First Australian isolation of epidemic *Clostridium difficile* PCR ribotype 027

Thomas V Riley, Sarah Thean, Graham Hool and Clayton L Golledge

We report the first isolation in Australia of a hypervirulent epidemic strain of Clostridium difficile, *PCR ribotype 027. It was isolated from a 43-year-old woman with a permanent ileostomy, who appears to have been infected while travelling in the United States. The isolate was positive for toxin A, toxin B and binary toxin, and resistant to fluoroquinolone antimicrobials, and had characteristic deletions in the* tcdC *gene. All diagnostic laboratories and health care facilities in Australia should now be on high alert for this organism.*

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Clinical record

A 43-year-old woman was admitted to a Perth hospital at the end of October 2008. She had been diagnosed with ulcerative colitis 8 years previously and, in 2002, underwent proctocolectomy and permanent ileostomy. Since then, she had experienced a number of stoma problems requiring surgical repair or local revision. On this occasion, computed tomography (CT) showed a parastomal small-bowel herniation, with a normal appearance on ileoscopy. The herniation was repaired with intraperitoneal mesh.

After the operation, the patient developed small-bowel ileus and was placed on total parenteral nutrition. She then developed a central-line infection, with both *Serratia marcescens* and *Staphylococcus epidermidis* isolated from a central venous catheter tip, for which she was treated with intravenous cefepime and vancomycin (each, 1 g 12-hourly) for 10 days. A subsequent ileoscopy did not show any mucosal abnormality. Culture and faecal cytotoxin testing of the stoma fluid for *Clostridium difficile* was negative before her discharge from hospital at the end of November 2008.

The patient subsequently travelled to the United States and, while in New York City on Christmas Day, became unwell with high ileostomy output, cramping abdominal pain and vomiting. Despite progressively worsening symptoms, she travelled to Hawaii via Vancouver, Canada. On arrival in Hawaii, she required hospitalisation and was admitted to an intensive care unit in Honolulu on 6 January 2009 with a diagnosis of complicated *C. difficile* infection with generalised sepsis and acute renal failure. An ileoscopy showed diffuse inflammation and ulceration of the ileal mucosa. She recovered slowly after treatment with oral vancomycin (250 mg 6-hourly), and was discharged after 14 days.

On her arrival back in Australia, the symptoms recurred. An ileoscopy on 4 February 2009 showed a single inflamed ulcerated area close to the stoma, and biopsy specimens of this area were reported as consistent with pseudomembranous enteritis and *C. difficile* infection (Box 1). Culture of the ileostomy fluid again resulted in the isolation of toxigenic *C. difficile*.

The isolate was determined to be positive for toxin A (tcdA), toxin B (tcdB), and binary toxin (CDT) by polymerase chain reaction (PCR) testing for toxin genes (*tcdA*, including the repetitive region, *tcdB*, and both the *cdtA* and *cdtB* binary toxin genes).[1,2]

The antimicrobial susceptibility profile of the isolate on E-strip testing (AB bioMérieux) indicated fluoroquinolone resistance:

- penicillin, susceptible (S) (minimum inhibitory concentration [MIC], 0.75 mg/L);
- clindamycin, S (MIC, 2 mg/L);
- metronidazole, S (MIC, 0.38 mg/L);
- levofloxacin, resistant (R) (MIC, > 32 mg/L);
- moxifloxacin, R (MIC, 16 mg/L); and
- vancomycin, S (MIC, 0.38 mg/L).

The *tcdC* gene (which encodes a negative regulator in toxin production) was sequenced and found to contain an 18-base-pair deletion, as well as a single nucleotide deletion at position 117,[3] characteristic of the epidemic *C. difficile* strain, PCR ribotype 027. On the basis of these findings, the patient's isolate was PCR ribotyped,[4] which confirmed it to be *C. difficile* PCR ribotype 027 (Box 2). Because of the severity of the patient's initial illness, she was treated with a further 14-day course of oral vancomycin (250 mg 6-hourly). Her condition improved, and she has remained well since, with two negative cultures for *C. difficile* since cessation of vancomycin.

