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Antimicrobial Susceptibility Testing of *Clostridium difficile* Ribotypes Infecting Canadian Patients: The Canadian *Clostridium difficile* Surveillance Study (CAN-DIFF) 2013-2015

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ABSTRACT

Background: Clinical microbiology laboratories (CMLs) do not routinely culture toxin-positive (TP) stool specimens for *C. difficile* (CD), nor do they perform antimicrobial susceptibility testing (AST) or ribotype TP-CD isolates. Periodic assessments of TP-CD isolate antibiograms and ribotypes may be informative.
Methods: We determined *in vitro* activities of routinely tested anti-anaerobic agents as well as fidaxomicin (FDX), its active metabolite OP-1118, and vancomycin (VAN) against genotyped isolates of TP-CD collected in 8 hospital CMLs across Canada from 2013 to 2015. 1,310 isolates of CD were cultured from TP stool specimens using CDMN agar and each isolate's identity confirmed by accepted laboratory methods. CLSI agar dilution AST (M11-A8, 2012) was performed and MICs interpreted using CLSI breakpoints (M100-S26, 2016) when available. The presence of *tcdA* (toxin A), *tcdB* (toxin B), and *cdtB* (binary toxin) were determined by PCR methods. Genotyping was performed using an internationally standardized, high-resolution, capillary gel-based electrophoresis PCR ribotyping protocol for CD.
Results: For agents with CLSI MIC breakpoints, percent susceptibilities were: metronidazole (MTZ; 100%), amoxicillin-clavulanate (99.9%), moxifloxacin (MXF; 68.9%), ceftriaxone (CRO; 11.3%), and clindamycin (CLI; 4.6%). For agents without CLSI MIC breakpoints, MIC ranges/MIC_{90}s (µg/mL) were: FDX (≤0.015-2/0.5), OP-1118 (0.25-32/16), and VAN (≤0.25-4/2). The most common ribotypes were 027 (24.5% of isolates), 014 (7.7%), 020 (6.6%), 106 (6.1%), and 002 (4.6%). 98.7% (1,293/1,310) of isolates were *tcdA*-positive; all isolates were *tcdB*-positive. 97.8% (314/321) of ribotype 027 isolates were *cdtB*-positive compared with only 18.7% (185/989) of isolates with non-027 ribotypes. Ribotype 027 accounted for 14.5, 21.0, and 37.1% of isolates from western (BC, AB, MB), central (ON), and eastern Canada (QC, NS), respectively, as well as for 15.4, 22.9, and 40.6% of isolates from patients aged ≤64, 65-79, and ≥80 years. Ribotype 027 isolates had ≥4-fold higher MIC_{90}s than non-027 ribotype isolates for MXF (>32 versus 8 µg/mL) and MTZ (4 versus 1 µg/mL) and 49.5% of ribotype 027 isolates were multidrug-resistant (MDR; resistant to MXF, CLI, CRO) compared to 4.4% for non-027 ribotypes.
Conclusion: Ribotype 027 was the most common TP-CD ribotype infecting patients in Canada. FDX demonstrated potent *in vitro* activity (MIC range, ≤0.015-2 µg/mL; MIC_{90}, 0.5 µg/mL) against all genotypes of TP-CD including ribotype 027 (NAP1/BI).

BACKGROUND

- *Clostridium difficile* is the most frequently identified infectious cause of nosocomial diarrhea. *C. difficile* infection (CDI) occurs primarily in patients previously receiving antimicrobial agents.
- Antimicrobial susceptibility testing of *C. difficile* is rarely performed in clinical laboratories because of its complexity, cost, and dubious clinical significance.
- Management of patients with CDI includes withdrawal of the predisposing antimicrobial agent, if possible, and empiric therapy most commonly with either metronidazole or oral vancomycin. However, the adequacy of both metronidazole and vancomycin as empiric therapies for CDI is suspect.
- Treatment failure and CDI recurrence in patients treated with metronidazole occur with considerable frequency (1-4) and the use of vancomycin to treat CDI in hospitals is discouraged to minimize the risk of vancomycin resistance in enterococci and staphylococci (5).
- As the epidemiology 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 the oral, narrow-spectrum macrocyclic antimicrobial agent fidaxomicin, its active metabolite OP-1118, and other investigational agents, are warranted.
- Fidaxomicin inhibits bacterial RNA polymerase.

MATERIALS & METHODS

Bacterial isolates studied
1,310 isolates of *C. difficile* were cultured on *C. difficile* Moxalactam Norfloxacin (CDMN) Selective Supplement agar (Oxoid Canada, Nepean, ON, Canada) from toxin-positive stool specimens (following an ethanol shock step) submitted by 8 hospital clinical microbiology laboratories across Canada to the clinical microbiology laboratory at 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 test, and chartreuse fluorescence under UV light (6). Chi-square testing was used to establish statistical significance (significance level, $P < 0.05$).

