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Recurrent *Clostridioides* (*Clostridium*) *difficile* infection in a patient suffering from inflammatory bowel disease and benefits of resistotyping

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ABSTRACT

Two-year *Clostridioides (Clostridium) difficile* recurrences in a boy with ulcerative colitis are described. Isolates were toxin A/B positive and nonhypervirulent, and resistotypes of 2017 isolates differed from those in 2016, suggesting a reinfection, later confirmed by multilocus sequence typing (ST49 and ST92, respectively). Resistotypes may show the need of genotypic analysis.

Clostridioides (*Clostridium*) *difficile* infection (CDI) involves antibiotic-associated diarrhea and pseudomembraneous colitis due to enterotoxin and/or cytotoxin and, by some strains, binary toxin production (Shields et al., 2015). Inflammatory bowel disease (IBD) patients have a 33% higher risk for recurrent CDI (rCDI) due to intestinal dysbiosis, frequent (≥2) hospitalizations within previous months, weakened mucosal immunity, and exposure to immunosuppressive drugs and antibiotics (Nasiri et al., 2018; Razik et al., 2016; Shields et al., 2015; Zilberberg et al., 2014).

The aim of the study was to evaluate the 2-year course of ulcerative colitis aggravated by rCDI in a 15-year-old boy and to distinguish between relapses and reinfections.

On April 2015, the patient had colic pains and mucus- and blood-containing stool. Ulcerative colitis was diagnosed by colonoscopy and histology. The patient was treated with mesalazine previously already for 1 year; his stool specimen sent on May 20, 2015, was *C. difficile* negative by both direct toxin test (DTT) and culture. In this regard, it is worth noticing the good results in most ulcerative colitis patients by anti-TNF therapy with infliximab or adalimumab following mesalazine use (Fredericks et al., 2018).

Most specimens were evaluated by DTT (RIDA®Quick *Clostridium difficile* Toxin A/B, R-biopharm AG, Germany), culture, and PCR. Following ethanol shock, the specimens were plated onto both selective medium (*Clostridium difficile* agar base, Liofilchem, Italy) with 5% sheep blood and selective supplement (Oxoid, UK) containing norfloxacin 12 mg/L and moxalactam 32 mg/L, and nonselective medium (Anaerobe basal agar, Oxoid), and were incubated anaerobically at 37 °C for 2–3 days. Isolates were identified by RapID ANA II (Oxoid). Metronidazole, tetracycline, clindamycin, levofloxacin, and vancomycin susceptibility was tested by E tests (Liofilchem). Susceptibility to metronidazole and vancomycin was read according to EUCAST breakpoints (EUCAST, 2018). Toxigenic culture was done for all *C. difficile* isolates.

PCR was performed using primers for *gluD* gene (*C. difficile* glutamate dehydrogenase) (Paltansing et al., 2007); *tcdA* (toxin A), *tcdB* (toxin B), *cdtA*, and *cdtB* (binary toxin) genes (Terhes et al., 2004); and *tcdC* region prone to in-frame deletions (Persson et al., 2008, 2011). Multilocus sequence typing (MLST) was performed for two 2016 isolates and two 2017 isolates as described by Griffiths et al. (2010) (Table 1). A few colonies were emulsified in TE (Tris-EDTA) buffer, heated at 100 °C for 10 min, and centrifuged at 13,000 rpm for 5 min, and the supernatant was used for the PCRs and MLST. We sequenced 7 *C. difficile* housekeeping genes (*adk*, *atp*, *dxr*, *glyA*, *rec*, *sodA* and *tpi*). Assignment to allele numbers, clade, and sequence types (ST) was performed according to PubMLST-*Clostridium difficile* MLST databases (http://pubmlst.org/cdifficile).

On April 25, 2016, owing to colic pains and mucus- and blood-containing stool, the patient was hospitalized and the specimen yielded

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Table 1
Evaluations of the stool specimens of the patient and *C. difficile* isolates.

Date	Antibiotic therapy[a]	Direct toxin test[b]	Toxigenic culture[b]	MICs[a] (mg/L)					MLST[c] type
				MET	TET	CLI	LEV	VAN	
May 20, 2015	None	Neg	Neg	NA	NA	NA	NA	NA	
April 11, 2016	None	NR	Pos	0.5	64	0.25	>32	0.75	92
April 25, 2016	MET IV 7j, oral MET, 500 mg daily for 7 days	NR	Pos	0.5	64	0.25	>32	0.75	
June 8, 2016	Oral MET, 500 mg daily for 13 days	Neg	Pos	0.5	64	0.25	>32	0.75	92
July, 8, 2016	None, control of therapy	Neg	Neg	NA	NA	NA	NA	NA	
October 18, 2016	Ceftriaxone IV, oral MET 500 mg twice daily	Neg	Neg	NA	NA	NA	NA	NA	
November 14, 2016	None	Neg	Neg	NA	NA	NA	NA	NA	
January 9, 2017	None	Pos	Pos	0.125	1	8	>32	0.38	49
March 4, 2017	Oral MET 500 mg twice daily for 1 month since January 9, 2017	Neg	Pos	0.125	1	8	>32	0.38	49
April 4, 2017	Oral VAN pulsed therapy for 6 weeks since March 4, 2017	Neg	Neg	NA	NA	NA	NA	NA	
May 3, 2017	None, control of therapy	Neg	Neg	NA	NA	NA	NA	NA	

[a] Antibiotics: MET = metronidazole, TET = tetracycline, CLI = clindamycin, LEV = levofloxacin, VAN = vancomycin. In addition, the patient was treated for the ulcerative colitis with mesalazine, corticosteroids (methylprednisolone), and azathioprine (see the text).
[b] Pos = positive, Neg = negative, NR = not required by the clinicians, NA = not appropriate.
[c] MLST = multilocus sequence typing.

a toxigenic *C. difficile* strain. The boy was treated with metronidazole parenterally for 7 days and then by oral metronidazole (500 mg daily) for 7 days.

