E-1680

In Vitro Activities of Fidaxomicin, Its Metabolite OP-1118, and Comparative Agents Against Clinical Isolates of Toxin-Positive *Clostridium difficile* Cultured from Diarrheal Stool Specimens in Canada

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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. Ongoing surveillance for antimicrobial resistance and NAP types may be useful as the epidemiology and pathogenicity of TP-CD evolves. The current study assessed the in vitro activities of 7 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 2006-2007.

Methods: Isolates of CD (*n* = 440 to date) were cultured on CDMN agar from TP stool specimens. Each isolate's identity was confirmed by Gram stain, typical odor, latex agglutination (Microgen), and chartreuse fluorescence under UV light. Antimicrobial susceptibility testing was performed using the agar dilution method recommended by CLSI (M11-A8, 2012). A modified method employing *Sma*I digested DNA separated by PFGE was performed to identify NAP types (Alfa *et al.* J Clin Microbiol 2000;38:2706-14).

Results: All CD isolates tested were susceptible to metronidazole, amoxicillin-clavulanate, and meropenem. Isolate percent susceptibility was 0.5, 13.4, and 59.9%, respectively, for ceftriaxone, clindamycin, and moxifloxacin. MIC ranges (µg/mL) were 0.12-1 for fidaxomicin, 0.25-16 for OP-1118, and 0.5-4 for vancomycin. The NAP2 genotype was associated with the highest levels of resistance to ceftriaxone, clindamycin, and moxifloxacin and was frequently multidrug-resistant (MDR); MIC distributions for fidaxomicin were indistinguishable for NAP2 and non-NAP isolates.

Conclusion: Fidaxomicin and its metabolite, OP-1118, demonstrated potent in vitro activity against TP-CD. Fidaxomicin was equally active versus various CD NAP types including isolates with MDR phenotypes.

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 and cost. Management of patients with *C. difficile* infection (CDI) includes withdrawal of the predisposing antimicrobial agent, if possible, and empiric therapy with either metronidazole or oral vancomycin. Recent publications have reported an increasing risk of treatment failure and CDI recurrence for patients treated with metronidazole (1-3) and have discouraged the use of vancomycin to treat CDI in hospitals to minimize the risk of vancomycin resistance in enterococci and staphylococci (4). As the adequacy or acceptability of current empiric therapies may be suspect and the epidemiology and pathogenicity of *C. difficile* evolves, routine surveillance of clinical isolates to determine their in vitro susceptibility profiles and studies determining the activities of newer and investigational agents is warranted.

REFERENCES

1. Musher DM, et al. 2005. Clin Infect Dis 40:1586-90.
2. Pepin J, et al. 2005. Clin Infect Dis 40:1591-7.
3. McDonald LC, et al. 2006. Emerg Infect Dis 12:409-15.
4. HICPAC. 1995. Infect Control Hosp Epidemiol 16:105-13.
5. Karlowsky JA, et al. 2008. Antimicrob Agents Chemother 11:4163-5.
6. National Committee for Clinical Laboratory Standards. 2002. M35-A.
7. Karlowsky JA, et al. 2012. J Med Microbiol 61:693-700.
8. Clinical and Laboratory Standards Institute. 2012. M11-A8.
9. Alfa MJ, et al. 2000. J Clin Microbiol 38:2706-14.
10. Miller M, et al. 2010. Clin Infect Dis 50:194-201.
11. Martin H, et al. 2008. J Clin Microbiol 46 :2999-3004.

RESULTS

Table 1. Antimicrobial susceptibility testing results for 440 toxin-positive isolates of *C. difficile*

	MIC (µg/ml)				MIC interpretation		
Antimicrobial agent	Range	Mode	MIC_{50}	MIC_{90}	% S	% I	% R
Fidaxomicin	0.12-1	0.5	0.25	1	NA	NA	NA
OP-1118	0.25-16	2	2	8	NA	NA	NA
Metronidazole	0.12-4	0.5	0.5	1	100	0	0
Vancomycin	0.5-4	0.5	0.5	1	NA	NA	NA
Amoxicillin-clavulanate	0.5-2	1	1	1	100	0	0
Meropenem	1-4	2	2	2	100	0	0
Clindamycin	1->16	>16	8	>16	13.2	36.1	50.7
Moxifloxacin	0.5->256	1	2	256	55.0	0	45.0
Ceftriaxone	16-256	32	32	128	0.7	65.4	33.9

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

Table 2. Distribution of MICs for antimicrobials tested against 440 toxin-positive isolates of *C. difficile*

	Number of isolates for which the antimicrobial agent MIC (µg/ml) was:										
Antimicrobial agent	0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64
Fidaxomicin		127	106	159	48						
OP-1118			6	72	49	131	126	49	7		
Metronidazole		1	54	244	123	15	3				
Vancomycin				226	196	17	1				
Amoxicillin-clavulanate				2	428	10					
Meropenem					63	376	1				
Clindamycin					12	46	159	36	1	186[a]	
Moxifloxacin				1	167	74		7	33	45	113[b]
Ceftriaxone									3	288	149[b]

[a] All 186 isolate MICs were >16 µg/ml.
[b] 105/113 (moxifloxacin) and 114/149 (ceftriaxone) isolate MICs were >64 µg/ml.

