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In Vitro Activities of Fidaxomicin and Its Metabolite OP-1118 Against Clinical Isolates of Toxin-Positive *Clostridium difficile* Cultured from Diarrheal Stool Specimens in Canada: CAN-DIFF 2013

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REVISED ABSTRACT

Background: Clinical microbiology laboratories do not routinely culture *C. difficile* (CD) toxin-positive (TP) stool specimens or perform antimicrobial susceptibility testing (AST) on these isolates. As the epidemiology, susceptibility, and pathogenicity of TP-CD evolves, periodic surveillance for antimicrobial resistance and ribotypes may be useful. The current study assessed the *in vitro* activities of 6 routinely tested anti-anaerobic agents and the new, oral, narrow-spectrum macrocyclic antimicrobial, fidaxomicin, and its active metabolite, OP-1118, against TP-CD isolates collected in Canada in 2013.
Methods: Isolates of CD (*n* = 379) were cultured on *Clostridium difficile* Moxalactam Norfloxacin (CDMN) agar from TP stool specimens. Each isolate's identity was confirmed by Gram stain, typical odor, latex agglutination or a positive L-proline aminopeptidase activity test, and chartreuse fluorescence under UV light. Antimicrobial susceptibility testing was performed using the agar dilution method recommended by CLSI (M11-A8, 2012). Genotyping was performed by PCR ribotyping.
Results: All CD isolates tested were susceptible to metronidazole and amoxicillin-clavulanate. Isolate percent susceptibilities were 9.5, 9.8, and 63.6%, respectively, for clindamycin, ceftriaxone, and moxifloxacin. MIC ranges (µg/mL) were ≤0.015-1 for fidaxomicin, ≤0.06-32 for OP-1118, and 0.5-4 for vancomycin. A significant difference in % susceptibility for ribotype 027 isolates (n = 22) compared with non-ribotype 027 isolates (n = 92) was only identified for moxifloxacin (P<0.001; 0% susceptible for ribotype 027 isolates versus 78% susceptible for non-ribotype 027 isolates). Fidaxomicin and OP-1118 had MIC_{50}s and MIC_{90}s one doubling-dilution higher for ribotype 027 isolates than for non-ribotype 027 isolates.
Conclusion: Fidaxomicin and its active metabolite, OP-1118, demonstrated potent *in vitro* activity against TP-CD, including ribotype 027 isolates.

BACKGROUND

Clostridium difficile is the most frequently identified infectious cause of nosocomial diarrhea, occurring primarily in patients previously receiving antimicrobial agents. Antimicrobial susceptibility testing is rarely performed for *C. difficile* because of its complexity, clinical significance, and cost. Management of patients with *C. difficile* infection (CDI) includes withdrawal of the predisposing antimicrobial agent, if possible, and empiric therapy most commonly with either metronidazole or oral vancomycin. Recent publications have reported an increasing risk of treatment failure and CDI recurrence for patients treated with metronidazole (1-4) and have discouraged the use of vancomycin to treat CDI in hospitals to minimize the risk of vancomycin resistance in enterococci and staphylococci (5). As the adequacy of metronidazole and vancomycin as empiric therapies may be suspect and the epidemiology, susceptibility, and pathogenesis of *C. difficile* evolves, routine surveillance of clinical isolates to determine their *in vitro* susceptibility profiles, and studies determining the activities of newer agents such as fidaxomicin and OP-1118, the active metabolite of fidaxomicin, as well as other investigational agents is warranted.

