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Comparison of Strain Typing Results for *Clostridium difficile* Isolates from North America▽

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Accurate strain typing is critical for understanding the changing epidemiology of *Clostridium difficile* infections. We typed 350 isolates of toxigenic *C. difficile* from 2008 to 2009 from seven laboratories in the United States and Canada. Typing was performed by PCR-ribotyping, pulsed-field gel electrophoresis (PFGE), and restriction endonuclease analysis (REA) of whole-cell DNA. The Cepheid Xpert *C. difficile* test for presumptive identification of 027/NAP1/BI isolates was also tested directly on original stool samples. Of 350 isolates, 244 (70%) were known PCR ribotypes, 224 (68%) were 1 of 8 common REA groups, and 187 (54%) were known PFGE types. Eighty-four isolates typed as 027, NAP1, and BI, and 83 of these were identified as presumptive 027/NAP1/BI by Xpert *C. difficile*. Eight additional isolates were called presumptive 027/NAP1/BI by Xpert *C. difficile*, of which three were ribotype 027. Five PCR ribotypes contained multiple REA groups, and three North American pulsed-field (NAP) profiles contained both multiple REA groups and PCR ribotypes. There was modest concordance of results among the three methods for *C. difficile* strains, including the J strain (ribotype 001 and PFGE NAP2), the toxin A-negative 017 strain (PFGE NAP9 and REA type CF), the 078 animal strain (PFGE NAP7 and REA type BK), and type 106 (PFGE NAP11 and REA type DH). PCR-ribotyping, REA, and PFGE provide different but overlapping patterns of strain clustering. Unlike the other methods, the Xpert *C. difficile* 027/NAP1/BI assay gave results directly from stool specimens, required only 45 min to complete, but was limited to detection of a single strain type.

Clostridium difficile is a Gram-positive, spore-forming bacillus that causes a range of clinical syndromes ranging from mild to severe diarrhea, toxic megacolon, and, in some cases, sepsis and death (5, 21). *C. difficile* infections (CDI) continue to spread worldwide (4, 24, 27, 33). Some strains, such as the 027/NAP1/BI (where NAP is North American pulsed-field) "epidemic" strain (33), appear to show increased virulence, particularly in outbreak settings (3, 33, 34, 35). Accurate strain typing is critical for understanding the changing epidemiology of this organism and for determining outbreaks of infection in hospitals (3, 25, 38). However, clinical laboratories, particularly in the United States, have limited options for typing *C. difficile* isolates since virtually all typing methods require culturing of the stool sample to recover the isolate before typing can be performed, and cultures for *C. difficile* are performed rarely in the United States (16). Even when *C. difficile* culture methods are available in the laboratory, the organisms usually must be sent to a reference laboratory for typing, and results are often not available for days to weeks.

Multiple techniques have been used to study the epidemiology of *C. difficile* infections including pulsed-field gel electrophoresis (PFGE) (22), restriction endonuclease analysis (REA) of total DNA (7, 19), PCR-ribotyping, multilocus sequence typing (MLST), and multilocus variable-number tandem repeat assays (30, 46). Multilocus sequence typing is less discriminatory than the other methods and has been used primarily for population studies of *C. difficile* (30). Strain typing data are particularly valuable for investigations of hospital outbreaks but often are not available in real time to guide infection control efforts (4). Strain typing data that could be generated in parallel with the identification of the *tcdB* (toxin B gene), which is the basis for several PCR-based commercial *C. difficile* assays, could be of value to infection control efforts to reduce the spread of CDI in hospitals, as noted in two recent studies reported by Huang et al. (15) and Babady et al. (2).

The goals of this study were the following: (i) to compare the results of three *C. difficile* strain typing methods, i.e., PCR-ribotyping, REA, and PFGE, performed on toxigenic isolates available in pure culture, to determine how frequently the results were in agreement for the identification of common strains of *C. difficile*, such as the J strain (19), the toxin A-negative type 017 strain (38), the type 027/NAP1/BI hyperviruent/epidemic strain (26, 33), the type 078 "animal" strain (17, 39), and the type 106 United Kingdom epidemic strain (41); (ii) to determine the frequency of isolation of these strains at various study sites in the United States and Canada; and (iii) to assess the accuracy of the Xpert *C. difficile* assay for identifying 027/NAP1/BI strains directly in stool samples versus the strain types determined by the three typing methods on isolates obtained in pure culture.

