Case report

Two cases of *Vibrio cholerae* non-O1/non-O139 septicaemia with favourable outcome in Lebanon

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Introduction

Vibrio cholerae species can be divided into 2 major groups: cholera-causing strains of the serogroups O1 and O139, and non-O1 *V. cholerae* (NOVC) [1]. Non-O1 *V. cholerae* (NOVC) typically does not elaborate cholera toxin. It is also antigenically distinct from the *V. cholerae* O1 and O139 strains, which cause epidemic cholera in many countries.

Although NOVC strains are usually associated with sporadic diarrhoea, some outbreaks have been reported. These strains have also been associated with skin infections, cholecystitis, meningitis and septicaemia. While gastrointestinal infections caused by NOVC usually have a favourable outcome, invasive infections may be fatal [2]. Immunocompromised patients, particularly those with hepatic cirrhosis or haematological malignancies, are at highest risk for septicaemia with NOVC [2,3]. In this paper, we report on 2 residents of Beirut who were diagnosed with NOVC and treated in a large public facility. They are, to our knowledge, the first reported cases of severe NOVC infection in Lebanon.

Background

In Lebanon, laboratory-confirmed cholera is a reportable disease by law. The serotype identification, however, is not required for this reporting (Epidemiological Surveillance Unit, www.public-health.gov.lb). Limited outbreaks usually associated with O139 Bengale serotype, have been historically described throughout the 20th century. In 1993, an epidemic, which started in neighbouring Syria, expanded into Lebanon causing a few deaths in Tripoli and Beirut and lasting for about 3 months [S.M. Adib, I. Hajj, E. Makarem. *Investigation of the cholera epidemic—Lebanon, 1993*, unpublished report]. Since then, and as post-civil war reconstruction efforts continued, no such large outbreaks were reported. However, cholera may still have limited niches of low endemicity, especially in Northern Lebanon, where sub-clinical cases may occur. An unknown number of summer acute diarrhoeas may actually be caused by various strains of cholera, yet no systematic bacteriological surveillance has been initiated to this day in the country. Moreover, infections due to non-O1/O139 and other *Vibrio* species do not have to be reported to the Ministry of Public Health.

Case 1

A 54-year-old man with a history of alcoholic liver cirrhosis was admitted with a 1-day history of abdominal pain, high-grade fever and fatigue. The patient had no diarrhoea. On examination, the patient was conscious, looking ill, dehydrated, with a temperature of 39.4 °C, pulse 110/min, and blood pressure 110/60 mmHg. Shifting dullness indicating an ascitis was present on abdominal examination. Initial laboratory results were notable for a white blood cell count of 9600/mm^3 with 85% neutrophils. Liver function tests were abnormal. The ascitic fluid revealed a white blood cell count of 270/µL with 90% neutrophils, but the culture was negative. Blood and urine specimens were submitted for culture prior to treatment with intravenous antibiotics and hydration. The patient received an initial regimen of ceftazidime and metronidazole to cover enteric organisms.

Anaerobic and aerobic blood cultures were positive on BACTEC 9240® system (Becton Dickinson Microbiology Systems) after 4 days of incubation. Colonies grew on MacConkey and chocolate agars. Gram stain revealed colonies of small, curved gram-negative rods. A subsequent subculture on thiosulfate citrate bile salt (TCBS) agar showed yellow, sucrose-fermenting, oxidase-positive colonies. Using a commercial system (API 20 E®, bioMerieux), our microbiology laboratory identified the isolate as *V. cholerae*. Using an automated identification system (Becton Dickinson Phoenix100®), we identified the organism as *V. cholerae*. Slide agglutination testing with polyvalent O1 antisera was negative.

The specimen was sent to the Centre National de Référence des Vibrions et du Choléra at the the Institut Pasteur (France) where non-toxigenic *V. cholerae* non-O1/non-O139 was identified. Using a disc diffusion testing according

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Received: 6/10/09; accepted: 08/03/10

to National Committee for Clinical Laboratory Standards guidelines 2008 [4], the organisms were found susceptible to ampicillin, ceftriaxone, tetracycline, chloramphenicol, cephalotin and trimethoprim-sulfamethoxazole, but resistant to nalidixic acid, polymixin, ciprofloxacin and colistin.

Meanwhile, fever, nausea, and abdominal pain resolved after 5 days in the hospital, and the patient was discharged in a good health status. Epidemiological investigation revealed no recent travel history and no exposure of skin to salted or fresh water. Additionally, the source of drinking water was bottled water, however, the patient revealed a consumption of fish and shrimps in the week prior to his admission.

Case 2

A female infant was born at 33 weeks gestation after a preterm, premature rupture of the membranes at 28 weeks gestation. The mother was admitted for chorioamnionitis and precipitated delivery.

