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Non-O1, non-O139 *Vibrio cholerae* bacteraemia in splenectomised thalassaemic patient from Malaysia

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Abstract. *Vibrio cholerae* infection is mainly caused acute diarrhoea disease. Bacteraemia due to non-O1 *V. cholerae* is rare and mainly reported in liver cirrhotic patients. We report one case of non-O1 *V. cholerae* bacteraemia in splenectomised thalassaemic patient who presented with septic shock secondary to abdominal sepsis. She had undergone emergency laporatomy and was managed in the intensive care unit for nine days. She was treated with meropenem and doxycyline and discharged well after fourteen days of admission. The *V. cholerae* was identified by API 20NE, serotype and polymerase chain reaction showed as non-O1, non-O139 strain. Besides known cholera-like toxin and El Tor hemolysin, with increasing reported cases of *V. cholerae* bacteraemia, there is possibility of other virulence factors that allow this organism to invade the bloodstream.

INTRODUCTION

Vibrio cholerae re-emerged and is becoming an increasingly important public health challenge in Malaysia (Lim, 2001). Its infection is usually caused by serogroup O1 or O139. The infection of serogroup non-O1 has been reported in Malaysia and mainly caused acute diarrhoeal disease. Bacteraemia due to non-O1 *Vibrio cholerae* is rare and mainly reported in cirrhotic patients (Ko *et al.*, 1998).

CASE REPORT

A 28 year-old lady married with one child, known to have thalassaemia major and hepatitis C infection with history of total splenectomy in 1996 and laparoscopic cholecystectomy in 2005, was referred from a district hospital to a tertiary hospital for septic shock. She had history of high grade fever for two days followed by upper abdominal pain, malaise and as well as per vaginal bleeding.

Upon arrival at the reference hospital, she was conscious, pale, icteric, dehydrated and tachypnoeic. Her temperature was 39°C. Her pulse rate was 120bpm and blood pressure was 90/60 mmHg with double inotropic support (noradrenaline and dobutamine). Her abdomen was tense. The total white count was 64.9 X 10^3/µL with

predominant neutrophils. Chest X-ray revealed no sign of bowel perforation. Her urinary pregnancy test was positive and ultrasound showed empty uterus with presence of fluid in pouch of Douglas.

Emergency laparotomy was performed for suspected ectopic pregnancy. Intraoperatively, the general surgical team was called in to assess the copious amount of golden yellow fluid material in peritoneal cavity and presence of large amount of cystic gelatinous sloughy material at subhepatic region, posterior abdominal wall and pelvis. No product of conceptus was identified. The small and large bowels were normal. There was no bile leak from the bile duct. Both the ovaries and fallopian tubes were normal.

Post operatively she was intensively nursed in intensive care unit. She was down with septic shock, coagulopathy, deranged liver function and pulmonary oedema. For the first few days she needed four inotropes. Intravenous meropenem was started intraoperatively. Histopathological report of the gelatinous sloughy material was consistent with chronic abscess however culture of golden yellow peritoneal fluid failed to grow any organism.

At day three post operatively, the blood culture grew Gram-negative bacilli which was non-lactose fermenter and showed haemolysis on blood agar. Culture on thiosulphate citrate bilesalt sucrose (TCBS) agar produced yellow (sucrose-fermenting) colonies. The API 20NE (bioMérieux, France) test turned out as *Vibrio cholerae* but negative serotyping for both O1 and O139. The organism was sensitive to doxycyline and this antibiotic was added. The patient improved with the intensive care management for nine days. She was discharged after fourteen days of admission. Two months later, she was seen in the surgical outpatient clinic. She was doing fine.

Further confirmation of the suspected *V. cholerae* sample at the molecular level was performed using polymerase chain reaction (PCR). The PCR primer mix contained a pair of internal control primers and eight sets of primers to identify the serogroup (O1, O139 or non-O1, non-O139), biotype (Classical or El Tor), virulence genes (ace, zot and/or ctx) and antibiotic resistance gene (tetracycline) of the suspected *V. cholerae* sample.