MATERIALS AND METHODS

Bacterial isolates. A total of 350 isolates of toxigenic *C. difficile* recovered from symptomatic patients from the eastern, midwestern, and western United States (including California, Illinois, Indiana, North Carolina, and Washington) and

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TABLE 1. Comparison of the most common PCR-ribotyping results to results of REA and PFGE

PCR ribotype (no. of isolates)	Corresponding REA group; other identified group(s) (no. of isolates)[a]	Corresponding PFGE type; other identified type(s) (no. of isolates)
001 (9)	J (7)	NAP2 (6)
002 (18)	G (17); DH	Undefined types only
014 (6)	Y (4)	NAP4 (4)
017 (15)	CF (10); BK, Y	NAP9 (11)
020 (8)	Y (8)	Primarily undefined types; NAP4 (1)
027 (92)[b]	BI (86); BK	NAP1 (89)
053 (13)	K (6); DH, Y	Undefined types only
078 (15)	BK (14)	NAP7 (6)
087 (4)	Unspecified group only	NAP12 (4)
104 (9)	G (1); primarily unspecified group	NAP11 (8)
106 (17)	DH (14)	NAP11 (13)

[a] Only eight major REA groups were identified in this study.
[b] A total of 89 of the 92 isolates were identified presumptively as 027/NAP1/BI by the Xpert *C. difficile* assay.

Canada (Quebec) were collected from November 2008 to January 2009, as previously described (43). Stool samples were collected, an aliquot was tested with the Xpert *C. difficile* assay on site (which included detection of *tcdB* [toxin B gene], *cdtA* [the binary toxin gene], and a single nucleotide deletion at base 117 in *tcdC*), and the remainder of the sample was transported to a central reference laboratory in anaerobic transport medium (Anaerobe Systems, Morgan Hill, CA). The samples were inoculated onto prereduced cycloserine-cefoxitin-fructose agar (CCFA) and into cycloserine-cefoxitin-mannitol broth with taurocholate-lysozyme-cysteine (CCMB-TAL) (both media were obtained from Anaerobe Systems, Morgan Hill, CA). The CCFA plate was incubated at 35°C to 37°C for 48 h; CCMB-TAL was incubated at 35°C to 37°C for 24 h and subcultured to CCFA for an additional 48 h. The presence of isobutyric, isocaproic, and isovaleric acids by gas-liquid chromatography as end products of glucose fermentation served as confirmation of *C. difficile* identification (18).

PCR-ribotyping. PCR-ribotyping was performed as previously described by Stubbs et al. (40) with minor modifications (42). Analysis was performed using BioNumerics, version 5.1 (Applied Maths, Belgium). PCR-ribotyping patterns were compared to a database containing >3,000 clinical isolates including *C. difficile* reference strains obtained from the Culture Collection, University of Göteborg, Sweden, and from Ed Kuijper, Leiden University Medical Center, Netherlands (i.e., the Cardiff-European Centre for Disease Prevention and Control [ECDC] *C. difficile* collection).

PFGE. For each isolate, DNA was prepared by *in situ* lysis of cells encased in agarose plugs and digested with SmaI, as described by Killgore et al. (22). Pulsed-field gel electrophoresis (PFGE) was performed using a Bio-Rad CHEF DR III System at 6 V/cm, 14°C, and 120° included angle, with switching from 5 to 15 s for 10 h, followed by switching from 15 to 60 s for 13 h. Images of gels stained with ethidium bromide or SYBR gold were archived using a Bio-Rad Gel Doc XR System. PFGE profiles were compared using BioNumerics with XbaI-digested *Salmonella enterica* serovar Braenderup H9812 DNA as a molecular size and gel normalization standard. PFGE patterns were categorized in comparison to known types using 80% similarity as determined by dendrogram analysis using Dice coefficients with the unweighted-pair group method using average linkages (UPGMA) as previously described by Killgore et al. (22). Analysis of PFGE patterns was performed using BioNumerics software, version 5.1 (Applied Maths, Belgium).

REA of chromosomal DNA. Analysis of chromosomal DNA after restriction with the frequent-cutting enzyme HindIII was performed as previously described by Clabots et al. (7). DNA banding patterns were systematically compared by visual analysis in 1-mm segments to an extensive library of known REA groups. A similarity index was calculated, and patterns exhibiting ≥90% relatedness were grouped together. For this study, only the eight most frequently isolated REA groups were reported, i.e., BI, BK, CF, DH, G, J, K, and Y. The remaining patterns were designated the "unspecified" REA group.