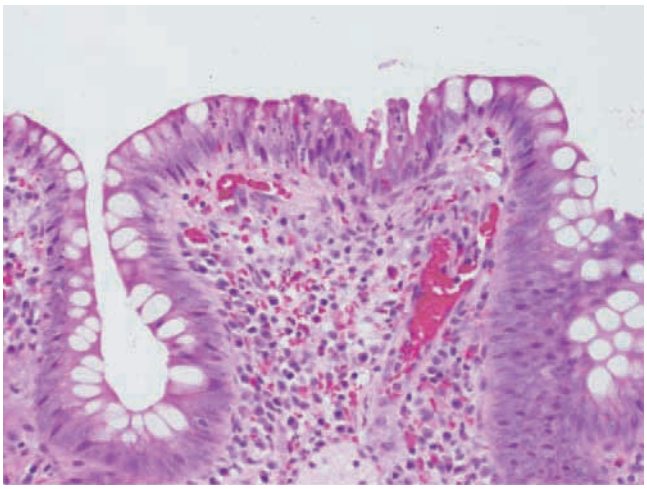
Discussion

A hypervirulent, epidemic strain of *C. difficile*, PCR ribotype 027, has been responsible for outbreaks of severe disease in North America and Europe. This organism is characterised by production of increased quantities of toxins A and B, plus an additional, binary toxin (actin-specific ADP-ribosyltransferase), and fluoroquinolone resistance. Overuse of fluoroquinolones is probably driving epidemic spread of this strain in North America and Europe, and attributable mortality in people aged over 60 years who are infected has been over 10%. There has been concern in Australia because of the lack of suitable surveillance systems to detect the entry of epidemic *C. difficile* into this country;[5] this is the first report of such an occurrence.

This case is unusual because infection apparently occurred while the patient was travelling. Travel-associated *C. difficile* infection is extremely rare and, to our knowledge, acquisition of *C. difficile* during travel has never been proven. However, *C. difficile* is known to cause diarrhoea in travellers, as a result of antibiotics given either as prophylaxis before the journey, or as treatment for traveller's diarrhoea afterwards. Indeed, in 1995, we reported three cases of laboratory-proven *C. difficile* infection following doxycycline administration for malaria prophylaxis.[6] All three patients apparently acquired the organism outside Australia, although this could not be proven as no cultures for *C. difficile* were performed before travel. Six cases of *C. difficile* infection were recently

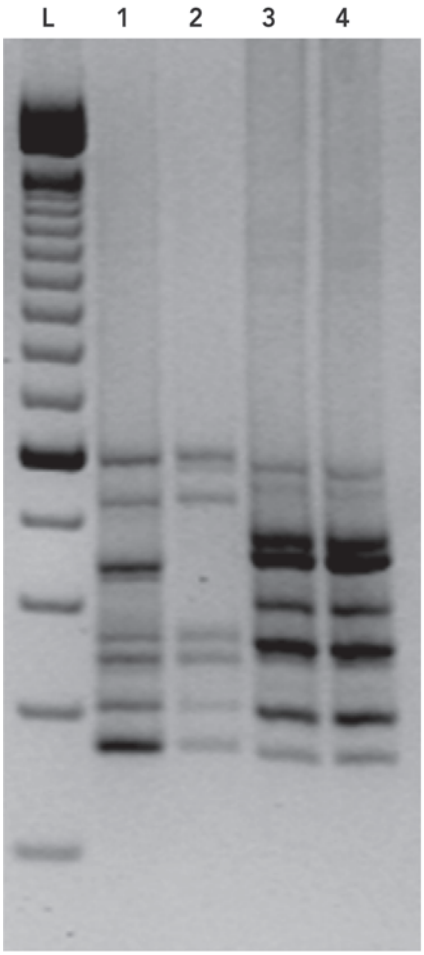
For editorial comment, see page 661.

