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CASE REPORT

Infection with Toxin A-Negative, Toxin B-Negative, Binary Toxin-Positive *Clostridium difficile* in a Young Patient with Ulcerative Colitis

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Large clostridial toxin-negative, binary toxin-positive (A^- B^- CDT^+) strains of *Clostridium difficile* are almost never associated with clinically significant *C. difficile* infection (CDI), possibly because such strains are not detected by most diagnostic methods. We report the isolation of an A^- B^- CDT^+ ribotype 033 (RT033) strain of *C. difficile* from a young patient with ulcerative colitis and severe diarrhea.

CASE REPORT

A 15-year-old boy from Perth, Western Australia, Australia, presented at the Fremantle Hospital emergency department (Fremantle, Western Australia, Australia) with a 4-week history of diarrhea and significant weight loss of approximately 7 kg. These symptoms had failed to respond to oral metronidazole and norfloxacin prescribed by his general practitioner. Hematological investigation revealed moderate thrombocytosis and a platelet count of 765×10^9/liter (normal range, 150 to 400 $\times 10^9$/liter) but no neutrophilia or hypoalbuminemia. A stool specimen was negative for *Clostridium difficile* glutamate dehydrogenase (GDH) antigen and toxins A and B by the *C. difficile* Quik Chek Complete rapid membrane enzyme immunoassay kit (Techlab Inc., Blacksburg, VA, USA), and no other enteric pathogens were detected, including norovirus, rotavirus, adenovirus, enterohemorrhagic *Escherichia coli*, salmonella, shigella, cryptosporidium, and parasitic ova and cysts. He was discharged home the same day but presented at the Princess Margaret Hospital for Children (PMH) emergency department in Perth nearly 1 week later with severe diarrhea (approximately 15 bowel movements a day), abdominal pain, further weight loss, fever, and lethargy. Fecal microscopy demonstrated leukocytes and erythrocytes but failed to identify any ova, cysts, or parasites, and again no other enteric pathogens were detected. Two stool specimens were negative for toxins by cell cytotoxin neutralization assay (CCNA) but grew *C. difficile* on cycloserine cefoxitin fructose agar (CCFA). Although *C. difficile* was isolated, it was considered nontoxigenic due to the lack of fecal cytotoxin. The patient's fecal calprotectin levels were high, 760 μg/g (normal reference range, < 100 μg/g), typical of intestinal inflammation in active inflammatory bowel disease (IBD), colon cancer, and infectious diarrhea. He had an erythrocyte sedimentation rate (ESR) of 63 mm/h (normal range, 1 to 15 mm/h) with moderate thrombocytosis, a platelet count of 602×10^9/liter, and a low hemoglobin level of 113 g/liter (normal range, 125 to 170 g/liter). Endoscopic investigation revealed erythematous, friable mucosa with superficial ulceration indicative of moderate to severe colitis, but no pseudomembranes. Histology showed mucin depletion and a dense inflammatory infiltrate with crypt abscess formation but without any granulomata, findings characteristic of ulcerative colitis (UC). Treatment was commenced with 45 mg oral prednisolone daily and 1 g oral mesalamine every 8 hours with subsequent symptomatic improvement.

In view of the patient's diarrheal symptoms, the *C. difficile*-positive stool specimens were forwarded to PathWest Laboratory Medicine (PathWest) for molecular typing. *C. difficile* was grown on ChromID media (bioMérieux) under anaerobic conditions (A35 anaerobic workstation; Don Whitley Scientific, Shipley, West Yorkshire, United Kingdom) and then characterized by toxin gene profiling and PCR ribotyping as previously described (1). Consistent with the earlier laboratory diagnostic tests, *C. difficile* ribotype 033 (RT033), negative for the toxin genes *tcdA* and *tcdB* but positive for binary toxin genes (*cdtAB*), was identified from the specimens.

Two weeks later, in parallel with decreasing steroid dose, the patient's symptoms flared and he was rehospitalized for 5 days for symptomatic control. Another stool sample was collected at PMH and tested for *C. difficile* with a newly acquired test platform, the Cepheid Xpert *C. difficile*/Epi. This assay detects *tcdB* (encoding toxin B) and *cdtA* (encoding the A subunit of binary toxin, or CDT). Both targets were detected in the patient's stool sample; however, only *C. difficile* RT014/020 was isolated at PathWest. This result was inconsistent with the result obtained with the Xpert *C. difficile*/Epi, which detected *cdtA* in the stool specimen. *C. difficile* RT014/020 produces toxins A and B only and not CDT. The variability in results suggested possible infection by two different RTs of *C. difficile*. *C. difficile* RT033 was previously detected in the patient's stool sample; thus, it was possible that this ribotype was responsible for the CDT-positive result obtained. Susceptibility testing using an agar dilution method and CLSI/EUCAST in-

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terpretive criteria (2) showed that both RTs were susceptible to clindamycin, moxifloxacin, metronidazole, and vancomycin. The MICs of fidaxomicin and rifaximin were 0.06 mg/liter and 0.004 mg/liter, respectively. Despite treatment with 400 mg oral metronidazole every 8 hours, the patient's symptoms worsened. He was rehospitalized, given 125 mg oral vancomycin every 6 hours for 5 days, and then commenced on 125 mg oral azathioprine daily because of steroid-resistant UC. His stool became culture negative for *C. difficile*, and vancomycin was ceased. Repeat tests a week later at PMH confirmed the absence of *C. difficile* as well as other enteric pathogens.