Table 3. *Sma*I PFGE NAP isolate type analysis

NAP type	n (% of all isolates)	Most common NAP subtypes (n, % of NAP type isolates)
NAP1	39 (8.9%)	0251 (8, 20.5%); 0006 (7, 17.9%); 0018 (6, 15.4%); 9 other subtypes[a] (18, 46.2%)
NAP2	124 (28.2%)	0003 (106, 85.4%); 0332 (9, 7.3%); 6 other subtypes (9, 7.3%)
NAP3	1 (0.2%)	0011 (1, 100%)
NAP4	32 (7.3%)	0033 (15, 46.9%); 0023 (6, 18.8%); 6 other subtypes (11, 34.4%)
NAP5	0	-
NAP6	29 (6.6%)	0024 (16, 55.2%); 8 other subtypes (13, 44.8%)
NAP7	3 (0.7%)	0153 (2, 67%); 0080 (1, 33%)
NAP8	0	-
Non-NAP	212 (48.2%)	0012 (24, 11.8%); 0122 (18, 8.5%); 0139 (13, 6.1%); 77 other subtypes (156, 73.6%)

[a] Only two 0001 subtype isolates (NAP1 ribotype 027) were identified among the 39 NAP1 isolates.

Table 4. Antimicrobial susceptibility testing results of toxin-positive isolates of *C. difficile* stratified according to NAP type

		MIC (µg/ml)				MIC interpretation [c]		
Antimicrobial agent [a]	NAP type [b]	Range	Mode	MIC_{50}	MIC_{90}	% S	% I	% R
Moxifloxacin	NAP1	1-128	1	1	2	94.9	0	5.1
	NAP2	1->256	256	32	256	7.3	0	92.7
	NAP4	1-256	2	2	16	87.5	0	12.5
	NAP6	1-32	1	1	2	93.1	0	6.9
	Non-NAP	0.5->256	1	2	256	64.6	0	35.4
Clindamycin	NAP1	1->16	4	4	8	28.2	48.7	23.1
	NAP2	1->16	>16	>16	>16	1.6	4.0	94.4
	NAP4	1->16	4	4	8	25.0	62.5	12.5
	NAP6	1->16	4	4	8	20.7	62.1	17.2
	Non-NAP	1->16	4	4	>16	14.2	44.8	41.0
Ceftriaxone	NAP1	32-64	32	32	32	0	94.9	5.1
	NAP2	32-128	128	128	128	0	9.7	90.3
	NAP4	32-128	32	32	64	0	75.0	25.0
	NAP6	32-256	32	32	32	0	96.6	3.4
	Non-NAP	16-256	32	32	64	1.4	86.8	11.8

[a] Metronidazole, vancomycin, amoxicillin-clavulanate, and meropenem were excluded from this table because there were no differences in isolate susceptibility when stratified by NAP type. [b] NAP1, 39 isolates ; NAP2, 124 isolates ; NAP4, 32 isolates ; NAP6, 29 isolates ; and Non-NAP types, 212 isolates. There were also three NAP7 isolates and one NAP3 isolate; data for these four isolates are not presented in the table. [c] S: susceptible, I: intermediate, R: resistant

MATERIALS & METHODS

Bacterial isolates studied. 440 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 to two tertiary-care clinical microbiology laboratories and to the provincial public health laboratory in Manitoba, Canada (Western Canada)(5). Each isolate's identity was confirmed by Gram stain, typical odor, latex agglutination (Microgen Bioproducts Ltd., Surrey, UK), and chartreuse fluorescence under UV light (6).

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing for fidaxomicin, OP-1118, and 7 additional agents was performed using the agar dilution method recommended by CLSI (7,8). Fidaxomicin and OP-1118 were supplied by Optimer Pharmaceuticals, Inc. (San Diego, CA, U.S.A.); the solvent for both compounds was DMSO; water was used as the diluent. *C. difficile* ATCC 700057 was used as the control strain; reference MIC ranges for this strain were 0.03-0.25 µg/ml for fidaxomicin. In vitro susceptibility testing interpretive criteria for fidaxomicin have not been determined.

Pulsed-field gel electrophoresis. A modified protocol employing *Sma*I-digested DNA separated by pulsed-field gel electrophoresis (PFGE) was used to type the isolates (9). The PFGE profiles of the clinical isolates were assigned NAP types using bioNumerics comparison to representative PFGE NAP types (10). Gel images were analyzed using Bionumerics software version 4.0 (Applied Maths, Austin, TX). Each gel was standardized using a band tolerance of 1%. Cluster analysis was performed using Dice coefficient and the unweighted-pair group method with arithmetic means (11). Isolates were considered to be of the same PFGE type if they demonstrated 80% homology (11). All PFGE testing was performed at the National Microbiology Laboratory, Public Health Agency of Canada.

ACKNOWLEDGEMENTS

The authors would like to thank the laboratory staff at the Health Sciences Centre, St. Boniface General Hospital, and the Cadham Provincial Laboratory for their assistance with specimen collection.

The study was supported in part by the Health Sciences Centre, University of Manitoba, National Microbiology Laboratory, and Optimer Pharmaceuticals.

CONCLUSIONS

- Fidaxomicin and its metabolite, OP-1118, demonstrated potent in vitro activity against TP-CD.
- Fidaxomicin demonstrated the narrowest MIC range of 0.1-1.0 µg/ml among antibiotics used in the treatment of CDAD.
- The highest MIC reported for fidaxomicin was 1 µg/ml compared with 2 µg/ml for amoxicillin-clavulanate and 4 µg/ml for metronidazole and vancomycin.
- MIC distributions for fidaxomicin were indistinguishable for MDR NAP2, NAP1, and non-NAP1/NAP2 isolates.

53rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, CO, September 10-13, 2013