Bacterial sample for PCR was prepared from a single colony of the organism using boiling method. Two µl of the boiled bacterial sample was added to 18 µl of PCR reaction mixture containing 1X PCR buffer (Fermentas, Lithuania), 3.75 mM $MgCl_2$, 0.25 mM dNTP; 2 U *Taq* DNA polymerase (Fermentas, Lithuania), 6.3 µl of primer mix (containing 9 primer pairs) and 1 ng of internal control plasmid. PCR was performed in a thermal cycler (PTC-200, MJ Research, USA) under the following conditions: 1 cycle at 95ºC for 5 min; 30 cycles, each consisting of 30s at 95ºC, 30s at 60ºC, and 30s at 72ºC; and a final round of extension for 30s at 60ºC and 5 min at 72ºC.

The amplified PCR product was separated by electrophoresis on a 2.0% (w/v) agarose gel and visualized by UV transillumination as shown in Figure 1. Agarose gel electrophoresis of the suspected *V. cholerae* sample (lanes 4 and 5) gave a single 519 bp band specific for the *lol*B gene of *V. cholerae* and a 150 bp internal control band.

This electrophoretic profile was similar to the *V. cholerae* non-O1, non-O139 control strain (lane 9). Therefore, the organism was identified as a non-O1, non-O139 strain of *V. cholerae* by PCR.

On further questioning, she claimed that her village was flooded four months prior to her current illness but she was not exposed directly to the flood. She took boiled water that was supplied from local safe water supply. There was no history of eating outside. The case was notified to local public health department and contact-tracing was negative.

DISCUSSION

Invasive *V. cholerae* infection has been reported world wide. These include bacteraemia, meningitis, spontaneous bacteria peritonitis, fasciatis, cholangitis and enteritis (Lee *et al.*, 2007). Bacteraemia was the commonest presentation in which fifteen of thirty non-O1, non-O139 *V. cholerae*

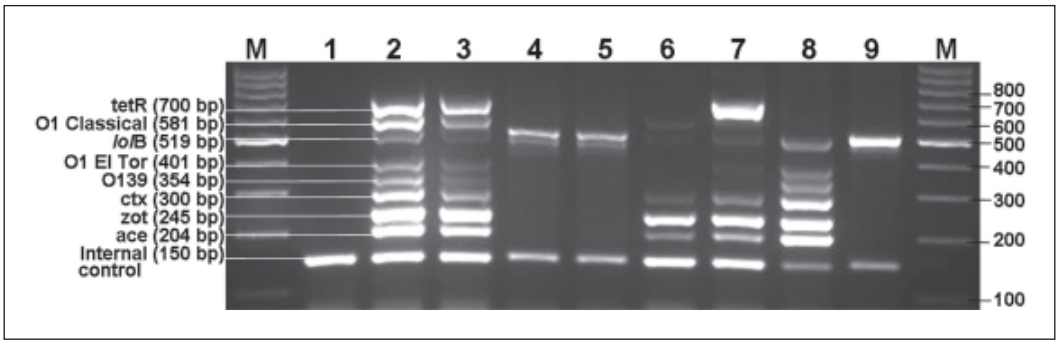

Figure 1. Agarose gel electrophoresis of the suspected *Vibrio cholerae* sample using primers specific for the serogroups, biotypes, virulence genes and tetracycline resistance gene of *V. cholerae*.
Lanes M: 100bp DNA marker; 1: Negative control; 2, 3: Positive control, 4, 5: Suspected *V. cholerae* sample (duplicates), 6: *V. cholerae* O1 Classical control strain, 7: *V. cholerae* O1 El Tor control strain (with tetracycline resistance gene); 8: *V. cholerae* O139 control strain, 9: *V. cholerae* non-O1, non-O139 control strain.

infected patient presented with bacteraemia (Ko *et al.*, 1998).