Three weeks later the patient re-presented with fever, bloody diarrhea, and increased stool frequency (15 to 20 times a day). Stool cultures once again grew no *C. difficile*. He was commenced on intravenous infliximab (5 mg/kg dose) and, with some clinical improvement, resumed outpatient care. After 2 months, a routine stool sample tested positive for *C. difficile* by culture on CCFA and a week later his diarrhea returned, with fever and increased stool frequency of 20 times a day. He was administered 500 mg intravenous metronidazole every 8 hours, and subsequent stool samples did not grow *C. difficile*. Tests for other enteric pathogens, including cytomegalovirus, were negative. Biopsies of the rectum and the sigmoid and ascending colon were performed and excluded cytomegalovirus colitis.

Nine months after his first presentation, he was again hospitalized with severe diarrhea and 20 bowel movements per day. Due to the frequency of presentations, severity of colitis, and lack of response to medical therapy, a colectomy was performed. Histopathology of the colon showed destruction of crypt architecture, focal crypt abscesses, inflammatory infiltrate in the lamina propria, and focal multinucleated giant (MNG) cells, but no pseudomembranes, ischemic changes, or granulomas, in keeping with UC.

The connection between CDI and IBD was initially demonstrated in 1980 (3). The association between the two diseases was related to the underlying dysbiosis in IBD patients, which favored colonization by *C. difficile* with symptomatic exacerbations and disease complications (3–5). Patients with UC and Crohn's disease have always been at a higher risk of developing CDI than non-IBD individuals and have a higher mortality rate and increased odds of colectomy following CDI (6).

An estimated cost of $68.2 million was attributed to hospitalization due to IBD-related CDI (IBD-CDI) in 2011 in the United States, compared to $8.7 million in 1997 (7). This increase may be due to improved assessment by attending physicians; however, diagnosis of CDI in IBD patients remains complex. Acute IBD and CDI have similar clinical features of diarrhea, abdominal pain, fever, leukocytosis, anemia, and hypoalbuminemia. Therefore, unless a test for *C. difficile* is specifically requested, IBD-CDI episodes often remain undetected (4, 6). This similarity in presentation is complicated by an overlap in histological features that make it difficult to determine the significance of *C. difficile* in stool specimens of IBD-CDI patients (5, 6). Also noteworthy is that prior antibiotic use and age, major risk factors in the development of CDI, are not prerequisites in IBD-CDI development (6). For these reasons, accurate diagnosis and surveillance of CDI within this population are essential for effective patient management.

The major virulence factors of *C. difficile* are the toxins A and B, the genes for which, *tcdA* and *tcdB*, are also the targets for most diagnostic methods. Consequently, most reported cases of IBD-CDI are due to A^+ B^+ *C. difficile* strains and rarely A^- B^- strains. CDT is a third toxin produced by some *C. difficile* strains (8). Since CDT was first reported in 1988, its role in CDI has been overlooked, and a possible role in IBD-CDI has never been considered. CDT is beginning to receive attention due to its increasing prevalence in human and animal isolates of *C. difficile* (1, 9). In the present case, A^- B^- CDT^+ *C. difficile* RT033 was isolated during recurrent episodes of severe diarrhea in the absence of other enteric pathogens.

Commonly associated with animal infection, *C. difficile* RT033 belongs to clade 5 of *C. difficile*, a clade that is known to cause significant mortality and that contains the "hypervirulent" RT078 strain (10). *C. difficile* RT033 is frequently isolated from calves and neonatal pigs in Australia and other parts of the world (1, 11). Its recent isolation from symptomatic patients suggests potential zoonotic transmission and the possibility of disease occurring when CDT only is produced (1, 9). Detection of *C. difficile* strains such as the RT033 strain in diagnostic laboratories is not common, because most of the current methods used, apart from culture, do not detect A^- B^- CDT^+ *C. difficile* strains (12). Unfortunately, our case was complicated by the detection of a RT014/020 strain of *C. difficile* in addition to RT033 from one of the patient's stool samples. This ribotype is highly prevalent in human infections around the world (13) and may have had a contributory role, although RT033 was the only enteric pathogen isolated during the initial diarrheal episodes. There are no clear guidelines for the therapy of IBD-CDI; however, it is recommended that patients receive treatment on the basis of the severity of disease, including recurrences, as with CDI patients (6). However, the management of CDI differs from the management of acute episodes of IBD, and it is important to specifically test for *C. difficile* in IBD patients presenting with diarrhea to avoid potentially exacerbating the CDI with immunosuppressant agents used to treat IBD.

The clinical challenge of distinguishing CDI and acute IBD episodes when *C. difficile* is detected is significant. Does the bacterium act as a pathogen triggering symptoms, does it merely represent carriage, or has there been a relapse of IBD? Despite the difficulty in attributing causality to either strain of *C. difficile* in our case, this report highlights three important issues: (i) increasing severe CDI in patients with IBD; (ii) the isolation of strains of possible animal origin; and (iii) the lack of diagnostic tests to detect A^- B^- strains of *C. difficile*.

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