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RESULTS

Table 1. Antimicrobial susceptibility testing results for 379 toxin-positive isolates of *C. difficile*

		MIC (µg/ml)			MIC interpretation		
Antimicrobial agent	Range	Mode	MIC_{50}	MIC_{90}	% S	% I	% R
Fidaxomicin	≤0.015-1	0.5	0.25	0.5	NA	NA	NA
OP-1118	≤0.06-32	8	8	16	NA	NA	NA
Metronidazole	0.25-8	0.5	0.5	2	100	0	0
Vancomycin	0.5-4	1	1	1	NA	NA	NA
Amoxicillin-clavulanate	≤0.25-8	1	1	2	100	0	0
Clindamycin	≤0.12->64	4	4	16	9.5	50.1	40.4
Moxifloxacin	≤0.25->32	1	2	>32	63.6	1.1	35.3
Ceftriaxone	≤0.5->128	32	32	64	9.8	56.7	33.5

NA – MIC interpretive breakpoints not available; S: susceptible, I: intermediate, R: resistant

Table 2. Distribution of MICs for antimicrobials tested against 379 toxin-positive isolates of *C. difficile*

	Number of isolates for which the antimicrobial agent MIC (µg/ml) was:												
Antimicrobial agent	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64
Fidaxomicin	6[a]	5	11	83	94	163	17						
OP-1118			8[a]		1	3	17	56	102	115	76	1	
Metronidazole					29	184	66	91	6	3			
Vancomycin						61	288	24	6				
Amoxicillin-clavulanate					10[a]	10	211	144	3	1			
Clindamycin				7[a]	3	1	1	24	190	113	2		38[b]
Moxifloxacin					6[a]	4	134	97	4	2	49	83[c]	
Ceftriaxone						8[a]	1		1	1	26	215	127[d]

[a] Isolate count shown for lowest the dilution tested; some MICs may be lower than the lowest dilution tested.
[b] 35/38 isolate MICs for clindamycin were >64 µg/ml.
[c] 64/83 isolate MICs for moxifloxacin were >32 µg/ml.
[d] 16/127 isolate MICs for cetriaxone were >128 µg/ml.

Table 3. PCR ribotyping analysis for 114 isolates

Ribotype	*n* (% of all isolates)
027	22 (19.3%)
014	10 (8.8%)
106	7 (6.1%)
020	6 (5.3%)
ns37	5 (4.4%)
002	4 (3.5%)
017	4 (3.5%)
087	4 (3.5%)
056	3 (2.6%)
072	3 (2.6%)
ns25	3 (2.6%)
015	2 (1.8%)
046	2 (1.8%)
075	2 (1.8%)
076	2 (1.8%)
ns18	2 (1.8%)
ns28	2 (1.8%)
ns36	2 (1.8%)
Singular ribotypes	29 (25.4%)

Table 4. Antimicrobial susceptibility testing results for 114 toxin-positive isolates of *Clostridium difficile* stratified according to PCR ribotype

		MIC (µg/ml)				MIC interpretation		
Antimicrobial agent	Ribotype	Range	Mode	MIC_{50}	MIC_{90}	% Susceptible	% Intermediate	% Resistant
Fidaxomicin	027	0.25-1	0.5	0.5	1	NA	NA	NA
	014	0.12-0.5	0.12	0.12	0.5	NA	NA	NA
	Non-027 Ribotypes	0.03-1	0.5	0.25	0.5	NA	NA	NA
OP-1118	027	8-16	8	8	16	NA	NA	NA
	014	1-8	2/4	2	4	NA	NA	NA
	Non-027 Ribotypes	0.5-16	4	4	8	NA	NA	NA
Metronidazole	027	1-2	2	2	2	100	0	0
	014	0.5-1	0.5/1	0.5	1	100	0	0
	Non-027 Ribotypes	0.25-2	0.5	0.5	2	100	0	0
Vancomycin	027	0.5-4	1	1	1	NA	NA	NA
	014	0.5-1	1	1	1	NA	NA	NA
	Non-027 Ribotypes	0.5-4	1	1	1	NA	NA	NA
Amoxicillin-clavulanate	027	0.5-4	2	2	2	100	0	0
	014	0.5-1	1	1	2	100	0	0
	Non-027 Ribotypes	0.5-4	1	1	2	100	0	0
Clindamycin	027	2->64	4	4	8	4.6	63.6	31.8
	014	4	4	4	4	0	100	0
	Non-027 Ribotypes	1->64	4	4	64	8.5	59.8	31.7
Moxifloxacin	027	16->32	>32	>32	>32	0	0	100
	014	1-2	2	2	2	100	0	0
	Non-027 Ribotypes	1->32	1	2	16	78	2.5	19.5
Ceftriaxone	027	32->64	64	64	64	0	9.1	90.9
	014	16-64	32	32	32	20	70	10
	Non-027 Ribotypes	16->64	32	32	64	6.1	73.2	20.7