Non-O1 *V. cholerae* invasive infection and bacteraemia were more common in Taiwan. More than thirty cases of non-O1 *V. cholerae* invasive infection and bacteraemia was reported or retrospectively reviewed in Taiwan (Lin *et al.*, 1996; Ko *et al.*, 1998; Liou *et al.*, 2001; Ou *et al.*, 2003; Chang-Chien, 2006;Yang *et al.*, 2008). Non-O1 *V. cholerae* bacteraemia was uncommon in other places. With the exclusion of cases reported from Taiwan, the selected reported cases of non-O1*V. cholerae* bacteraemia is summarized in Table 1.

The first case of non-O1 *V. cholerae* septicaemia/bacteraemia in Malaysia was reported from Penang (Tan *et al.*, 1994). The patient was an 8-year-old child who presented with bloody diarrhoea, fever and severe dehydration. Non-O1 *V. cholerae* was isolated from blood and stool cultures. The clinical course was uneventful after starting appropriate rehydration and supportive therapy.

This is the second case of non-O1 *V. cholerae* septicaemia/bacteraemia reported from Malaysia. We confirmed *V. cholerae* using polymerase chain reaction (PCR) method. It showed similar electrophoretic profile to the *V. cholerae* non-O1, non-O139 control strain. Presence of *lol*B gene support the confirmation of *V.cholerae*. The *lol*B PCR is a common marker and detected all O1, O139 and non-O1, non-O139 serogroup and biotypes of *V. cholerae* (Lalitha *et al.*, 2008).

The common risk factors are liver cirrhosis and haematological malignancy. This is the second *V. cholera* bacteraemia case reported associated to splenectomy. The first case was reported earlier in Bangkok, Thailand (Thisyakorn & Reinprayoon, 1990). The patient was a 15-year-old girl who had β-thalassemia/ haemoglobin E disease. She had undergone a splenectomy 3 years prior to the problem. She presented with primary peritonitis and septicaemia of 2-days duration. Exploratory laparatomy disclosed 50ml of peritoneal fluid which failed to grow any organism. The blood culture isolated sensitive strain of non-O1 *V. cholerae*. The patient's postoperative course was uneventful, and she became afebrile on the fifth day of hospitalization. Compared to this case, our patient did not have any symptoms of acute gastroentritis such as vomiting or diarrhoea.

Thallassaemic patients have higher risk of infection due to immune abnormalities, splenectomised and iron overload (Vento *et al.*, 2006). Numerous immune abnormalities have been described including greater numbers and activity of CD8 suppressor cells, decreased CD4/CD8 ratio, impaired differentiation of B lymphocytes, increased levels of antibodies but defects in neutrophils and macrophages chemotaxis and phagocytosis. The factors behind these

Table 1. Cases of non-O1 *Vibrio cholerae* bacteraemia reported in the English literature (excluding cases reported from Taiwan) that were available as full article by medline search of 'cholera and bacteraemia'

