Strain Types and Antimicrobial Resistance Patterns of *Clostridium difficile* Isolates from the United States, 2011 to 2013

Isabella A. Tickler,^a Richard V. Goering,^b Joseph D. Whitmore,^a Ashley N. W. Lynn,^b David H. Persing,^a Fred C. Tenover,^a for the Healthcare Associated Infection Consortium

Cepheid, Sunnyvale, California, USA^a; Creighton University, Omaha, Nebraska, USA^b

We determined the PCR ribotypes and antimicrobial susceptibility patterns of 508 toxigenic *Clostridium difficile* isolates collected between 2011 and 2013 from 32 U.S. hospitals. Of the 29 PCR ribotypes identified, the 027 strain type was the most common (28.1%), although the rates varied by geographic region. Ribotype 014/020 isolates appear to be emerging. Clindamycin and moxifloxacin resistances (36.8% and 35.8%, respectively) were the most frequent resistance phenotypes observed. Reduced susceptibility to vancomycin was observed in 39.1% of 027 isolates.

The epidemiology of *Clostridium difficile* infection (CDI) continues to evolve; however, there are relatively few reports on strain types and antimicrobial susceptibility test (AST) patterns for *C. difficile* isolates from the United States. This makes assessing changes in epidemiology difficult. This study examined the PCR ribotypes and the AST patterns of a convenience sample of 508 *C. difficile* isolates from the United States and compared them to results from recent studies (1–5).

(These data were presented in part at the 2013 Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, CO.)

Stool samples containing toxigenic *C. difficile* isolates were collected between May 2011 and April 2013 from 508 unique patients at 32 hospitals in the U.S. Northeast ($n = 180$), South ($n = 84$), Midwest ($n = 78$), and West ($n = 166$) and then shipped in anaerobic transport medium (Anaerobe Systems, Morgan Hill, CA) to a central laboratory. The samples were inoculated into cycloserine-cefoxitin-mannitol broth with taurocholate and lysozyme (CCMB-TAL) and subcultured to prereduced anaerobically sterilized (PRAS) *Brucella* blood agar plates (Anaerobe Systems). The cultures were incubated anaerobically at 35°C, and the organisms were identified as previously described (3, 6).

The isolates were tested for susceptibility to clindamycin, metronidazole, moxifloxacin, rifampin, tetracycline, and vancomycin, using Etest strips (bioMérieux, Marcy-l'Étoile, France) (7), as previously described (3). The MIC results were interpreted using Clinical and Laboratory Standards Institute (CLSI) document M11-A8 (8). Tetracycline resistance was defined as an MIC of ≥ 16 $\mu\text{g/ml}$ (9). Reduced susceptibility to vancomycin was defined as an MIC of > 2 $\mu\text{g/ml}$ (10). Agar dilution testing using the CLSI reference method (8) was performed to confirm reduced susceptibility to vancomycin. We tested for the presence of *vanA* and *vanB* using the primers and probes in the Xpert *vanA/vanB* assay (Cepheid, Sunnyvale, CA) (11). PCR ribotyping was performed as previously described (2). The results were compared to those from a subset of 43 type strains from the Cardiff ribotype collection. Ribotypes 014 and 020 and ribotypes 078 and 126 were grouped together as previously reported (5).

Overall, 29 previously defined ribotypes were observed among 423 (83.3%) of the 508 isolates of *C. difficile* received in 2011 to 2013 (Table 1); 85 (16.7%) of the isolates had unnamed patterns. Twenty-three of the unnamed patterns grouped into 12 clusters,

TABLE 1 Frequency of PCR ribotypes among *C. difficile* isolates from the United States, 2011–2013 ($n = 508$)

