Severe infection with *Clostridium difficile* PCR ribotype 027 acquired in Melbourne, Australia

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We report the first recognised case of infection with *Clostridium difficile* PCR ribotype 027 acquired in Australia. This pathogen has caused significant morbidity and mortality in widespread hospital-based outbreaks in the northern hemisphere. Clinicians need to be aware of the clinical picture, limitations of diagnostic tests, availability of further testing for epidemic strains, new therapeutic approaches, and in-hospital control strategies for this infection. (MJA 2011; 194: 369-371)

Clinical record

An 83-year-old Latvian man underwent an aortic valve replacement for aortic stenosis in late January 2010 at a hospital in Melbourne, Australia. He had a history of hypertension and chronic renal failure. He lived alone in his own home, and had not travelled outside Australia since September 2009 when he returned from a 3-month trip to Latvia. Between his return to Australia and the surgery, he had not received any antibiotics except for a single preoperative dose of cephalothin. His regular medications included various supplements, but no proton-pump inhibitor. He was admitted to the hospital the day before surgery. Two days after the surgery, he developed severe sepsis from a urinary tract infection, for which he received ticarcillin–clavulanate and a noradrenaline infusion. A coagulase-negative *Staphylococcus* was isolated from blood cultures, and he was given vancomycin. He later developed an infiltrate at the left lung base, but no change was made to his therapy.

Five days after the surgery, he developed watery diarrhoea. *Clostridium difficile* was isolated from stool samples, although the results of enzyme-linked fluorescent assays (VIDAS, bioMérieux, Sydney, NSW) for *C. difficile* toxins were negative at this time. His leukocyte count was 9.5×10⁹/L (reference range, 4.0–11.0×10⁹/L) and his serum albumin concentration was 42 g/L (reference range, 35–50 g/L). Therapy with metronidazole (400 mg orally, 8-hourly) was commenced along with vancomycin. A coagulase-negative *Staphylococcus* was isolated from stool samples, although the results of enzyme-linked fluorescent assays (VIDAS, bioMérieux, Sydney, NSW) for *C. difficile* toxins were negative at this time. His leukocyte count was 9.5×10⁹/L (reference range, 4.0–11.0×10⁹/L) and his serum albumin concentration was 42 g/L (reference range, 35–50 g/L). Therapy with metronidazole (400 mg orally, 8-hourly) was commenced for presumed *C. difficile* infection (CDI), and the patient was placed under contact precautions. Alcohol-based hand rub was replaced with traditional soap and water hand washing (see below). Therapy with ticarcillin–clavulanate was subsequently ceased.

After 9 days of metronidazole therapy, the diarrhoea became more frequent and vancomycin concentration of >32 μg/L. The stool sample was positive by real-time polymerase chain reaction (PCR, GenXpert, Cepheid, Sunnyvale, Calif, USA) when tested for the presence of *C. difficile* organisms carrying genes for toxin B (tdcB), binary toxin (cdtB) and an 18-base-pair deletion within the tcdC gene that is characteristic of the PCR ribotype 027 strain. These findings were confirmed by sequencing the tcdC gene, and this also identified a point mutation at nucleotide position 117, which is also characteristic of this strain. PCR ribotyping was undertaken using a previously published method¹ that confirmed the isolate as PCR ribotype 027 (Box).

Nineteen days after surgery, the patient’s condition deteriorated further. His temperature was 39.2°C, his leukocyte count was 31.2×10⁹/L and his serum albumin concentration was 25 g/L. The diarrhoeal frequency fell to a single bowel action per day, and an abdominal x-ray showed a distended right colon. The oral vancomycin dose was increased to 500 mg, 6-hourly, and therapy with intravenous metronidazole was commenced along with vancomycin enemas (500 mg in 500 mL normal saline, 6-hourly). Ticarcillin–clavulanate therapy was recommenced. A surgical opinion was sought and subtotal colectomy discussed. As there was felt to be a high risk of mortality with surgery, medical management was preferred.

After 5 days, the fever and diarrhoea improved. The enemas were ceased after 8 days and metronidazole therapy after 14 days. The patient subsequently recovered, and the diarrhoea had not recurred at 3-month follow-up.

**Discussion**

An epidemic strain of *C. difficile* (PCR ribotype 027) was first identified in Quebec Province in Canada in 2005, as a cause of hospital

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outbreaks of severe infection with high mortality rates.\(^5\) Retrospective analyses suggested that this strain had caused outbreaks across North America since 2000.\(^3\) The organism later spread to Europe, and cases have now been described in Asia and Central America.\(^4\) Increased toxin production by *C. difficile* PCR ribotype 027 may be responsible for its increased virulence,\(^5\) and fluoroquinolone resistance is likely to be contributing to its spread.\(^6\) Infection with this strain more often leads to severe disease, and is associated with more recurrences and a greater risk of death.\(^2\) Until now, only one case has been described in Australia in a patient who was thought to have acquired the infection in North America.\(^7\)

This is the first case of hypervirulent CDI diagnosed in Australia with apparent local acquisition. Several factors support the conclusion that the infection was not acquired overseas. First, although the patient had travelled to Latvia 4 months before being admitted, the possibility that he acquired *C. difficile* PCR ribotype 027 then and remained colonised is remote. *C. difficile* does not colonise the normal adult gastrointestinal tract, and the patient received no antibiotics that may have disrupted his gut flora in the time between returning from Latvia and admission to hospital. Second, a recent publication from Latvia indicates that *C. difficile* ribotype 027 was not present in the country when our patient was there.\(^8\) Finally, there were at least two other subsequently confirmed cases of infection with *C. difficile* PCR ribotype 027 in the hospital at the time the patient developed symptoms of infection (it is not known where these cases were acquired).

