones for improved patient outcomes (Anderson *et al.*, 2004). On the other hand, in a case report from Wiwatworapan and Insiripong (2008), a patient who had *V. cholerae* non-O1/ non-O139 peritonitis and septicemia was successfully treated with intravenous cefotaxime for one week, followed by oral fluoroquinolones for another week (Wiwatworapan & Insiripong, 2008). As for our patient, he completed a seven-day course of intravenous ceftriaxone, followed by oral ciprofloxacin for another seven days. He recovered well, with no residual symptoms.

In conclusion, *V. cholerae* non-O1/non-O139 infection may be considered as a potential causative agent of SBP in patients with cirrhosis. Although rare, early diagnosis and intervention would prevent complications including septicaemia, and thus preventing mortality attributed to this infection. The use of a molecular diagnostic tool would be useful for the detection of difficult cases.

ACKNOWLEDGMENT

The first author (Engku Nur Syafirah, E.A.R.) is financially supported by Universiti Sains Malaysia (USM) via the USM Fellowship Scheme.

Conflicts of Interest

All the authors have declared that there is no conflict of interest regarding the publication of this paper.

Role of funding

This work was supported by Universiti Sains Malaysia (USM) through the Short Term Grant Scheme (304/PPSP/6315459) awarded to Chan Yean Yean.

REFERENCE

Alaniz, C. & Regal, R.E. (2009). Spontaneous bacterial peritonitis: a review of treatment options. *P & T : A Peer-Reviewed Journal for Formulary Management* **34**(4): 204-210.

Ali, M., Nelson, A.R., Lopez, A.L. & Sack, D.A. (2015). Updated global burden of cholera in endemic countries. *PLoS Neglected Tropical Diseases* **9**(6): e0003832. https://doi.org/10.1371/journal.pntd.0003832

Anderson, A.M.L., Varkey, J.B., Petti, C.A., Liddle, R.A., Frothingham, R. & Woods, C.W. (2004). Non-O1 *Vibrio cholerae* septicemia: Case report, discussion of literature, and relevance to bioterrorism. *Diagnostic Microbiology and Infectious Disease* **49**(4): 295-297. https://doi.org/10.1016/j.diagmicrobio.2004.04.016

Ang, G.Y., Yu, C.Y., Balqis, K., Elina, H.T., Azura, H., Hani, M.H. & Yean, C.Y. (2010). Molecular evidence of cholera outbreak caused by a toxigenic *Vibrio cholerae* O1 El tor variant strain in Kelantan, Malaysia. *Journal of Clinical Microbiology* **48**(11): 3963-3969. https://doi.org/10.1128/JCM.01086-10

Cavuoti, D., Fogli, M., Quinton, R., Gander, R.M. & Southern, P.M. (2002). Splenic abscess with *Vibrio cholerae* masking pancreatic cancer. *Diagnostic Microbiology and Infectious Disease* **43**(4): 311-313. https://doi.org/10.1016/S0732-8893(02)00412-1

Center for Disease Control and Prevention, C. (2014). Non-O1, and non-O139 *Vibrio cholerae* Infections. Retrieved from https://www.cdc.gov/cholera/non-01-0139-infections.html

Chen, Y.-T., Tang, H.-J., Chao, C.-M. & Lai, C.-C. (2015). Clinical manifestations of non-O1 *Vibrio cholerae* infections. *PloS One* **10**(1): e0116904. https://doi.org/10.1371/journal.pone.0116904

Deris, Z.Z., Leow, V.M., Wan Hassan, W.M.N., Nik Lah, N.A.Z., Lee, S.Y., Siti Hawa, H. & Ravichandran, M. (2009). Non-O1, non-O139 *Vibrio cholerae* bacteraemia in splenectomised thalassaemic patient from Malaysia. *Tropical Biomedicine* **26**(3): 320-325.

Díaz-Menéndez, M., Alguacil-Guillén, M., Bloise, I., García-Pallarés, M. & Mingorance, J. (2018). A case of otitis externa caused by non-01/non-0139 *Vibrio cholerae* after exposure at a Mediterranean bathing site. *Revista espanola de quimioterapia : publicacion oficial de la Sociedad Espanola de Quimioterapia* **31**(3): 295-297.