PCR ribotype	No. of isolates	% of isolates
027	143	28.1
014/020	63	12.4
106	31	6.1
001	22	4.3
053	21	4.1
002	18	3.5
056	14	2.8
046	12	2.4
017	11	2.2
015	10	2.0
078/126	10	2.0
023	8	1.6
057	8	1.6
095	8	1.6
012	7	1.4
029	5	1.0
054	5	1.0
220	5	1.0
087	4	0.8
103	4	0.8
005	2	0.4
010	2	0.4
026	2	0.4
081	2	0.4
356	2	0.4
011	1	0.2
013	1	0.2
039	1	0.2
131	1	0.2
Unnamed	85	16.7

Received 20 December 2013 Returned for modification 5 February 2014

Accepted 14 April 2014

Published ahead of print 21 April 2014

Address correspondence to Isabella A. Tickler, isabella.tickler@cepheid.com.

Copyright © 2014, American Society for Microbiology. All Rights Reserved.

doi:10.1128/AAC.02775-13

FIG 1 Seven most prevalent PCR ribotypes in the United States by region (2011 to 2013).

some of which were present in multiple geographic regions. Ribotype 027 was the predominant strain type (28.1% of isolates), although its prevalence varied by geographic region and was the highest in the Northeast (40.0%) and the South (38.1%) (Fig. 1). The next most frequent ribotypes were 014/020, 106, 001, 053, and 002. In the West, isolates of ribotype 014/020 were more common than those of ribotype 027 (Fig. 1). Ribotype 106 was primarily found in the Northeast and the West but was sporadically found in the Midwest and the South. Ribotype 001 represented 9.6% of the isolates from the West but was rare elsewhere.

Approximately 36% of the isolates were resistant to either clindamycin or moxifloxacin. Ribotype 027 isolates had the highest rate of moxifloxacin resistance (92.3%), followed by ribotypes 053 and 001 (Table 2). Rifampin resistance was observed in 11.2% of the isolates, including 35.7% of the ribotype 027 isolates. Ribotype 053 had the highest proportion of clindamycin resistance. Tetracycline resistance was observed in only 6.7% of the isolates, primarily in ribotypes 046 and 017. Decreased susceptibility to vancomycin was observed in 13.2% of the isolates tested, including 39.1% of the ribotype 027 isolates. The vancomycin Etest results were confirmed via agar dilution on a subset of 10 isolates; all of the results showed vancomycin MICs of 4 µg/ml. Neither the *vanA* nor the *vanB* gene was present in any of the isolates with decreased vancomycin susceptibility. Resistance to ≥3 antimicro-

bial agents was noted among ribotypes 027, 053, 001, 017, 046, and 023. Overall, 30.8% of 027 strains were resistant to moxifloxacin, clindamycin, and rifampin, and 18.2% were resistant (or nonsusceptible) to 4 antimicrobial agents, including tetracycline and/or vancomycin. Each of the 508 isolates was susceptible to metronidazole, although the Etest may not be the optimal method for assessing resistance to this drug (12).

Using a statistical model (Fisher's exact binomial, two-proportion model), we compared the results of our 2008-2009 study from the midwestern and western regions of the United States with the ribotype distributions of the *C. difficile* isolates observed in the present study in the same regions (data from the Northeast and the South were lacking in the 2008-2009 survey). Our data show a significant change in the overall proportion of ribotypes observed ($P < 0.0001$). This is driven primarily by changes in ribotype 027, the prevalence of which decreased significantly in the West ($P < 0.007$) while remaining unchanged in the Midwest ($P = 0.219$), and ribotype 014/020, the prevalence of which increased significantly in both the West ($P < 0.0001$) and the Midwest ($P < 0.046$). The observed frequency of ribotype 014/020 isolates in our current study is consistent with the data from a 2011 study reported by Waslawski et al. (1) (Table 3). Interestingly, data from the Nationwide Inpatient Sample of the Healthcare Cost and Utilization Project (see www.hcup-us.ahrq.gov/nisoverview.jsp)

TABLE 2 Antimicrobial resistance rates of *C. difficile* isolates of the seven most common PCR ribotypes