The baby had an Apgar score of 8 at birth and was admitted to neonatal intensive care for prematurity. A few hours post-delivery, physical examination revealed a baby in distress with a temperature of 37 °C, a heart rate of 143 beats/min, a respiratory rate of 89 breaths/min, and a blood pressure of 41/25 mm Hg. The total white blood cell count was 22500/mm^3 with 62% neutrophils, the INR (international normalized ratio) was 3.2 with APTT (activated partial thromboplastin time) of 89 s. The blood pH fell to 7.11 and the CO_2 to 10 mmol/L. Blood and cerebrospinal fluid (CSF) cultures were taken and the baby was intubated and started on vasopressors (dopamine) and antibiotics (cefotaxime chloride, gentamicin and ampicillin). Two days later, the baby developed renal failure which forced the replacement of gentamicin and cefotaxime chloride by ciprofloxacin and imipenem/cilastin. She started having seizures and brain ultra sound revealed hydrocephalus and intraventricular haemorrhage. Meanwhile, CSF cultures revealed no growth, but isolates were recovered from the Standard Aerobic/F and Anaerobic/F blood culture bottles using the BACTEC 9240® system (Becton Dickinson Microbiology Systems). Colourless, lactose-negative colonies subsequently developed on MacConkey agar and grew on TCBS. Microscopically, the organisms were curved, Gram-negative bacilli. Biochemically, the isolates were oxidase-positive and were further identified by Api system (API 20 E®, bioMerieux), and by BD identification system (Becton Dickinson Phoenix®) as *V. cholerae*. The agglutination test for *Vibrio* O1 was negative. The isolate was referred to the Centre National de Référence des Vibrions et du Choléra of the Institut Pasteur in France, where non-toxigenic *V. cholerae* non-O1 non-O139 was identified. The isolate was sensitive to ampicillin, tetracycline, chloramphenicol, nalidixic acid, cephalotin, ciprofloxacin. It was resistant to erythromycin, trimethoprim-sulfamethoxazole and colistin.

Epidemiological investigation revealed no illness with diarrhoea in the patient's mother or other close contacts. During the second week post-delivery, this infant's condition started improving progressively under treatment. She gained weight, was extubated and antibiotics were discontinued 1 month later. She recovered completely and was discharged after 2 months of hospitalization.

Discussion

There are more than 200 serotypes of the bacterium *V. cholera*: O1 and O139 serogroups are toxin-producing and cause classical cholera with profuse, watery diarrhoea. The non-O1, non-O139 serogroups are usually non-epidemic strains reported as sporadic cases [5]. They can cause infections such as gastroenteritis, septicaemia, wound infection, meningitis and cholecystitis. [6–8]. Septicaemia in adults occurs mainly in patients with underlying liver cirrhosis [9,10] or other conditions associated with immunodeficiency, haematological malignancies, diabetes, AIDS or lymphoma [11].

Case 1 is likely to be the first case of NOVC which caused spontaneous peritonitis in a cirrhotic patient in Lebanon. Additionally, the infant case presented is one of only a handful of paediatric NOVC cases ever described, sometimes associated with meningitis [7,12].

Seawater seems to be the main natural reservoir for non-O1 non-O139 *V. cholerae* since it requires trace amounts of sodium chloride for growth. However, it can also grow in fresh water [13]. These organisms have been isolated from surface water in several locations around the world [14–16]. Additionally, the association of NOVC with marine plankton has been demonstrated in the Mediterranean. However, no reports are available of isolation of NOVC from coastal waters nor of surface water contamination in Lebanon.

Generally, the most common source of NOVC infection is consumption of contaminated raw or undercooked seafood. Contamination can also occur due to direct invasion through abraded skin or wound [5]. In the 2 cases presented here, the source of the infection could not be determined. In the adult case, consumption of local fish and seafood (shrimps) in the week prior to admission was revealed. It was not possible, however, to obtain samples of the food items consumed. Nor was it possible to investigate the persons who shared these meals with the patient because the initial exposure occurred a while before that patient reached the hospital.

No apparent source of infection could be identified in the neonatal case although the contamination probably occurred during or before delivery. The

mother denied any consumption of seafood or contact with seawater. At the time of delivery, the mother was free of NOVC contamination. Since the patient lives in a low socioeconomic community in the coastal area of the southern suburbs of Beirut, it seems possible that drinking water drawn from wells in the area may be contaminated by seawater backflow.

To date, there are no published guidelines for antibiotic therapy of NOVC infection [17]. In our cases, the pattern of sensitivity to antibiotics varied only slightly, suggesting a common source of contamination. Both strains were sensitive to ampicillin and cephalosporins. The first strain was resistant to ciprofloxacin and nalidixic acid, and both were resistant to colistin.

Prognosis in NOVC bacteraemia is less favourable than in isolated NOVC gastroenteritis. Two separate reviews of NOVC gastroenteritis showed a 100% survival rate [18,19]. In contrast, NOVC bacteraemia is a potentially fatal disease. The case-fatality in a series of 15 cases in Taiwan was 47% [2]. Another review of 20 cases from Florida reported a 25% case-fatality rate [19]. Therefore, it was gratifying to obtain a total recovery in the 2 cases described in this report.

Among infectious disease agents, *Vibrio* species cause a potential threat to public health. NOVC strains, which contaminate the marine environment near coastal lines, should be monitored carefully to detect a risk of outbreaks. Today, NOVC infection may be more common than toxigenic *V. cholerae* O1 or O139 in Lebanon. This situation may justify the addition of NOVC infections to the list of diseases with mandatory notification to the Ministry of Public Health. National surveillance for all *Vibrio* species would increase our knowledge of the burden and epidemiology of these potential pathogens and provide important measures for assessing the effectiveness of interventions to control *Vibrio* illness. Additionally, monitoring the plankton on algal blooms in the Lebanese seawater is important since NOVC survives on those blooms and can contaminate local fish and seafood.

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