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, hydronephrosis of the left kidney, urethral stenosis and uraemic pericarditis treated by pericardiotomy 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 °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 hypocondrium 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 *tcpI*), 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 *hlyAET*⁺ *stn/sto*⁻ *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

Strain characteristics	Result	Method	Positive control strain	Negative control strain	Reference
Proteolytic activity	Positive	Single diffusion method	—	—	Iyer <i>et al.</i> (2000)
Presence of gene for cholera toxin (<i>ctx</i>)	Negative	PCR	<i>V. cholerae</i> 569B classical Inaba <i>V. cholerae</i> O1 strain from a 1994 Italian outbreak	<i>V. cholerae</i> O1 non-toxigenic Italian environmental isolate	Fields <i>et al.</i> (1992)
Presence of gene for heat-stable toxin (<i>stn/sto</i>)	Negative	PCR	<i>V. cholerae</i> VCC043	<i>V. cholerae</i> strain VC032	Guglielmetti <i>et al.</i> (1994)
Presence of gene for <i>V. cholerae</i> O1 classical biotype haemolysin (<i>hlyA</i>)	Negative	PCR	<i>V. cholerae</i> O1 ATCC 9459		Rivera <i>et al.</i> (2001)
Presence of gene for <i>V. cholerae</i> O1 El Tor haemolysin (<i>hlyA</i>)	Positive	PCR	<i>V. cholerae</i> O1 authors' collection		Rivera <i>et al.</i> (2001)
Presence of gene for toxin co-regulated pilus (<i>tcpA</i>)	Negative	PCR	<i>V. cholerae</i> O1 classical biotype ATCC 9459 <i>V. cholerae</i> O1 El Tor biotype (<i>ctxA</i> and <i>zot</i> +) authors' collection		Rivera <i>et al.</i> (2001)
Presence of gene for toxin co-regulated pilus (<i>tcpI</i>) and for zonula occludens toxin (<i>zot</i>)	Negative	PCR	<i>V. cholerae</i> O1 classical biotype ATCC 9459		Rivera <i>et al.</i> (2001)
Production of haemolysin	Negative	Culture in AKI medium	—	—	Saha <i>et al.</i> (1996)
Induction of intestinal colonization	Positive	Suckling mouse model	—	—	Angelichio <i>et al.</i> (1999)
Production of Shiga toxin	Negative	Reverse passive latex agglutination test for VT1-2	—	—	VTEC-RPLA (Oxoid)
Cytotoxic factor for Hep-2 and Vero cells	Positive	Cytotoxicity testing on cellular lines	—	—	Luzzi <i>et al.</i> (1992)

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Angelichio, M. J., Spector, J., Waldor, M. K. & Camilli, A. (1999). *Vibrio cholerae* intestinal population dynamics in the suckling mouse model of infection. *Infect Immun* **67**, 3733–3739.

CLSI (2006). *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently*

Isolated or Fastidious Bacteria. Approved Standard M45-A. Wayne, PA: Clinical and Laboratory Standards Institute.

Farina, C., Luzzi, I. & Lorenzi, N. (1999). *Vibrio cholerae* O2 sepsis in a patient with AIDS. *Eur J Clin Microbiol Infect Dis* **18**, 203–205.

Farina, C., Gneccchi, F., Luzzi, I. & Vailati, F. (2000). *Vibrio cholerae* O2 as a cause of a skin lesion in a tourist returning from Tunisia. *J Travel Med* **7**, 92–94.

Fields, P. I., Popovic, T., Wachsmuth, K. & Olvich, O. (1992). Use of polymerase chain reaction for detection of toxigenic *Vibrio cholerae* O1 strains from the Latin America cholera epidemic. *J Clin Microbiol* **30**, 2118–2121.

Guglielmetti, P., Bravo, L., Zanchi, A., Montè, R., Lombardi, G. & Rossolini, G. M. (1994). Detection of the *Vibrio cholerae* heat-stable enterotoxin gene by polymerase chain reaction. *Mol Cell Probes* **8**, 39–44.

- Iyer, L., Vadivelu, J. & Puthucheary, S. D. (2000). Detection of virulence associated genes, haemolysin and protease amongst *Vibrio cholerae* isolated in Malaysia. *Epidemiol Infect* **125**, 27–34.
- Lukinmaa, S., Mattila, K., Lehtinen, V., Hakkinen, M., Koskela, M. & Siitonen, A. (2006). Territorial waters of the Baltic Sea as a source of infections caused by *Vibrio cholerae* non-O1, non-O139: report of 3 hospitalized cases. *Diagn Microbiol Infect Dis* **54**, 1–6.
- Luzzi, I., Minelli, F., Gianviti, A. & Caprioli, A. (1992). Impaired detection of faecal verocytotoxin in the presence of *Clostridium difficile* cytotoxin in patients with haemolytic uraemic syndrome. *Eur J Clin Microbiol Infect Dis* **11**, 934–936.
- Ottaviani, D., Leoni, F., Rocchegiani, E., Santarelli, S., Masini, L., Di Trani, V., Canonico, C., Pianetti, A., Tega, L. & Carraturo, A. (2009). Prevalence and virulence properties of non-O1 non-O139 *Vibrio cholerae* strains from seafood and clinical samples collected in Italy. *Int J Food Microbiol* **132**, 47–53.
- Phetsouvanh, R., Nakatsu, M., Arakawa, E., Davong, V., Vongsouvath, M., Lattana, O., Moore, C. E., Nakamura, S. & Newton, P. N. (2008). Fatal bacteremia due to immotile *Vibrio cholerae* serogroup O21 in Vientiane, Laos – a case report. *Ann Clin Microbiol Antimicrob* **7**, 10.
- Piersimoni, C., Morbiducci, V. & Scalise, G. (1991). Non-O1 *Vibrio cholerae* gastroenteritis and bacteraemia. *Lancet* **337**, 791–792.
- Restrepo, D., Huprikar, S. S., VanHorn, K. & Bottone, E. J. (2006). O1 and non-O1 *Vibrio cholerae* bacteremia produced by haemolytic strains. *Diagn Microbiol Infect Dis* **54**, 145–148.
- Rivera, I. N. G., Chun, J., Huq, A., Sack, R. B. & Colwell, R. R. (2001). Genotypes associated with virulence in environmental isolates of *Vibrio cholerae*. *Appl Environ Microbiol* **67**, 2421–2429.
- Saha, P. K., Koley, H. & Nair, G. P. (1996). Purification and characterisation of an extracellular secretogenic non membrane damaging cytotoxin produced by clinical strains of *Vibrio cholerae* stable enterotoxin gene by polymerase chain reaction. *Infect Immun* **64**, 3101–3108.