PCR ribotype (no. of isolates) and parameter	Value for:					
	Clindamycin	Metronidazole	Moxifloxacin	Rifampin	Tetracycline	Vancomycin ^a
027 (143)						
MIC range ^b	2 to >256	0.25–2	1 to >32	≤0.002 to >32	0.06–64	1–4 (87)
MIC ₅₀	8	0.25	>32	≤0.002	0.12	1
MIC ₉₀	>256	0.5	>32	>32	4	4
No. R (%)	72 (50.3)	0 (0)	132 (92.3)	51 (35.7)	9 (6.3)	34 (39.1)
014/020 (63)						
MIC range	2 to >256	0.12–1	2 to >32	≤0.0020–0.004	0.06–0.5	1–2 (42)
MIC ₅₀	4	0.25	2	≤0.002	0.12	1
MIC ₉₀	8	0.5	2	≤0.002	0.12	2
No. R (%)	22 (34.9)	0 (0)	3 (4.8)	0 (0)	0 (0)	0 (0)
106 (31)						
MIC range	2–8	0.25–1	1 to >32	≤0.0020–0.004	0.06–0.5	1–2 (17)
MIC ₅₀	4	0.5	2	≤0.002	0.12	1
MIC ₉₀	4	0.5	2	0.004	0.12	2
No. R (%)	1 (3.2)	0 (0)	3 (9.7)	0 (0)	0 (0)	0 (0)
001 (22)						
MIC range	2 to >256	0.25–1	1 to >32	≤0.002–0.004	0.06–4	1–4 (9)
MIC ₅₀	4	0.25	2	≤0.002	0.12	2
MIC ₉₀	>256	0.5	>32	≤0.002	4	2
No. R (%)	10 (45.5)	0 (0)	10 (45.5)	0 (0)	0 (0)	1 (11.1)
053 (21)						
MIC range	2 to >256	0.12–0.5	1 to >32	≤0.002–0.004	0.06–16	2–4 (7)
MIC ₅₀	>256	0.25	>32	≤0.002	0.12	2
MIC ₉₀	>256	0.5	>32	≤0.002	4	2
No. R (%)	16 (76.2)	0 (0)	12 (57.1)	0 (0)	2 (9.5)	1 (14.3)
002 (18)						
MIC range	2–8	0.25–0.5	1 to >32	≤0.002–0.004	0.06–1	1–4 (10)
MIC ₅₀	4	0.25	2	≤0.002	0.12	1
MIC ₉₀	8	0.25	2	≤0.002	0.12	2
No. R (%)	3 (16.7)	0 (0)	1 (5.6)	0 (0)	0 (0)	1 (10.0)
056 (14)						
MIC range	2 to >256	0.25–2	2 to >32	≤0.002–0.004	0.06–0.25	0.5–2 (10)
MIC ₅₀	4	0.25	2	≤0.002	0.12	1
MIC ₉₀	4	0.5	>32	≤0.002	0.12	2
No. R (%)	2 (14.3)	0 (0)	2 (14.3)	0 (0)	0 (0)	0 (0)

^a Not all isolates were tested for vancomycin susceptibility. The number tested for each ribotype is listed in parentheses. For vancomycin only, resistance is defined as an MIC of 4 µg/ml.

^b All MIC, MIC₅₀, and MIC₉₀ values are given in µg/ml.

^c R, resistant.

showed that patients in the West and Midwest who were discharged with a primary diagnosis of CDI in 2011 had lower mortality rates and lengths of stay than did patients discharged in the South and Northeast, where ribotype 027 isolates are more common, according to our data and those of Waslawski et al. (1). This is consistent with recent reports of 027 strains causing more severe disease (13), although a direct relationship has not been determined.

The increased prevalence of ribotype 014/020 isolates in our study is also consistent with a recent report from England by Wilcox et al. (5), where 014/020 isolates increased from the 6th most common ribotype in 2008–2009 to the 2nd most common ribotype in 2009–2010, replacing ribotype 106. Ribotype 027 remained the most prevalent ribotype in the United Kingdom.

