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Antimicrobial-Resistant Strains of *Clostridium difficile* from North America

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A total of 316 toxigenic *Clostridium difficile* clinical isolates of known PCR ribotypes from patients in North America were screened for resistance to clindamycin, metronidazole, moxifloxacin, and rifampin. Clindamycin resistance was observed among 16 different ribotypes, with ribotypes 017, 053, and 078 showing the highest proportions of resistance. All isolates were susceptible to metronidazole. Moxifloxacin resistance was present in >90% of PCR-ribotype 027 and 053 isolates but was less common among other ribotypes. Only 7.9% of the *C. difficile* isolates were resistant to rifampin. Multidrug resistance (clindamycin, moxifloxacin, and rifampin) was present in 27.5% of PCR-ribotype 027 strains but was rare in other ribotypes. These results suggest that antimicrobial resistance in North American isolates of *C. difficile* varies by strain type and parallels rates of resistance reported from Europe and the Far East.

Clostridium difficile is a major health care-associated pathogen that is responsible for a wide spectrum of disease, ranging from mild diarrhea to life-threatening complications, such as pseudomembranous colitis and toxic megacolon (19). The severity and outcome of *C. difficile* infection (CDI) is influenced by a multiplicity of factors, including patient demographics, such as age and immune status, length of hospitalization, and, most of all, receipt of antimicrobial therapy (23, 25). Antimicrobial therapy, often given for treatment of other infectious diseases, can render the patient susceptible to CDI if the patient is exposed to a toxigenic strain of the organism. When CDI was first reported in the 1970s, prior use of clindamycin was established as a significant risk factor. However, by 1980, cephalosporins replaced clindamycin as the major risk factor (4). In the next 20 years, expanded- and extended-spectrum cephalosporins became associated with a high risk of CDI (6). More recently, fluoroquinolones have been linked to CDI and to severe epidemics, particularly those caused by PCR-ribotype 027 (27). Between 5% and 30% of patients receiving antimicrobial agents may develop health care-associated diarrhea, with *C. difficile* causing up to 30% of those cases (22). Antimicrobial stewardship programs can assist in curtailing these selective pressures (17, 18). The emergence of *C. difficile* strains that are resistant to multiple antimicrobial agents can complicate prevention programs and potentially, in the case of metronidazole, treatment (13). Thus, knowing the prevalence of antimicrobial-resistant strains in an institution can be helpful for optimizing antimicrobial stewardship programs.

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MATERIALS AND METHODS

Bacterial isolates. A total of 316 toxigenic clinical isolates of *C. difficile* were received from 7 hospitals in the United States (including hospitals located in California, Illinois, Indiana, North Carolina, and Washington) and Canada (Quebec) during 2008 and 2009. These isolates have been described elsewhere (32). Organisms were isolated using broth-enrichment toxigenic culture and typed by PCR-ribotyping (PCR-R), pulsed-field gel electrophoresis (PFGE), and restriction endonuclease analysis of whole-cell DNA (REA). The isolates were preserved at −80°C in 15% glycerol-*Brucella* broth and were subcultured twice on prereduced anaerobically sterilized (PRAS) *Brucella* blood agar (Anaerobe Systems, Morgan Hill, CA) prior to antimicrobial susceptibility testing.

TABLE 1 MIC range, MIC_{50}, and MIC_{90} for antimicrobial agents tested against 316 *C. difficile* isolates

Drug	MIC (μg/ml)			No. resistant	% resistant
	Range	50%	90%		
Clindamycin	0.25–>256	4	>256	131	41.50
Metronidazole	0.125–4	0.25	0.5	0	0
Rifampin	≤0.002–>32	≤0.002	≤0.002	25	7.90
Moxifloxacin	0.5–>32	2	>32	120	38.00