The case illustrates important features of hypervirulent CDI. The identification of severe disease is critical in guiding management. For surveillance purposes, severe disease may be simply identified as infections requiring ICU admission or surgery, or infections resulting in death, or a diagnosis of toxic megacolon.\(^9\) More sensitive diagnostic criteria for severe disease in addition to those above are required to guide patient care. While no such criteria have yet been prospectively validated, proposed markers of severe disease include age greater than 65 years, leukocytosis greater than 20 × 10\(^9\) cells/L, deterioration of renal function, temperature greater than 38.3°C, serum albumin concentration less than 25 g/L and an elevated serum lactate concentration.\(^10\) Our patient met all these criteria except for the serum lactate concentration, which was not recorded.

Although metronidazole remains the recommended first-line agent for mild to moderate CDI, oral vancomycin is now recommended for severe disease.\(^9,10\) Although there is no evidence that high-dose oral vancomycin (500 mg, 6-hourly) is any better than standard doses of 125 mg 6-hourly, higher doses are favoured by many clinicians. Evidence of benefit for vancomycin enemas is limited to case series; eight of nine patients with refractory severe disease had complete resolution with this therapy.\(^11\) In the setting of ileus with toxic megacolon, oral vancomycin will not reach the colon and intravenous delivery of metronidazole is preferable.\(^12\)

Surgery should be considered if severe disease is unresponsive to medical therapy after 48 hours, or if there is bowel perforation or multiorgan failure.\(^13\) Elevation of plasma lactate to between 2.2 and 4.9 mmol/L has been identified in a retrospective review of a selected group of severely ill patients as a guide to when colectomy is most beneficial.\(^13\) Other strategies requiring further investigation for use in severe disease include intravenous immunoglobulin, alternative antibiotics such as tigecycline, and monoclonal antibodies.

*C. difficile* spores are highly resistant to killing by alcohol and most other disinfectants. In outbreaks of CDI, health care workers should be instructed to wash their hands with soap and water in addition to using alcohol-based hand disinfection when caring for infected patients. Patients should be isolated and contact precautions with gowns and gloves are recommended. Environmental cleaning with hypochlorite-based solutions is necessary to eliminate the spores.\(^9\) Antibiotic stewardship is also an important element in control strategies, with studies of antibiotic restriction showing benefit.\(^14\)

In Australia, laboratory diagnosis of CDI is most commonly made through detection of *C. difficile* toxins A and B using enzyme immunoassay (ELA) kits. ELA kits have reported sensitivities of 75%–95%, but most of the evaluations reporting these sensitivities use faecal cytotoxin detection (a flawed test) as the gold standard.\(^15\) In addition, the positive predictive value of these tests declines markedly in situations where the prevalence of disease is low.\(^16\) Poor sensitivity of the assay is the likely explanation for the initial negative result of the enzyme-linked fluorescent assay in our case. Despite this limitation, ELA kits remain widely used because of their simplicity and relatively low cost. Commercial real-time PCR testing for toxin genes (usually tcdB), has better sensitivity (93%) and specificity (97%),\(^16\) and is now available in several Australian laboratories. Toxigenic culture — isolation of the organism followed by toxin testing of the isolate — is extremely sensitive, but it is labour intensive and takes at least 3 days.\(^16\) There is currently great debate about the value of an algorithmic approach to diagnosing CDI, whereby a sensitive screening test is used to screen out negatives, thus improving the positive predictive value of a secondary test, particularly when the prevalence of infection is low.\(^17\)

Distinguishing *C. difficile* PCR ribotype 027 from other strains of *C. difficile* does not affect individual patient management, but is important for surveillance purposes. Some commercially available PCR methods can presumptively identify PCR ribotype 027 based on detection of binary toxin genes and the characteristic 18-base-pair deletion in the tcdC gene. An alternative, less expensive, approach is to screen for moxifloxacin resistance by using a 5 μg moxifloxacin disc on a lawn culture of *C. difficile* on Mueller-Hinton agar. Worldwide, most PCR ribotype 027 isolates are resistant to moxifloxacin,\(^2\) while, in Australia, the prevalence of resistance in all strains of *C. difficile* is 1%. (TV Riley, B Elliot and colleagues, unpublished data). Zones of inhibition for resistant strains (potentially PCR ribotype 027) are >16 mm while for susceptible strains, they are ≥16 mm (TV Riley and colleagues, unpublished data). Isolates of moxifloxacin-resistant *C. difficile* identified in this way can then be sent for further typing. Given the arrival of *C. difficile* PCR ribotype 027 in this country, periodic PCR ribotyping of a representative sample of isolates now needs to be performed at designated reference laboratories Australia-wide, and recurrent funding is required for this task. This will be an important adjunct to local screening measures, and will also detect the emergence of virulent PCR ribotypes other than 027. An Australian Commission on Safety and Quality in Healthcare recommendation for hospital surveillance programs in all states and territories to monitor *C. difficile* was approved by Australian Health Ministers in November 2008. As yet, the states and territories have not implemented this recommendation, and there has been no collation or analysis of national surveillance data.

With the identification of the first case of PCR ribotype 027 *C. difficile* infection acquired locally, it is important that clinicians in Australia are aware of the clinical picture, limitations of diagnostic tests, availability of further testing for PCR ribotype 027, new therapeutic approaches,\(^18\) and in-hospital control strategies for this infection.\(^19\) The solution to the bigger problem of the emergence of virulent strains of *C. difficile* continues to lie in the basics of surveillance, antimicrobial stewardship, infection control and environmental cleanliness.
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Competing interests
Thomas Riley has undertaken contract research for Cepheid.

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