The overall proportions of resistance in 2011 to 2013 were similar to those in our previous study, although clindamycin resistance decreased significantly for ribotypes 017 and 078/126 (data not shown). Of the 302 isolates tested with vancomycin in our study, 40 (13.2%) showed reduced susceptibility, similar to that shown in other recent studies (14–16).

The ribotype 014/020 isolates in our study were typically susceptible to most antimicrobial agents, similar to data reported from European studies (17). The low levels of resistance of 014/020 strains (18) raise the question of what other factors are responsible for its increased frequency. Janezic et al. (19) showed that 014/020 was the most frequently isolated strain from humans, animals, and the environment between 2008 and 2010, while Pasquale et al. (20) reported that *C. difficile* from edible mollusks also

TABLE 3 Published reports of PCR ribotype frequency

Location (date)	No. of isolates	Frequency (%) of ribotype:							Reference or source
		027	017	002	001	053	014/020	078/126	
United States (2011-2013)	508	28.2	2.2	3.5	4.3	4.1	12.4	1.9	This study
United States (2011)	720	25.1	2.8	4.6	3.6	5.0 ^a	11.9	3.1	1
United States (2008-2009)	304	24.7	4.3	4.9	2.6	3.9	4.3	4.6	2
United States and Canada (2005-2007)	443	36.1 ^b	1.1	5.0	11.3	2.3	9.0 ^c	2.0 ^d	4

^a Ribotypes 053 and 163.^b REA group BI.^c REA group Y.^d REA group BK.

included 014/020 strains. In addition, both Wetterwik et al. (21) and Schneeberg et al. (22) isolated ribotypes 014/020 from dogs and cats. These reports suggest that a large reservoir of 014/020 isolates may now be moving into human populations.

Our study has several limitations. First, the 508 isolates examined represent a convenience sample in which different diagnostic procedures at the collaborating hospitals may have enriched certain strains over the others. Additionally, our 2008-2009 study was limited to isolates primarily from 6 hospitals in the western and midwestern United States, while the current study included isolates from 32 hospitals. While there are now >400 ribotypes described (23), we had access to only 43 control strains. Even so, all but 16.7% of our isolates provided known ribotypes. The availability of additional ribotype controls in the future will be important for further defining our unknown ribotypes, some of which are clearly represented throughout the United States.

In conclusion, our typing results are similar to results reported from recent studies in the United States and suggest that ribotype 027 remains a significant cause of CDI. However, other strain types, particularly ribotype 014/020, are gaining in prevalence.

ACKNOWLEDGMENTS

We thank Diane Citron for performing the agar dilution vancomycin MIC testing for this study. We also thank Ferric Fang, J. Stacey Klutts, Stephen Brecher, and Allan Truant for their assistance with editing the manuscript.

This study was supported by Cepheid.

I. A. Tickler, J. D. Whitmore, D. H. Persing, and F. C. Tenover are employees and shareholders of Cepheid, and R. V. Goering has received research funding from Cepheid. A. N. W. Lynn has no conflicts to report.

Healthcare Associated Infections Consortium members include Niaz Banaei, Matthew Bankowski, Stephen Brecher, Adam Bressler, Eileen Burd, Kimberle Chapin, Young Choi, Diane Citron, Steven Dallas, Phyllis Della-Latta, Diane Dryja, Ferric Fang, Mary Jane Ferraro, Paul Granato, Diane Halstead, Mary Ann Henthorne, J. Stacey Klutts, Margie Morgan, Christopher Polage, Albert Rojzman, Paul Schreckenberger, Susan Sharp, David Tison, and Allan Truant.