Antimicrobial susceptibility testing. Isolates were tested for susceptibility to clindamycin, metronidazole, moxifloxacin, and rifampin using Etest strips (bioMérieux, Marcy-l'Étoile, France), as described in the Etest technical guide. The MIC results were rounded up to the next doubling dilution and interpreted using Clinical and Laboratory Standards Institute (CLSI) breakpoints for susceptibility testing of anaerobic bacteria (8). Individual colonies from 24- to 48-h *Brucella* blood agar plates were suspended in *Brucella* broth to the turbidity of 1.0 McFarland standard, and the inoculum was applied to 150-mm *Brucella* blood agar plates (Anaerobe Systems, Morgan Hill, CA). Plates were incubated anaerobically at 35°C in a Bactron anaerobic chamber (Sheldon Manufacturing Inc., Cornelius, OR). MICs were read and recorded at 24 h, and clindamycin results were confirmed after 48 h of incubation to ensure detection of inducible resistance (as per the Etest package insert). *Bacteroides fragilis* ATCC

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TABLE 2 MIC range, MIC_{50}, and MIC_{90} among isolates of the seven most frequent PCR ribotypes

PCR ribotype (no. of isolates)	Measure[b]	Result[a]			
		Clindamycin	Metronidazole	Moxifloxacin	Rifampin
002 (17)	MIC_{50}	4	0.25	2	≤0.002
	MIC_{90}	8	0.5	>32	≤0.002
	No. R	1	0	3	0
	% R	5.9	0	17.7	0
017 (13)	MIC_{50}	>256	0.5	2	≤0.002
	MIC_{90}	>256	0.5	>32	0.004
	No. R	13	0	3	1
	% R	100	0	23.1	7.7
027 (80)	MIC_{50}	4	0.5	>32	≤0.002
	MIC_{90}	>256	1	>32	>32
	No. R	38	0	78	22
	% R	47.5	0	97.5	27.5
053 (12)	MIC_{50}	>256	0.5	>32	≤0.002
	MIC_{90}	>256	0.5	>32	≤0.002
	No. R	11	0	11	0
	% R	91.7	0	91.7	0
078 (14)	MIC_{50}	8	0.25	1	≤0.002
	MIC_{90}	>256	0.5	2	0.003
	No. R	10	0	1	0
	% R	71.4	0	7.1	0
104 (9)	MIC_{50}	6	0.5	2	≤0.002
	MIC_{90}	6	0.50	2	≤0.002
	No. R	1	0	0	0
	% R	11.1	0	0	0
106 (17)	MIC_{50}	4	0.50	2	0.003
	MIC_{90}	8	0.50	2	0.003
	No. R	4	0	1	0
	% R	23.5	0	5.8	0

[a] MIC results are in μg/ml.
[b] No. R and % R are the number and percentage of resistant isolates, respectively.

25285, *Bacteroides thetaiotaomicron* ATCC 29741, and *Eubacterium lentum* ATCC 43055 were used for quality control (9). All control results were within acceptable ranges.

RESULTS

The MIC_{50} and MIC_{90} results for clindamycin, metronidazole, moxifloxacin, and rifampin when tested against 316 *C. difficile* strains are presented in Table 1. Clindamycin resistance was present in 41.5% of isolates, while moxifloxacin resistance was present in 38.0% of isolates; only 7.9% of isolates were resistant to rifampin. All isolates were susceptible to metronidazole. There were no inner colonies suggestive of heteroresistance within the zones of inhibition around the metronidazole Etest strips. Table 2 shows the MIC data for the seven most common PCR ribotypes observed among the study isolates. Moxifloxacin resistance was present in >90% of PCR-ribotype 027 and 053 isolates but was less common among other ribotypes (Fig. 1). Clindamycin resistance was observed among several ribotypes, with ribotypes 017, 053, and 078 showing the highest proportions of resistance (100%, 91.7%, and 71.4%, respectively). Rifampin resistance (MIC ≥ 32 μg/ml) was observed in 27.5% of ribotype 027 isolates and sporadically among ribotypes 017 and 012 isolates (Fig. 1). Overall, 27.5% of ribotype 027 isolates were resistant to clindamycin, moxifloxacin, and rifampin. This multidrug resistance pattern was also observed among several isolates of ribotype 017.

A comparison of the proportions of resistance observed in this study and those reported in 15 other studies of *C. difficile* resistance is shown in Table 3. Moxifloxacin resistance ranged from 2 to 87%. A report of 82% resistance was from a single site, where 69% of isolates were pulsed-field gel electrophoresis types NAP1 or NAP2 (7). Clindamycin resistance ranged from 15 to 97% (the latter in a study of 1,613 isolates from Scotland). Rifampin resistance was reported infrequently, and only a single metronidazole-resistant isolate was reported in these other studies.