Antimicrobial susceptibility testing
Antimicrobial susceptibility testing for fidaxomicin, OP-1118, and 6 additional agents was performed using the agar dilution reference method defined by CLSI (7). Fidaxomicin and OP-1118 were supplied by Merck & Co., Inc.; the solvent for each of these compounds was DMSO and water was used as the diluent. *C. difficile* ATCC 700057 was used as the control strain; the reference minimum inhibitory concentration (MIC) range for this strain was 0.06-0.25 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
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).

PCR for toxin genes
DNA extraction was performed using a commercial kit (InstaGene Matrix; Bio-Rad, Richmond, CA). The presence of the genes coding for toxin A (*tcdA*), toxin B (*tcdB*), and binary toxin (*cdtB*) were determined for each cultured isolate using previously described PCR methods (10-12). PCR products were separated by electrophoresis on a 1.5% agarose gel and visualized with ethidium bromide staining, and images captured using Alpha Imager software (Alpha Innotech Corp., San Leandro, CA). All PCR testing was performed at the National Microbiology Laboratory, Public Health Agency of Canada.

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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. Johnson S, et al. Clin Infect Dis. 2014 59:345-54.
5. HICPAC. 1995. Infect Control Hosp Epidemiol 16:105-13.
6. CLSI. 2008. M35-A2.
7. CLSI. 2015. M11-A10.
8. CLSI. 2016. M100-S26.
9. Fawley WN, et al., 2015. PLOS One DOI:10.1371/journal.pone.0118150 Feb 13, 2015:1-14.
10. Kato H, et al. 1998. J Clin Microbiol 36:2178-82.
11. Lemee L, et al. 2004. J Clin Microbiol 42:5710-4.
12. Stubb S, et al. 2000. FEMS Microbiol Lett 186:307-12.

RESULTS

Table 1. Antimicrobial susceptibility testing results for 1,310 toxin-positive isolates of *C. difficile*

Antimicrobial agent	MIC (µg/ml) Range	Mode	MIC_{50}	MIC_{90}	MIC interpretation % S	% I	% R
Fidaxomicin	≤0.015-2	0.25	0.25	0.5	NA	NA	NA
OP-1118	0.25-32	4	4	16	NA	NA	NA
Metronidazole	0.12-4	0.5	0.5	2	100	0	0
Vancomycin	≤0.25-4	1	1	2	NA	NA	NA
Amoxicillin-clavulanate	≤0.25-8	1	1	2	99.9	0.1	0
Clindamycin	≤0.12->64	8	8	>64	4.6	32.1	63.6
Moxifloxacin	0.5->32	1	2	>32	68.9	0.9	30.2
Ceftriaxone	8->128	32	32	64	11.3	60.6	28.1

NA – CLSI MIC interpretative breakpoints not available.

Table 2. Distribution of MICs for antimicrobials tested against 1,310 toxin-positive isolates of *C. difficile*

Antimicrobial agent	Number of isolates for which the antimicrobial agent MIC (µg/ml) was: 0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64
Fidaxomicin	3[a]	26	58	324	422	437	39	1					
OP-1118					5	27	73	214	385	347	258	1	
Metronidazole				4	150	532	281	285	58				
Vancomycin					1[a]	173	892	228	16				
Amoxicillin-clavulanate					4[a]	72	743	481	9	1			
Clindamycin				1[a]			11	44	421	649	12	2	170[b]
Moxifloxacin						2	506	383	10	19	150	240[c]	
Ceftriaxone										3	120	781	406[d]

[a] Isolate count shown for lowest the dilution tested; some MICs may be lower than the lowest dilution tested. [b] 163/170 isolate MICs for clindamycin were >64 µg/ml. [c] 158/240 isolate MICs for moxifloxacin were >32 µg/ml. [d] 60/406 isolate MICs for ceftriaxone were >64 µg/ml.

Table 3. PCR ribotyping analysis for 1,310 isolates[a]

Ribotype	*n* (% of all isolates)
027	321 (24.5%)
014	101 (7.7%)
020	86 (6.6%)
106	80 (6.1%)
002	60 (4.6%)
056	43 (3.3%)
072	36 (2.7%)
078	35 (2.7%)
015	32 (2.4%)
057	30 (2.3%)
087	21 (1.6%)
012	21 (1.6%)
176	19 (1.5%)
054	18 (1.4%)
076	18 (1.4%)
017	17 (1.3%)
005	17 (1.3%)
019	16 (1.2%)
Ribotypes with <15 isolates	239 (25.9%)
Total number of different ribotypes	141

[a] 1,293/1,310 (98.7%) isolates were positive for *tcdA* (toxin A); 17/1,310 (1.3%) isolates demonstrated toxin A deletion (negative for *tcdA*). All isolates (1,310/1,310) were positive for *tcdB* (toxin B). 499/1,310 (38.1%) isolates were positive for *cdtB* (binary toxin). 314/321 (97.8%) ribotype 027 isolates were positive for *cdtB*; 185/989 (18.7%) non-ribotype 027 isolates were positive for *cdtB*.