REFERENCES

- Waslawski S, Lo ES, Ewing SA, Young VB, Aronoff DM, Sharp SE, Novak-Weekley SM, Crist AE Jr, Dunne WM, Hoppe-Bauer J, Johnson M, Brecher SM, Newton DW, Walk ST. 2013. *Clostridium difficile* ribotype diversity at six health care institutions in the United States. J. Clin. Microbiol. 51:1938–1941. <http://dx.doi.org/10.1128/JCM.00056-13>.
- Tenover FC, Akerlund T, Gerding DN, Goering RV, Bostrom T, Jonsson AM, Wong E, Wortman AT, Persing DH. 2011. Comparison of strain typing results for *Clostridium difficile* isolates from North America. J. Clin. Microbiol. 49:1831–1837. <http://dx.doi.org/10.1128/JCM.02446-10>.
- Tenover FC, Tickler IA, Persing DH. 2012. Antimicrobial-resistant strains of *Clostridium difficile* from North America. Antimicrob. Agents Chemother. 56:2929–2932. <http://dx.doi.org/10.1128/AAC.00220-12>.
- Cheknis AK, Sambol SP, Davidson DM, Nagaro KJ, Mancini MC, Hidalgo-Arroyo GA, Brazier JS, Johnson S, Gerding DN. 2009. Distribution of *Clostridium difficile* strains from a North American, European and Australian trial of treatment for *C. difficile* infections: 2005-2007. Anaerobe 15:230–233. <http://dx.doi.org/10.1016/j.anaerobe.2009.09.001>.
- Wilcox MH, Shetty N, Fawley WN, Shemko M, Coen P, Birtles A, Cairns M, Curran MD, Dodgson KJ, Green SM, Hardy KJ, Hawkey PM, Magee JG, Sails AD, Wren MW. 2012. Changing epidemiology of *Clostridium difficile* infection following the introduction of a national ribotyping-based surveillance scheme in England. Clin. Infect. Dis. 55:1056–1063. <http://dx.doi.org/10.1093/cid/cis614>.
- Jousimies-Somer H, Summanen P, Citron DM, Baron EJ, Wexler HM, Finegold SM. 2002. Wadsworth-KTL anaerobic bacteriology manual, 6th ed. Star Publishing Company, Belmont, CA.
- Citron DM, Ostovari MI, Karlsson A, Goldstein EJ. 1991. Evaluation of the E test for susceptibility testing of anaerobic bacteria. J. Clin. Microbiol. 29:2197–2203.
- Clinical and Laboratory Standards Institute. 2012. Methods for antimicrobial susceptibility testing of anaerobic bacteria; approved standard—8th ed. CLSI document M11-A8. Clinical and Laboratory Standards Institute, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2013. Performance standards for antimicrobial susceptibility testing; 23rd informational supplement. CLSI M100-S23. Clinical and Laboratory Standards Institute, Wayne, PA.
- European Committee on Antimicrobial Susceptibility Testing. 1 January 2013. Clinical breakpoint tables, version 4.0. European Committee on Antimicrobial Susceptibility Testing, London, United Kingdom. http://www.eucast.org/clinical_breakpoints/.
- Marnier ES, Wolk DM, Carr J, Hewitt C, Dominguez LL, Kovacs T, Johnson DR, Hayden RT. 2011. Diagnostic accuracy of the Cepheid GeneXpert *vanA/vanB* assay ver. 1.0 to detect the *vanA* and *vanB* vancomycin resistance genes in *Enterococcus* from perianal specimens. Diagn. Microbiol. Infect. Dis. 69:382–389. <http://dx.doi.org/10.1016/j.diagmicrobio.2010.11.005>.
- Poillane I, Cruaud P, Torlotin JC, Collignon A. 2000. Comparison of the E test to the reference agar dilution method for antibiotic susceptibility testing of *Clostridium difficile*. Clin. Microbiol. Infect. 6:155–156. <http://dx.doi.org/10.1046/j.1469-0691.2000.00034-4.x>.
- Walker AS, Eyre DW, Wyllie DH, Dingle KE, Griffiths D, Shine B, Oakley S, O'Connor L, Finney J, Vaughan A, Crook DW, Wilcox MH, Peto TE, Infections in Oxfordshire Research Database. 2013. Relationship between bacterial strain type, host biomarkers, and mortality in *Clostridium difficile* infection. Clin. Infect. Dis. 56:1589–1600. <http://dx.doi.org/10.1093/cid/cit127>.
- Hecht DW, Galang MA, Sambol SP, Osmolski JR, Johnson S, Gerding DN. 2007. In vitro activities of 15 antimicrobial agents against 110 toxigenic *Clostridium difficile* clinical isolates collected from 1983 to 2004. Antimicrob. Agents Chemother. 51:2716–2719. <http://dx.doi.org/10.1128/AAC.01623-06>.
- Pelaez T, Alcalá L, Alonso R, Rodriguez-Creixems M, Garcia-Lechuz JM, Bouza E. 2002. Reassessment of *Clostridium difficile* susceptibility to metronidazole and vancomycin. Antimicrob. Agents Chemother. 46:1647–1650. <http://dx.doi.org/10.1128/AAC.46.6.1647-1650.2002>.
- Dong D, Zhang L, Chen X, Jiang C, Yu B, Wang X, Peng Y. 2013. Antimicrobial susceptibility and resistance mechanisms of clinical *Clos-*

