A fatal *Vibrio cholerae* O37 enteritis

Isolates of non-O1 and non-O139 *Vibrio cholerae* are associated with sporadic diarrhoeal disorders, limited enteritis outbreaks or extraintestinal infections, especially in immunocompromised patients with haematological malignancies, ascites, cirrhosis and renal failure. In Italy, very few cases of intestinal and extraintestinal infections caused by non-O1 and non-O139 *V. cholerae* have been described in the past, as reported by Piersimoni *et al.* (1991), Farina *et al.* (1999, 2000) and Ottaviani *et al.* (2009).

A 54-year-old Egyptian man living in northern Italy was admitted on 5 August 2008 to the Dialysis Unit of the AO ‘Ospedale San Carlo Borromeo’, Milano, Italy, to be suddenly submitted to haemodialysis (blood urea nitrogen 323 mg dl$^{-1}$). The patient, with a well-known history of asthma, hypertension, hydrenephrosis of the left kidney, urethral stenosis and uraemic pericarditis treated by pericardiectomy and *Salmonella* orchitis 1 year previously, had been regularly treated for 3 years with haemodialysis three times per week because of chronic renal failure. At admission, he declared his recent return from Alexandria, Egypt, where he stayed with his family for 3 weeks. Two days before coming back, he consumed undercooked fish and vegetables along the Nile river, and a few hours later he presented with vomiting and nausea.

A few hours after haemodialysis, the patient presented with greenish diarrhoea and vomiting, abdominal tenderness and fever (temperature 38.5 $^\circ$C). He had no skin lesions. Blood analysis showed a leukocyte count of 9690 ml$^{-1}$ (neutrophils 73.3 %). Blood chemistry values were as follows: blood urea nitrogen, 164 mg dl$^{-1}$; creatinine, 17.38 mg dl$^{-1}$; troponin I, 0.55 ng ml$^{-1}$; sodium, 137 mmol l$^{-1}$; potassium, 4.94 mmol l$^{-1}$. Prothrombin time international normalized ratio was 1.50, glutamic oxaloacetic transaminase was 55 U l$^{-1}$ and glutamic pyruvic transaminase was 40 U l$^{-1}$. The C-reactive protein level was 24.90 mg dl$^{-1}$ and the erythrocyte sedimentation rate was 82 mm h$^{-1}$.

He was treated with parenteral rehydration, acidosis correction and antibiotic therapy (ampicillin, then imipenem plus sulfamethoxazole–trimethoprim). An abdominal examination revealed a diffuse tenderness in the right hypochondrium but no resistance to palpation. Abdominal ultrasound and abdominal tomography demonstrated a reduction of the renal cortex, a normal liver, but no ascites or splenomegaly.

Nine blood samples were negative. Faeces were collected daily for 10 days: two samples yielded non-O1 and non-O139 *V. cholerae*, finally serotyped as *V. cholerae* O37. The strain was susceptible in vitro to several antimicrobial agents, including ampicillin, first-, second- and third-generation cephalosporins, imipenem, gentamicin, chloramphenicol, sulfamethoxazole–trimethoprim and quinolones, when tested by the agar disc diffusion method according to CLSI (2006) guidelines.

The patient’s condition became worse, and he died on 16 August because of hypovolaemic shock and ventricular fibrillation. The autopsy revealed end-stage kidney disease with haemodialysis-associated amyloidosis and left ureteral stenosis with stent. The gastrointestinal tract showed congestion and only a moderate infiltration of mononuclear inflammatory cells with focal superficial necrosis not directly related to *V. cholerae*. In addition, shock-related focal myocardial necrosis, diffuse vascular congestion, lung oedema, chronic uraemic pericarditis and endomyocarditis were observed. Culture of all tissues was negative for *V. cholerae*. Pus collected from the kidney and bladder yielded *Salmonella enterica* group C.

The *V. cholerae* O37 strain was further characterized for different virulence properties by using phenotypic tests and PCR assays to detect the presence of toxin genes (Table 1).

Cholera (the duba in Arabic) is caused by *V. cholerae* strains belonging only to serovars O1 and O139: it is well known in the Mediterranean area where it has been endemic since the 19th century when it was first described in Egypt (1832).