1 Ileal biopsy specimen from the patient, February 2009



Mucosa adjacent to the ulcer showed acute inflammation, with neutrophils invading the surface mucosa (stain, haematoxylin and eosin; original magnification, × 400). ◆

2 PCR ribotyping of the *Clostridium difficile* strain isolated from the patient in February 2009



Polymerase chain reaction (PCR) amplification of ribosomal RNA intergenic spacer regions results in specific banding patterns (ribotypes), which can be used to genetically fingerprint strains of Clostridium difficile.

Ribotyping showed the similarity between the patient's isolate (lane 4) and the epidemic 027 strain (lane 3).

Key to lanes:
L *= molecular weight ladder*
1 *= reference strain, VPI 10463*
2 *= ribotype 014 (the most common ribotype in Australia [unpublished data])*
3 *= ribotype 027 (epidemic strain)*
4 *= patient's isolate.* ◆

reported in Spain in travellers who took antibiotics to treat an acute diarrhoeal episode and subsequently presented with prolonged or recurrent gastrointestinal symptoms, including diarrhoea.[7] Interestingly, the first isolation of *C. difficile* PCR ribotype 027 in Austria was from a British tourist who was admitted to a hospital in Tyrol with a 5-day history of nausea, watery diarrhoea and lower abdominal pain. She was reportedly taking antibiotics prescribed by her physician to treat bronchitis, and the authors believed she acquired the strain in Great Britain before travel.[8]

We believe our patient most likely acquired *C. difficile* infection in New York City. According to the US Centers for Disease Control and Prevention, *C. difficile* PCR ribotype 027 has now been detected in 40 US states, including New York.[9] It is less likely that she was infected while passing through Canada, even though PCR ribotype 027 is thought to be endemic in the western part of that country.[10] In either case, as she had not been in contact with the health care system at that stage of the trip, community acquisition is most likely. Recent reports suggest that community acquisition of *C. difficile* is increasing worldwide.[11]

The lesions associated with *C. difficile* infection in humans are generally restricted to the colon, but pseudomembrane formation has been described in patients with an ileostomy.[12] Our patient may have been at risk of *C. difficile* infection for two reasons. First, in ulcerative colitis, the intestine is known to be more readily colonised by *C. difficile*, and much of this colonisation occurs in the community rather than the health care setting.[13] To what extent that risk is modified by colectomy is not known. The upper gastrointestinal tract microflora in patients with an ileostomy is similar to that of the colon 1 to 3 weeks after the ileostomy.[14] Second, our patient had completed a course of antibiotics about 1 month earlier, and the "normal" microflora may not yet have re-established enough to provide any protection.

We were fortunate that this patient was seen at an institution that routinely cultures for *C. difficile*. Currently, most laboratories in Australia do not culture for *C. difficile*, instead relying on either enzyme immunoassays or PCR tests. As molecular typing is required to identify *C. difficile* PCR ribotype 027, at a minimum, all patients with severe, suspected *C. difficile* infection should have specimens cultured, and any isolates should be sent to a laboratory with expertise in identifying epidemic strains. General practioners and diagnostic laboratories need to be reminded that patients presenting with community-acquired or travel-related diarrhoea may have *C. difficile* infection. Periodic targeted surveillance with molecular typing of *C. difficile* isolates should be funded by government, as suggested previously,[5] until more rapid molecular diagnostic tests to identify epidemic strains are developed.

We were also fortunate that the patient was seen as an outpatient on her return to Australia, and did not require hospital admission, minimising the possibility of contamination and spread of epidemic *C. difficile* within the hospital setting. However, this case exemplifies the ease with which this organism could be introduced into Australia. The conservative policies on fluoroquinolone use in this country may afford some protection against the establishment of epidemic *C. difficile*. Ciprofloxacin and moxifloxacin are the only fluoroquinolones available in Australia; levofloxacin, gatifloxacin and others are not. Nonetheless, all diagnostic laboratories and health care facilities in Australia should now be on high alert for the epidemic strain of *C. difficile*.

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