- tridium difficile* from a Chinese tertiary hospital. Int. J. Antimicrob. Agents 41:80–84. <http://dx.doi.org/10.1016/j.ijantimicag.2012.08.011>.
17. Pirs T, Avbersek J, Zdovc I, Krt B, Andlovic A, Lejko-Zupanc T, Rupnik M, Ocepek M. 2013. Antimicrobial susceptibility of animal and human isolates of *Clostridium difficile* by broth microdilution. J. Med. Microbiol. 62:1478–1485. <http://dx.doi.org/10.1099/jmm.0.058875-0>.
18. Bauer MP, Notermans DW, van Benthem BH, Brazier JS, Wilcox MH, Rupnik M, Monnet DL, van Dissel JT, Kuijper EJ, Group ES. 2011. *Clostridium difficile* infection in Europe: a hospital-based survey. Lancet 377:63–73. [http://dx.doi.org/10.1016/S0140-6736\(10\)61266-4](http://dx.doi.org/10.1016/S0140-6736(10)61266-4).
19. Janezic S, Ocepek M, Zidaric V, Rupnik M. 2012. *Clostridium difficile* genotypes other than ribotype 078 that are prevalent among human, animal and environmental isolates. BMC Microbiol. 12:48. <http://dx.doi.org/10.1186/1471-2180-12-48>.
20. Pasquale V, Romano V, Rupnik M, Capuano F, Bove D, Aliberti F, Krovacek K, Dumontet S. 2012. Occurrence of toxigenic *Clostridium difficile* in edible bivalve molluscs. Food Microbiol. 31:309–312. <http://dx.doi.org/10.1016/j.fm.2012.03.001>.
21. Wetterwik KJ, Trowald-Wigh G, Fernstrom LL, Krovacek K. 2013. *Clostridium difficile* in faeces from healthy dogs and dogs with diarrhea. Acta Vet. Scand. 55:23. <http://dx.doi.org/10.1186/1751-0147-55-23>.
22. Schneeberg A, Rupnik M, Neubauer H, Seyboldt C. 2012. Prevalence and distribution of *Clostridium difficile* PCR ribotypes in cats and dogs from animal shelters in Thuringia, Germany. Anaerobe 18:484–488. <http://dx.doi.org/10.1016/j.anaerobe.2012.08.002>.
23. Stabler RA, Dawson LF, Valiente E, Cairns MD, Martin MJ, Donahue EH, Riley TV, Songer JG, Kuijper EJ, Dingle KE, Wren BW. 2012. Macro and micro diversity of *Clostridium difficile* isolates from diverse sources and geographical locations. PLoS One 7:e31559. <http://dx.doi.org/10.1371/journal.pone.0031559>.