*V. cholerae* non-O1 and non-O139 strains have been shown to cause gastroenteritis in humans often presenting with an underlying hepatic, renal or haematological chronic disease. The mortality of non-O1, non-O139 *V. cholerae* systemic infections is high at 24–62 % (Phetsouvanh *et al.*, 2008).

The patient in the case presented here had a long history of chronic renal failure and steroid treatment, and may have contracted the non-O1, non-O139 *V. cholerae* infection from undercooked fish and vegetables consumed along the Nile river.

Previous studies (Lukinmaa *et al.*, 2006) demonstrated that, in the absence of cholera toxin, toxin co-regulated pilus (*tcpA* and *tefP*), zonula occludens toxin (*zot*) and non-O1 *V. cholerae* heat-stable enterotoxin (NAG-ST), non-O1, non-O139 *V. cholerae* still has the ability to cause diarrhoea by a mechanism entirely different from that of the toxigenic *V. cholerae* strains. In these cases, haemolysis, cytotoxicity and proteolytic activity were demonstrated to be the putative virulence factors (Iyer *et al.*, 2000; Restrepo *et al.*, 2006).

In agreement, our isolate, characterized by the genotype *hlyA*ET$^+$ *stru/stov* $^{-}$ *ctx* $^{+}$ *tcpA* $^{+}$ *zot* $^{-}$ *hlyA* class $^{-}$, was cytotoxic, showed haemolytic and proteolytic activities and was able to colonize the intestine of a suckling mouse. This report confirms that non-toxigenic, non-O1, non-O139, O37 *V. cholerae* can be isolated from patients suffering from a cholera-like syndrome after consumption of contaminated food.
Table 1. Virulence properties of the isolate and the control strains

<table>
<thead>
<tr>
<th>Strain characteristics</th>
<th>Result</th>
<th>Method</th>
<th>Positive control strain</th>
<th>Negative control strain</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of gene for cholera toxin (ctx)</td>
<td>Negative</td>
<td>PCR</td>
<td>V. cholerae O1 non-toxigenic Italian environmental isolate</td>
<td>V. cholerae O1 classical biotype ATCC 9459</td>
<td>Fields et al. (1992)</td>
</tr>
<tr>
<td>Presence of gene for heat-stable toxin (stn/sto)</td>
<td>Negative</td>
<td>PCR</td>
<td>V. cholerae O1 classical biotype ATCC 9459</td>
<td>V. cholerae VCC043</td>
<td>Guglielmetti et al. (1994)</td>
</tr>
<tr>
<td>Presence of gene for V. cholerae O1 classical biotype haemolysin (hlyA)</td>
<td>Positive</td>
<td>PCR</td>
<td>V. cholerae O1 El Tor strain from a 1994 Italian outbreak</td>
<td>–</td>
<td>Rivera et al. (2001)</td>
</tr>
<tr>
<td>Presence of gene for toxin co-regulated pilus (tcpA)</td>
<td>Negative</td>
<td>PCR</td>
<td>V. cholerae O1 classical biotype ATCC 9459</td>
<td>V. cholerae O1 classical biotype ATCC 9459</td>
<td>Rivera et al. (2001)</td>
</tr>
<tr>
<td>Presence of gene for toxin co-regulated pilus (tcpI) and for zonula occludens toxin (zot)</td>
<td>Negative</td>
<td>Culture in AKI medium</td>
<td>–</td>
<td>–</td>
<td>Saha et al. (1996)</td>
</tr>
<tr>
<td>Production of haemolysin</td>
<td>Positive</td>
<td>Suckling mouse model</td>
<td>–</td>
<td>–</td>
<td>Angelichio et al. (1999)</td>
</tr>
<tr>
<td>Induction of intestinal colonization</td>
<td>Positive</td>
<td>Reverse passive latex agglutination test for VT1-2</td>
<td>–</td>
<td>–</td>
<td>VTEC-RPLA (Oxoid)</td>
</tr>
<tr>
<td>Production of Shiga toxin</td>
<td>Negative</td>
<td>Cytoxicity testing on cellular lines</td>
<td>–</td>
<td>–</td>
<td>Luzzi et al. (1992)</td>
</tr>
</tbody>
</table>

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