Xpert *C. difficile* assay. The Xpert *C. difficile* assay results were generated directly from stool samples at each of the seven study sites using the Xpert *C. difficile* cartridge, as described by the manufacturer (Cepheid, Sunnyvale, CA). Stool samples that were positive for the *tcdB*, *cdtA*, and a single nucleotide deletion at base 117 in *tcdC* (33) were reported as presumptive 027/NAP1/BI. Samples that were positive only for *tcdB* were reported as 027/NAP1/BI negative.

Analysis of strain typing results. The congruence of the strain typing results generated by PCR-ribotyping, PFGE, and REA were determined by calculating the adjusted Rand and Wallace coefficients as described by Carrico et al. (6) and Pinto et al. (37) using the Comparing Partitions website (http://darwin.phyloviz.net/ComparingPartitions/index.php?link=Tool). Pairwise comparisons were made on data sets in which missing data (e.g., where one culture was contaminated and a strain type could not be determined) were deleted. Simpson's index of diversity was also determined for the three typing methods.

Statistical methods. Fisher's exact test and a chi-square test were used for statistical analysis where appropriate.

RESULTS

PCR-ribotyping results. All 350 toxigenic isolates obtained from symptomatic patients in six U.S. sites and one Canadian site were tested by PCR-ribotyping; 244 (69.7%) were assigned to 1 of 18 different PCR ribotypes (using the Cardiff-ECDC nomenclature). A total of 106 isolates did not match established ribotypes. Simpson's index of diversity for PCR-ribotyping was calculated to be 82.71 (95% confidence interval [CI], 80.02 to 85.40). The five most frequent ribotypes were 027 (92 isolates), 002 (18 isolates), 106 (17 isolates), 017 (15 isolates), and 078 (15 isolates) (Table 1). Isolates of all five of the most common ribotypes were recovered from eastern, midwestern, and western U.S. study sites, confirming the widespread dissemination of these strains.

Of the 92 PCR ribotype 027 strains, 89 were reported by PFGE as NAP1, and 86 were reported as BI by REA typing (Table 1 and Fig. 1). Five 027 isolates did not have REA types determined, and the other isolate belonged to the BK group. There were two undefined PFGE patterns, and the remaining culture was contaminated. PCR ribotype 002 correlated strongly with the REA G group (17 of 18 isolates), but there was no corresponding PFGE NAP type for this group of isolates. The 17 PCR ribotype 106 isolates were usually REA

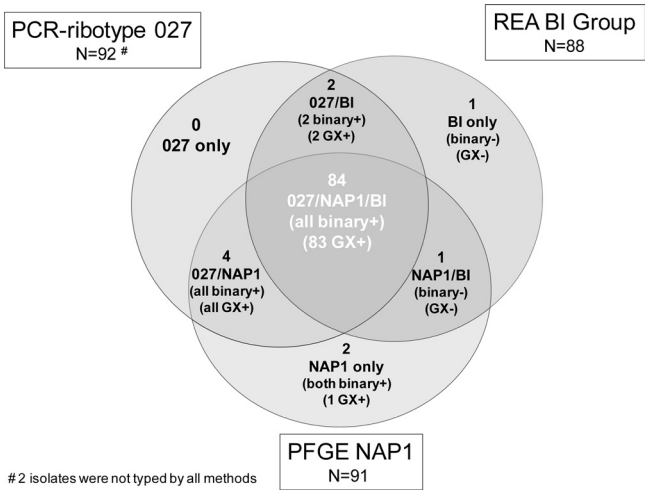

FIG. 1. Venn diagram displaying the results for the three typing methods (REA, PFGE, and PCR-ribotyping) used on isolated colonies and the Xpert *C. difficile* assay, which was performed directly on stool, for the 027/NAP1/BI strain. GX, Xpert *C. difficile* assay. Four of the 92 isolates designated PCR ribotype 027 did not have corresponding REA data and 1 isolate did not have a PFGE type due to strain contamination during shipment.