DISCUSSION

Multidrug resistance (i.e., resistance to clindamycin, moxifloxacin, and rifampin) was present in 22 of 80 (27.5%) *C. difficile* PCR-ribotype 027 isolates from the United States and Canada but was unusual among other ribotypes (Fig. 1). All rifampin-resistant strains were also resistant to clindamycin and moxifloxacin. This is in contrast to the report of Curry et al. (12), in which 81.5% of ribotype 027 isolates from a hospital in Pittsburgh were resistant to rifampin. The range of antibiograms observed in our study indicates that not all ribotype 027 isolates, which were obtained from seven laboratories across the United States and Canada, are clonal. This is consistent with the data presented by Killgore et al. (20), which indicated that subtypes could be defined within ribotype 027 isolates by other typing techniques. Unfortunately, rifampin resistance is rarely reported in other resistance surveys of *C. difficile* isolates, limiting comparisons to other data sets (Table 3). Although clindamycin and moxifloxacin resistance were both relatively common among our isolates (41.5% and 38.0% of isolates, respectively), clindamycin resistance was observed in all but two of the 16 ribotypes in our study (where there were at least 4 isolates of that ribotype tested), while moxifloxacin resistance was limited to 10 strain types. Yet, only in ribotype 053 isolates did the two resistances appear tightly linked (i.e., both were present in 11/12 isolates). The ribotype 053 strains came from four different laboratories located in California, Indiana, Illinois, and North Carolina and thus did not appear to be associated with a clonal outbreak of CDI. Overall, though, no resistance pattern was consistent enough to be a useful strain marker.

A review of antimicrobial resistance among *C. difficile* isolates by Huang et al. in 2009 (15) did not contain data specifically on ribotype 053 strains but did confirm high rates of clindamycin resistance in a variety of ribotypes, including ribotype 017 isolates from Europe (there were no data on ribotypes 012 or 046 reported). Among other surveys of *C. difficile* resistance not covered by Huang et al. (i.e., those cited in Table 3), only 7 of 15 reports showed higher rates of moxifloxacin resistance than was observed in our study, but 11 of 15 showed higher rates of clindamycin resistance. PCR ribotype 001 was the most common ribotype reported in these surveys, followed by ribotypes 014, 027, and 106. Modest levels of resistance were seen among our isolates of these ribotypes as well.

Resistance to the antimicrobial agents most commonly used to treat *C. difficile* infections, i.e., vancomycin and metronidazole (5,