Two weeks later, a toxigenic isolate was detected again, showing the same resistotype and PCR patterns. The strain was detected again on June 8, 2016; however, the DTT was negative, while the toxigenic culture and PCR were positive. Oral metronidazole (500 mg daily) was applied for 13 days. The patient was also treated by methylprednisolone and azathioprine for the ulcerative colitis. Control stool specimens taken on July 8, October 18, and November 14, 2016, were *C. difficile* negative by both DTT and culture.

Antibiotic minimal inhibitory concentrations (MICs) of the 2016 isolates are given in Table 1. The 3 isolates, obtained in 2016, were PCR positive for *C. difficile gluD* gene and *cdtA* and *cdtB* genes, and were binary toxin (*cdtA* and *cdtB*) gene negative. No major *tcdC* deletions were detected by PCR.

On January 9, 2017, the boy had symptom worsening, and a toxigenic *C. difficile* isolate (toxin A and B positive, binary toxin negative) was detected by DTT, toxigenic culture, and PCR. However, the resistotype of the isolate differed from those of the 2016 isolates. The patient was treated by oral metronidazole (500 mg twice daily) from January 9 to February 13, 2017. Nevertheless, at the end of February, symptoms and blood in the stool reappeared, and a toxigenic *C. difficile* isolate was found by toxigenic culture on March 4, 2017. The tetracycline MICs of the 2017 isolates were 64-fold lower and clindamycin MICs were 32-fold higher than those of the 2016 isolates (64 and 0.25 mg/L, respectively). There were also differences between MICs of metronidazole and vancomycin of the isolates from 2016 and those from 2017. Most importantly, the 2016 and 2017 strains showed different MLST types; the 2016 isolates were ST92 (*adk*-1,*atp*-1,*dxr*-2, *glyA*-3, *rec*-1,*sodA*-5,*tpi*-1) and the 2017 isolates were ST49 (*adk*-1,*atp*-1,*dxr*-2, *glyA*-1, *rec*-5,*sodA*-3,*tpi*-3), and both STs were clustered in clade I.

Because of the previous metronidazole treatments, pulsed-tapered vancomycin therapy was started on March 4, 2017. The therapy involved oral vancomycin for 6 weeks (40 mg/kg/day, divided into 4 doses for 14 days, followed by 10 mg/kg/day twice daily for 1 week, 10 mg/kg/day once daily for a week and, finally, 10 mg/kg/day 3 times weekly for 2 weeks). Control stool specimens on April 4 and May 3, 2017, were *C. difficile* negative by both DTT and toxigenic culture.

In this work, the ulcerative colitis, immunosuppressive treatments, and frequent hospitalizations were risk factors for CDI. Direct A/B toxin test was negative twice, when toxigenic culture was positive. Therefore, performing toxigenic culture following a negative DTT is important in IBD patients. All isolates were binary toxin negative according to PCR for *cdtA* and *cdtB* genes and the lack of *tcdC* deletions. This shows that, in the present case, the isolates were not hypervirulent. Despite the repetitive metronidazole treatments, all isolates were susceptible to the drug.

Although it is difficult to distinguish reinfection from relapse without performing ribotyping, CDI in 2017 appeared half a year following that in April 2016, suggesting a reinfection rather than a relapse. Reinfections usually occur later than relapses and are linked to frequent hospitalizations in IBD patients (Barbut et al., 2000).

Notably, in our study, the 2017 isolates exhibited different resistotypes compared with those in 2016, and the reinfection was confirmed by MLST, revealing 2 different (ST92 and ST49) MLST types. Therefore, resistotypes can indicate a reinfection and the subsequent need of ribotyping or MLST of isolates as well as searching for the infection source.

Recommendations for rCDI involve treatment with oral vancomycin or fidaxomicin, pulsed-tapered therapy with vancomycin or fidaxomicin, and fecal microbiota transplantation (Shields et al., 2015). In addition to the rCDI, fecal transplantation has been considered as a possible treatment option also for IBD with a 75% success rate within 2 months (Cho et al., 2018; Davidovics et al., 2019).

Metronidazole, if administered intravenously, reaches too low concentrations and can explain the failure of therapy (Hernández et al., 2017). Pulsed dosing vancomycin regimens have been more successful to treat rCDI compared with standard nonpulsed therapy (Shields et al., 2015) and should be considered for IBD patients. In the present case, although all isolates were metronidazole susceptible and nonhypervirulent, vancomycin was more effective compared with metronidazole.

In conclusion, the ulcerative colitis, immunosuppressive treatments, and multiple hospitalizations were associated with rCDI and symptom aggravation in the patient. It was important to test for CDI in IBD patients by culture and toxigenic culture. Multiple metronidazole treatments did not affect *C. difficile* susceptibility to the agent. Comparing *C. difficile* resistotypes by susceptibility testing to multiple antibiotics can suggest a possible reinfection and the requirement of following genotypic analysis and evaluation of the infection source. As stressed by Burke and Lamont (2014), CDI is a serious worldwide problem requiring increasing awareness of both clinicians and laboratory staff.

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