TABLE 2. Comparison of most common REA results to results of PCR-ribotyping and PFGE

REA group (no. of isolates)	Corresponding PCR ribotype (no. of isolates); other identified type(s)	Corresponding PFGE type (no. of isolates); other identified type(s)
BI (88)[a]	027 (86)	NAP1 (85)
BK (17)	078 (14); 027	NAP7 (6); NAP1
CF (10)	017 (10)	NAP9 (9)
DH (19)	106 (14); 002, 046, 053	NAP11 (12)
G (20)	002 (17); 104	Undefined types only
J (22)	001 (7); 046, 056	NAP2 (7)
K (8)	053 (6)	Undefined types only
Y (40)	020 (8); 014, 017, 053, 087	NAP4 (26)

[a] A total of 85 of the 88 isolates were identified presumptively as 027/NAP1/BI by the Xpert *C. difficile* assay.

TABLE 3. Comparison of PFGE results to results of PCR-ribotyping and REA

PFGE type (no. of isolates)	Corresponding PCR ribotype(s) (no. of isolates); other identified type(s)	Corresponding REA group (no. of isolates); other identified group(s)
NAP1 (91)[a]	027 (88)	BI (84); BK
NAP2 (8)	001 (6)	J (7)
NAP4 (31)	Primarily undefined types; 014 (1), 020 (1)	Y (26); DH, G
NAP7 (6)	078 (6)	BK (6)
NAP9 (11)	017 (11)	CF (9); BK, Y
NAP11 (24)	106 (13); 104	DH (12); G
NAP12 (7)	087 (4); 015	Unspecified group only

[a] A total of 88 of the 91 isolates were identified presumptively as 027/NAP1/BI by the Xpert *C. difficile* assay.

group DH and PFGE type NAP11 although most of the PCR ribotype 104 isolates were also NAP11 but were an unspecified REA group. Of the 15 PCR ribotype 017 isolates, 10 were REA group CF, and 11 were PFGE type NAP9. The PCR ribotype 078 isolates were usually REA group BK (14 of 15), but less than half had an identified PFGE type; of those that did, 6 of 15 were NAP7. Finally, among the nine PCR ribotype 001 isolates, seven belonged to the REA J group, and six of those were NAP2.

REA typing results. A total of 224 (67.9%) of the 330 isolates available for REA typing were assigned to one of eight major REA groups including BI (88 isolates), Y (40 isolates), J (22 isolates), G (20 isolates), DH (19 isolates), BK (17 isolates), CF (10 isolates), and K (8 isolates) (Table 2). For this study, the REA subtypes within the major groups (e.g., BI7) were not used for analysis. Other isolates were assigned to a unspecified REA group. Simpson's index of diversity for REA typing was calculated to be 78.25 (95% CI, 75.50 to 81.00). All but 2 of the 88 isolates reported as belonging to the BI group were ribotype 027, and neither of these was designated NAP1. Two additional BI isolates were not NAP1, but both were PCR ribotype 027. Of the 40 Y group isolates, 26 were NAP4, and the remaining isolates were an undefined pattern. The PCR ribotypes from group Y isolates were diverse; eight were 020, four were 014, and the other isolates were types 017, 053, 087, or unknown. The 22 J group isolates also showed diverse PCR ribotypes and PFGE types. Only seven J group isolates were type 001, and of those only five were NAP2. Two other J group isolates were NAP2, but most were other unnamed PFGE types. Aside from one isolate each of types 046 and 056, the remaining PCR ribotypes for the J group isolates were unnamed. All but three of the 17 BK group isolates were PCR ribotype 078, but only six were NAP7 (the others were an undesignated PFGE type or, in one case, NAP1). All of the CF group isolates were PCR ribotype 017, and nine of those were NAP9. Of the 19 DH group isolates, 14 were PCR ribotype 106, and the remaining isolates were 002, 046, or 053. The 20 group G isolates included 17 PCR ribotype 002, 1 type 104, and 2 other types. The majority of the G group isolates did not have an identified PFGE type although there was one isolate each of NAP4 and NAP11.