Table 4. Antimicrobial susceptibility testing results for 1,310 toxin-positive isolates of *C. difficile*, stratified according to PCR ribotype

Antimicrobial agent	Ribotype	MIC (µg/ml) Range	Mode	MIC_{50}	MIC_{90}	MIC interpretation[a] % S	% I	% R
Fidaxomicin	027	0.12-1	0.5	0.5	1	NA	NA	NA
	Non-027 Ribotypes	≤0.015-2	0.25	0.25	0.5	NA	NA	NA
OP-1118	027	1-32	16	16	16	NA	NA	NA
	Non-027 Ribotypes	0.25-16	4	4	8	NA	NA	NA
Metronidazole	027	0.25-4	2	2	4	100	0	0
	Non-027 Ribotypes	0.12-4	0.5	0.5	1	100	0	0
Vancomycin	027	≤0.25-4	1	1	2	NA	NA	NA
	Non-027 Ribotypes	0.5-4	1	1	2	NA	NA	NA
Amoxicillin-clavulanate	027	≤0.25-2	2	2	2	100	0	0
	Non-027 Ribotypes	≤0.25-8	1	1	2	99.9	0.1	0
Clindamycin	027	1->64	8	8	>64	3.4	30.6	66.0
	Non-027 Ribotypes	≤0.12->64	8	8	>64	4.6	32.6	62.8
Moxifloxacin	027	1->32	>32	32	>32	7.2	0.6	92.2
	Non-027 Ribotypes	0.5->32	1	1	8	87.8	1.0	11.2
Ceftriaxone	027	8->128	64	64	64	1.6	20.2	78.2
	Non-027 Ribotypes	8->128	32	32	64	11.9	72.4	15.7

[a] 49.5% (159/321) of ribotype 027 isolates were multidrug-resistant (MDR; resistant to ceftriaxone, clindamycin, and moxifloxacin) compared to 4.4% (43/989) for non-027 ribotypes.

Table 5. PCR ribotyping results stratified by Canadian geographic region[a]

Geographic Region (n)	Ribotype n (% of total from geographic region) 027	014	020	106	002
West (469)	68 (14.5%)	37 (7.9%)	29 (6.2%)	27 (5.8%)	24 (5.1%)
Central (367)	77 (21.0%)	29 (7.9%)	26 (7.1%)	23 (6.3%)	15 (4.1%)
East (474)	176 (37.1%)	35 (7.4%)	31 (6.5%)	30 (6.3%)	21 (4.4%)

[a] West (British Columbia, Alberta, Manitoba; 3 sites), Central (Ontario; 2-3 sites), and East (Quebec, Nova Scotia; 3 sites).

Table 6. Annual prevalence of Ribotype 027 by Canadian geographic region

Year (n)	Ribotype n (% of total from region/year) West	Central	East
2013 (411)	27/155 (17.4%)	25/99 (25.3%)	61/157 (38.9%)
2014 (414)	21/158 (13.3%)	31/102 (30.4%)	47/154 (30.5%)
2015 (485)	20/156 (12.8%)	21/166 (12.7%)	68/163 (41.7%)

Table 7. PCR ribotyping results stratified by patient age[a]

Age Group (n)	Ribotype n (% of total from patient age group) 027	014	020	106	002
≤ 64 yrs (584)	90 (15.4%)	44 (7.5%)	46 (7.9%)	43 (7.4%)	25 (4.3%)
65-79 yrs (363)	83 (22.9%)	30 (8.3%)	21 (5.8%)	19 (5.2%)	16 (4.4%)
≥ 80 yrs (360)	146 (40.6%)	27 (7.5%)	19 (5.3%)	18 (5.0%)	19 (5.3%)

[a] Patient age unknown for 3/1,310 (0.2%) isolates.

CONCLUSIONS

- Fidaxomicin demonstrated the highest potency (MIC_{90}, 0.5 µg/ml; maximum MIC, 2 µg/ml) of the 8 antimicrobial agents tested against toxin-positive clinical isolates of *C. difficile*. In comparison, vancomycin and metronidazole MIC_{90}s and maximum MICs were both 2 and 4 µg/ml, respectively.
- All isolates were susceptible to metronidazole (MIC, ≤8 µg/ml).
- There was tremendous ribotype diversity among the isolates of *C. difficile* tested, with ribotype 027 being the most frequently identified ribotype (accounting for 24.5% of isolates).
- Fidaxomicin MIC values were 2-fold higher for ribotype 027 isolates than for non-ribotype 027 isolates. OP-1118 (2-4-fold higher), metronidazole (4-fold higher), and moxifloxacin (8-32-fold higher) had even higher MIC values for ribotype 027 isolates than for non-ribotype 027 isolates. Vancomycin MIC values were identical for both ribotype 027 and non-ribotype 027 isolates.
- The prevalence of ribotype 027 was lowest in western Canada and highest in eastern Canada ($P<0.00001$).
- The prevalence of ribotype 027 was similar across the three years of the study ($P=0.208$).
- The prevalence of ribotype 027 was lowest in patients ≤64 years of age and highest in patients ≥80 years of age ($P<0.00001$).
- Fidaxomicin and its active metabolite OP-1118 demonstrated potent *in vitro* activity against toxin-positive *C. difficile*, including ribotype 027 isolates.