Jabeen, K., Siddiqui, J., Zafar, A., Shakoor, S., Ali, N. & Zaidi, A.K.M. (2010). *Vibrio cholerae* O1 bacteremia in Pakistan: analysis of eight cases. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **104**(8): 563-565. https://doi.org/10.1016/j.trstmh.2010.04.004

Jikal, M., Riduan, T., Aarifin, R., Jeffree, M.S. & Ahmed, K. (2019). Cholera outbreak by Sea Gypsies in Sabah, Malaysia: A challenge in North Borneo. *International Journal of Infectious Diseases* **83**: 83-85. https://doi.org/10.1016/j.ijid.2019.04.008

Johnson, R.A., Uddin, T., Aktar, A., Mohasin, M., Alam, M.M., Chowdhury, F. & Ryan, E.T. (2012). Comparison of immune responses to the O-specific polysaccharide and lipopolysaccharide of *Vibrio cholerae* O1 in Bangladeshi adult patients with cholera. *Clinical and Vaccine Immunology: CVI* **19**(11): 1712-1721. https://doi.org/10.1128/CVI.00321-12

Lai, C.-C., Liu, W.-L., Chiu, Y.-H., Gau, S.-J. & Hsueh, P.-R. (2012). Spontaneous bacterial empyema due to non-O1, non-O139 *Vibrio cholerae* in a cirrhotic patient with hepatocellular carcinoma. *Diagnostic Microbiology and Infectious Disease* **73**(1): 84-85. https://doi.org/10.1016/j.diagmicrobio.2012.01.011

Lalitha, P., Siti Suraiya, M.N., Lim, K.L., Lee, S.Y., Nur Haslindawaty, A.R., Chan, Y.Y. & Ravichandran, M. (2008). Analysis of lolB gene sequence and its use in the development of a PCR assay for the detection of *Vibrio cholerae*. *Journal of Microbiological Methods* **75**(1): 142-144. https://doi.org/10.1016/j.mimet.2008.05.001

Lata, J., Stiburek, O. & Kopacova, M. (2009). Spontaneous bacterial peritonitis: a severe complication of liver cirrhosis. *World Journal of Gastroenterology* **15**(44): 5505-5510. https://doi.org/10.3748/wjg.15.5505

Nusrat, S., Khan, M.S., Fazili, J. & Madhoun, M.F. (2014). Cirrhosis and its complications: evidence based treatment. *World Journal of Gastroenterology* **20**(18): 5442-5460. https://doi.org/10.3748/wjg.v20.i18.5442

Setoyama, H., Tanaka, M. & Sasaki, Y. (2019). *Diagnosis of Spontaneous Bacterial Peritonitis*. In *Clinical Investigation of Portal Hypertension.* pp. 511-516.

Teh, C.S.J., Suhaili, Z., Lim, K.T., Khamaruddin, M.A., Yahya, F., Sajili, M.H. & Thong, K.L. (2012). Outbreak-associated *Vibrio cholerae* genotypes with identical pulsotypes, Malaysia, 2009. *Emerging Infectious Diseases* **18**(7): 1177-1179. https://doi.org/10.3201/eid1807.111656

Wahab, H., Shokry, M., Eleyoun, S., Sayed, M. & Hassouna, S. (2018). Evaluation of the role of culture, PCR, and sequencing in the diagnosis of spontaneous bacterial peritonitis in patients with ascites. *International Journal of Current Microbiology and Applied Sciences* **7**: 182-192.

Wiwatworapan, W. & Insiripong, S. (2008). Non-O1/non-O139 *Vibrio cholerae* septicemia with peritonitis. *The Southeast Asian Journal of Tropical Medicine and Public Health* **39**(6): 1098-1101.

Zaw, M.T., Emran, N.A., Ibrahim, M.Y., Suleiman, M., Awang Mohd, T.A., Yusuff, A.S. & Lin, Z. (2019). Genetic diversity of toxigenic *Vibrio cholerae* O1 from Sabah, Malaysia 2015. *Journal of Microbiology, Immunology and Infection* **52**(4): 563-570. https://doi.org/10.1016/j.jmii.2018.01.003