Pulsed-field gel electrophoresis. Although all but 8 of the 342 isolates could be analyzed by PFGE, only 187 (54.7%) gave patterns that could be assigned to 1 of the 12 described NAP types. The patterns identified in the study were NAP1, NAP2, NAP4, NAP7, NAP9, NAP11, and NAP12 (Table 3). Simpson's index of diversity for PFGE was calculated to be 65.30 (95% CI, 61.29 to 69.32), which was the lowest of the three typing methods. Of the 91 isolates designated NAP1, 88 were PCR ribotype type 027, and 85 were REA group BI. Two isolates did not have a named PCR ribotype, while one was type REA type BK, one was an unspecified REA type, and four isolates were nontypeable or contaminated. Two NAP1 isolates were neither ribotype 027 nor REA group BI. Seven of the eight NAP2 isolates were the J group by REA, but only six were PCR ribotype 001. Of the 31 NAP4 isolates, 26 were REA group Y, but most did not have a named PCR ribotype. The four that did were PCR ribotype 014. The six NAP7 isolates all were PCR ribotype 078 and REA type BK. Of the 11 NAP9 isolates, all 11 were PCR ribotype 017, but only nine were REA group CF; the other two were group BK or Y. Isolates showing the NAP11 PFGE pattern encompassed both ribotypes 104 and 106, but only half were REA type DH (mostly those that also were type 106). The remaining isolates were not in a specified REA group.

Presumptive identification of 027/NAP1/BI strains using Xpert *C. difficile*. A total of 96 (27.4%) *C. difficile* isolates were designated presumptive 027/NAP1/BI by the Xpert *C. difficile* assay. Of these, 83 were PCR ribotype 027, REA group BI and PFGE type NAP1. Eight additional isolates were PCR ribotype 027, of which five were also NAP1 but not BI (four had REA groups other than the eight major groups reported), and two were BI but not NAP1. The remaining isolate did not have a PFGE or REA group reported due to culture contamination. One additional isolate was type 053/NAP undefined/K. The four remaining Xpert presumptive 027/NAP1/BI-positive isolates (of the 96 total) were neither 027, BI, nor NAP1. None of the four isolates, which came from three geographically diverse locations, had a known PCR ribotype, NAP type, or REA group identified. The two from the same location were isolated several months apart. Overall, 91 of 96 (94.8%) of the Xpert *C. difficile* presumptive 027/NAP1/BI designations were confirmed by at least two typing methods as being in the 027/NAP1/BI cluster. There were no significant differences between the sensitivities, specificities, or positive or negative predictive values of the results obtained with the Xpert *C. difficile* test and the results of PCR-ribotyping, REA, or PFGE ($P > 0.05$ for all comparisons) (Table 4). The presumptive

TABLE 4. Results of Xpert *C. difficile* 027/NAP1/BI test compared to results of standard typing methods

Parameter[a]	No. of isolates of the indicated type/no. of isolates tested (%)[b]			*P* value[c]
	PCR ribotype 027 (n = 350)	PFGE type NAP1 (n = 342)	REA type BI (n = 330)	
Sensitivity	91/92 (98.9)	88/91 (96.7)	85/88 (96.6)	0.5739
Specificity	253/258 (98.1)	244/251(97.2)	236/242 (97.5)	0.8343
PPV	91/96 (94.8)	88/95 (92.6)	85/91 (93.4)	0.8287
NPV	253/254 (99.6%)	244/247 (98.8)	236/239 (98.7)	0.5779

[a] PPV, positive predictive value; NPV, negative predictive value.
[b] Typing was determined by the indicated method. The denominator changes because not all isolates were available for typing by each method. *n*, total number of isolates.
[c] Calculated using Fisher's exact test.

identification of the 027/NAP1/BI strain using the Xpert cartridge was available within 45 min of initiating the testing of the stool sample.

Distribution of known *C. difficile* strains among study sites and analysis of congruence. The results of the three typing methods (PCR ribotype, PFGE type, and REA group) were combined to give a composite strain type for five well-characterized strains previously identified in the literature in an effort to better understand their geographic distribution. This included the J strain (001/NAP2/J) (19, 47), the toxin A-negative toxin B-positive ribotype 017 strain (017/NAP9/CF) (38), the hypervirulent/epidemic 027/NAP1/BI strain (33), the PCR ribotype 078 animal strain (078/NAP7/BK) (13, 17, 39), and the PCR ribotype 106 United Kingdom outbreak strain (106/NAP11/DH) (Table 5) (41). The 027/NAP1/BI strain was the most widely disseminated of the five strain types among the study sites and represented an overall mean of 25% of isolates recovered at each site (range, 11 to 54%) (data not shown). With the exception of the 001/NAP2/J strain, which was not reported by the East Coast study site, the other strains were present all across the United States but not in Canada. The 017/NAP9/CF results formed the tightest cluster and showed the most overlap of the three typing methods (after the 027/NAP1/BI cluster), while the J strain had many non-001 PCR ribotype results and non-NAP2 PFGE results, forming the loosest clustering of results.