METHODS

Bacterial isolates studied. 379 isolates of *C. difficile* were cultured on *Clostridium difficile* Moxalactam Norfloxacin (CDMN) Selective Supplement agar (Oxoid Canada, Nepean, ON, Canada) from TP stool specimens (following an ethanol shock step) submitted by 8 hospital clinical microbiology laboratories across Canada to the Winnipeg Health Sciences Centre. Each isolate's identity was confirmed by Gram stain, typical odor, latex agglutination (Microgen Bioproducts Ltd., Surrey, UK) or a positive L-proline aminopeptidase activity test, and chartreuse fluorescence under UV light (6).
Antimicrobial susceptibility testing. Antimicrobial susceptibility testing for fidaxomicin, OP-1118, and 6 additional agents was performed using the agar dilution method recommended by CLSI (7). Fidaxomicin and OP-1118 were supplied by Cubist Pharmaceuticals, Inc.; the solvent for both compounds was DMSO; water was used as the diluent. *C. difficile* ATCC 700057 was used as the control strain; the reference MIC range for this strain was 0.03-0.25 µg/ml for fidaxomicin. *In vitro* susceptibility testing interpretive criteria for fidaxomicin have not been determined; CLSI breakpoints were used to interpret MICs for the other antimicrobial agents tested (8).
PCR Ribotyping. 114 isolates were ribotyped at the National Microbiology Laboratory-Public Health Agency of Canada, using an internationally-standardized, high-resolution capillary gel-based electrophoresis PCR-ribotyping protocol for *C. difficile* (9).

CONCLUSIONS

- All TP *C. difficile* isolates tested were susceptible to metronidazole and amoxicillin-clavulanate; 9.5, 9.8, and 63.6% of isolates were susceptible to clindamycin, ceftriaxone, and moxifloxacin, respectively. The MIC_{90}s for fidaxomicin and vancomycin were 0.5 and 1 µg/ml, respectively (Table 1).
- Against TP clinical isolates of *C. difficile*, the potencies of the 8 agents tested, based upon MIC_{90}s, were: fidaxomicin > vancomycin > metronidazole = amoxicillin-clavulanate >> OP-1118 = clindamycin >> ceftriaxone = moxifloxacin (Table 1).
- The highest MIC reported for fidaxomicin was 1 µg/ml compared with 4 ug/ml for vancomycin and 8 µg/ml for amoxicillin-clavulanate and metronidazole (Table 2).
- There was tremendous ribotype diversity among the isolates of *C. difficile* tested (Table 3).
- Ribotype 027 was the most frequent identified ribotype, accounting for approximately 20% of isolates; all ribotype 027 isolates were resistant to moxifloxacin (Table 3).
- Ribotype 014 isolates were more susceptible to moxifloxacin, clindamycin, and ceftriaxone than were other ribotypes (Table 4).
- Fidaxomicin demonstrated greater *in vitro* potency than vancomycin, metronidazole, and amoxicillin-clavulanate based upon MIC_{90}s and had a lower maximum MIC (1 µg/ml) than did the three other agents (4-8 µg/ml).
- A significant difference in % susceptibility for ribotype 027 isolates (n = 22) compared with non-ribotype 027 isolates (n = 92) was only identified for moxifloxacin (P < 0.001; 0% susceptible for ribotype 027 isolates versus 78% susceptible for non-ribotype 027 isolates).
- Fidaxomicin and OP-1118 had MIC_{50}s and MIC_{90}s one doubling-dilution higher for ribotype 027 isolates than for non-ribotype 027 isolates.
- Fidaxomicin and its active metabolite, OP-1118, demonstrated potent *in vitro* activity against TP-CD, including ribotype 027 isolates.

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