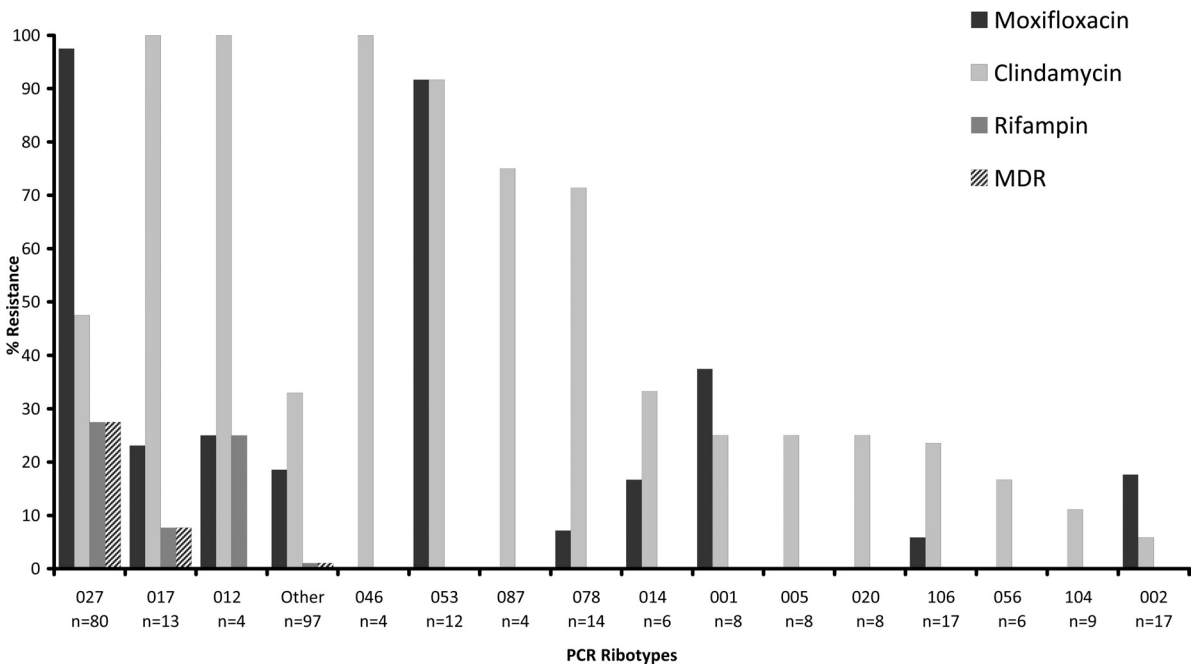

FIG 1 Proportions of antimicrobial resistance among 307 isolates of *C. difficile* according to their PCR ribotypes. The graph excludes a total of nine isolates of PCR ribotypes 003 and 075 for which no resistance was observed.

10), is reported rarely in the literature (15). However, both metronidazole heteroresistance (26) and reduced susceptibility to metronidazole have been reported (2). We did not observe either of these phenomena in our study, but this may have been due to the limited number of medical centers that contributed isolates to this study.

In summary, while resistance to clindamycin and moxifloxacin is widespread in *C. difficile* isolates from North America, multidrug resistance (i.e., resistance to clindamycin, moxifloxacin, and rifampin) was limited primarily to ribotype 027 isolates. Although these antimicrobial agents are not used for therapy (with the possible exception of rifaximin, which is in an antimicrobial agent class related to rifampin), they may still play a key role in enabling patients taking these antimicrobial agents to become colonized and infected with *C. difficile* if exposed to these resistant strains (11).

TABLE 3 Published reports of antimicrobial resistance rates in *C. difficile*[a]

Yr isolated, location	No.	% resistant to:				AST method(s) used	Most common ribotypes	% that were PCR ribotype:			Reference
		Mox	Clinda	Met	Rif			027	078	017	
2008–2009, U.S. and Canada	316	38	41	0	8	Etest	027, 106, 002	25	4	4	This study
2001–2009, Taiwan	113	16	46	0		Agar dilution	NA	0	0		21
2002–2004, Germany	317	40	65	0		Etest	NA				30
2004, Quebec, Canada	258	82	15	0	0	Agar dilution	NA[b]	69			7
2004–2006, Poland (hospital 1)	153	39	54	0		Etest	NA				28
2004–2006, Poland (hospital 2)	177	38	48	0		Etest	NA				28
2005, 14 EU countries	349	38	46	0		Etest	001, 014, 027	6	~2	5	3
2005, Scotland	116	87	63	0		Agar dilution	001, 106	0	0	0	24
2006–2007, Austria	142	38	57	1[c]		Etest	AI-5,[d] 014, 053	1	0		16
2007–2009, Scotland	1613	64	97	0		Agar dilution	106, 001, 027	13	3	0	33
2008, Sweden	585	20	16	0		Disk diffusion and Etest	012, SE37,[d] 017	<1	~4		1
2009, Sweden	364	16	16	0		Disk diffusion and Etest	SE21,[d] 001, 020	0	~5		1
2009, Ireland	133	57	22			Etest	027, 001, 106	19	11		31
2009, New Zealand	101	2	61	0		Agar dilution	014, 002, 005	0	1		29
2009, Shanghai, China	75	45	85	0	17	Agar dilution	017, 012, A	0	0	19	14
2009, Stockholm, Sweden	80	15	65	0	4	Agar dilution	005, 014, 023	0	0		14

[a] Mox, moxifloxacin; Clinda, clindamycin; Met, metronidazole; Rif, rifampin; AST, antimicrobial susceptibility testing method; NA, not available.
[b] These are primarily isolates of pulsed-field types NAP1 and NAP2.
[c] Isolate reverted to metronidazole susceptibility after storage.
[d] AI-5, SE21, and SE37 are locally defined strain types.

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