Place (Year) Serogroup	Age	Risk Factors	Presentation	Treatment and Outcome	Reference
Kuwait (1989) Non-O1	50-year-old women	Liver cirrhosis	Presented in comatose	IV ampicillin-cloxacillin with gentamicin. Died.	(Dhar *et al.*, 1989)
	31-year-old man	Liver cirrhosis	Fever and jaundice	Treated at another hospital	
Tel Aviv (1990) Non-O1	66-year-old man	Gastrectomy	Fever, watery diarrhoea, and diffuse abdominal pain	IV chloramphenicol. Recovered.	(Toeg *et al.*, 1990)
Pensylvania, USA (2000) Non-O1, non-O139	49-year-old man	No identified risk factor beside taking raw seafood	Severe profuse watery diarrhoea, nausea and vomiting	IV ciprofloxacin. Recovered.	(Namdari *et al.*, 2000)
Kuwait (2001) Non-O1	Neonate		Poor feeding, cyanosis and generalized tonic-clonic convulsion. The baby developed brain abscess.	Ampicillin and cefotaxime. Died.	(Ismail *et al.*, 2001)
Bangkok (2001) Non-O1	41-year-old man	Chronic liver disease	Fever, chills and right leg pain. Develop cerebritis after admission	IV ciprofloxacin. Recovered.	(Suankratay *et al.*, 2001)
Brussels (2002) Non- O1	78-year-old man	Rectal lung cancer	Abdominal cramp and constipation	IV ciprofloxacin for 10 days. Patient died after one month at home and no cause identify.	(Berghmans *et al.*, 2002)
Durham (2004) Non-O1	53-year-old man	Gastrointestinal surgeries for peptic ulcer disease	Fever, nausea and mild abdominal pain	IV ciprofloxacin. Recovered.	(Anderson *et al.*, 2004)
Slovania (2005) Non-O1, non-O139	20-year-old man	Not stated	Vomiting, diarrhoea and fever. Having complication of multiple liver and spleen abscesses.	Ampicillin, doxycycline and ciprofloxacin. Recovered.	(Štrumbelj *et al.*, 2005)
Poland (2006) Non-O1, non-O139	49-year-old man	Near drowning	High fever and diarrhoea.	Died suddenly.	(Stypulkowska-Misiurewicz *et al.*, 2006)
	79-year-old man	Not stated	High fever, diarrhoea and severe abdominal cramps.	Amoxicillin with clavulanic acid and ciprofloxacin. Recovered.	
Doha (2006) Non-O1, non-O139	47-year-old man	Liver cirrhosis	Abdominal pain, high fever and diarrhoea	IV piperacillin-tazobactams. Patient died two days after admission.	(El-Hiday *et al.*, 2006)
New York (2006) Non-O1	59-year-old man	End-stage liver disease	Fever, watery diarrhoea and malaise	IV doxycycline after failed IV cefepime and ciprofloxacin. Recovered.	(Restrepo *et al.*, 2006)
Vientiane (2008) O21	20-year-old woman	No identified risk factor beside taking undercooked snails	Abdominal pain, fever and watery diarrhoea	IV ampicillin and gentamicin. Died.	(Phetsouvanh *et al.*, 2008)
Arizona (2009) Non-O1	50-year-old woman	Liver cirrhosis	Myalgia and later unresponsiveness	Cefotaxime and doxycycline.	(Patel *et al.*, 2009)

immune alterations are poorly understood, but iron overload may have a prominent role. Whereas splenectomy can cause reduced immune clearance, alterations in complement activation, reduced IgM synthesis, lower specific antibody responses, and further reduced CD4/CD8 lymphocyte ratio. Post-splenectomised patients were found to have severe infections caused by encapsulated bacteria (*Streptococcus pneumoniae*, *Haemophilus influenzae* type B, *Neisseria meningitidis*) as well as other pathogens including *Escherichia coli*, *Pseudomonas aeruginosa*, group B streptococci, *Enterococcus* spp, *Ehrlichia* spp, and *Plasmodium* spp (Vento *et al.*, 2006).

The virulence factors that allow *V. cholerae* to invade the bloodstream are not well elucidated. Cholera-like toxin and El Tor hemolysin may play a role in the disease process (Safrin *et al.*, 1988). Other researchers suggest that the haemolytic property of *V. cholerae* isolates may have contributed to their ability to invade the bloodstream in immunocompromised hosts such as in our cirrhotic patient (Restrepo *et al.*, 2006). The strain isolated in this report also showed beta-haemolysis on blood agar culture.

In this case, the patient probably contracted *V. cholerae* during the floods in her village. *Vibrio cholerae* invaded the mucosa layer of the intestinal tract that led to primary peritonitis similar to previously reported cases (Thisyakorn & Reinprayoon, 1990). Primary spontaneous bacteria peritonitis is a known presentation of invasive non-O1 cholera infection (Lee *et al.*, 2007). Because of reduced capability of immune clearance in thallasemic and splenectomised patients, this patient was unable to clear the organism from the peritoneum cavity. This leads to formation of chronic abscess in the peritoneum. Once the immune system is further compromised due to any factor, this haemolytic strain of non-O1 *V. cholerae* will invade the blood vessel to cause systemic sepsis. The patient presented to our hospital four months after exposure to the organism with full-blown sepsis and septicaemic shock.

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