The adjusted Rand coefficients, which indicate the overall congruence between the results of two typing methods, were 0.546 for PCR-ribotyping and PFGE, 0.584 for PFGE and REA, and 0.686 for PCR-ribotyping and REA. This indicates a slightly better matching of PCR-ribotyping and REA results (the closer the number is to 1, the higher the congruence of results), but none of the pairs of results generated by two typing method was highly correlated. The Wallace coefficients, which indicate the likelihood that a strain type from one method can predict the strain type from a second method or, alternatively, that adding a second method will provide additional strain discrimination, are presented in Table 6. The high coefficients for PCR-ribotyping and PFGE (0.902) and for PCR-ribotyping and REA (0.834) indicate that the addition of either PFGE or REA groups to PCR-ribotyping adds little additional strain discrimination. However, adding PCR-ribotyping to PFGE results (0.488) has better utility (since the number is closer to zero) than adding PCR-ribotyping to REA (0.658).

TABLE 5. Geographic distribution of commonly recognized strains by three typing methods

Strain (PCR ribotype/ PFGE/REA)	No. of isolates	Locations
001/NAP2/J[a]	6	U.S. Midwest and West
017/NAP9/CF	9	U.S. East Coast, Midwest, and West
027/NAP1/BI[b]	84	U.S. East Coast, Midwest, Pacific Northwest, and West; Canada
078/NAP7/BK[c]	5	U.S. East Coast, Midwest, and West
106/NAP11/DH[d]	11	U.S. East Coast, Midwest, and West

[a] Published as J strain by Johnson et al. (19).
[b] See McDonald et al. (33).
[c] See Debast et al. (10).
[d] See Sundram et al. (41).

TABLE 6. Congruence of strain typing results as indicated by Wallace coefficients

Method	Wallace coefficient (95% CI)[a]		
	PCR-ribotyping	PFGE	REA
PCR-ribotyping		0.902 (0.852–0.951)	0.834 (0.795–0.872)
PFGE	0.488 (0.460–0.516)		0.588 (0.547–0.629)
REA	0.658 (0.618–0.698)	0.792 (0.733–0.852)	

[a] Data were generated using the Comparing Partitions website (http://darwin.phyloviz.net/ComparingPartitions/index.php?link=Tool). CI, confidence interval.

DISCUSSION

C. difficile strains can be differentiated from one another by a variety of different phenotyping and genotyping methods. PCR-ribotyping, PFGE, and REA are among the most commonly used genotypic methods for outbreak analysis and epidemiologic studies of *C. difficile* strains. MLST is reserved primarily for population studies as its level of strain discrimination is too low for studies of outbreaks (30). Although Killgore et al. (22) examined the ability of multiple genotypic methods to cluster isolates of the hypervirulent/epidemic 027/NAP1/BI strain from around the world accurately, the study was limited to 42 isolates and did not rigorously explore the accuracy of the methods for other *C. difficile* strains. Nonetheless, Wilson et al. used the report of Killgore and colleagues as a basis for extrapolating REA results to PCR ribotypes (45). In that study, REA group DH strains were presumed to be ribotype 106, and group J strains were presumed to be ribotype 001. Our data indicate that group DH strains could be ribotypes 002, 046, and 053 in addition to 106, while group J strains could be ribotype 046 in addition to ribotype 001, potentially altering the interpretation of the data. Martin and colleagues typed 1,080 *C. difficile* isolates from Ontario, Canada, by multiple methods, including PCR-ribotyping, but performed PFGE and toxinotyping only on selected isolates (32). While the study did not provide adequate information about the congruence of the typing methods, the authors did note that NAP1 isolates could be assigned one of three ribotypes and that NAP2 isolates could be divided into four distinct ribotypes. This prompted our interest in whether one typing method, such as PCR-ribotyping, could be used to identify strains that were initially described using another typing tech-

nique, such as the J strain (representing REA grouping) (19) or the toxinotype V/NAP7 strain from animals (identified by PFGE), which would later be identified as PCR ribotype 078 (17).

Among the 350 isolates we examined by three methods, PFGE, which had the lowest calculated index of diversity at 65.30, yielded an established pattern for only 54% of the isolates tested while PCR-ribotyping (index of diversity of 82.71) provided a named ribotype for approximately 70% of the isolates. Both typing methods usually generated readable banding patterns, but many of the patterns had not yet been assigned a strain type. This is less of a problem with REA, which has more extensively defined patterns in its database (7), although for simplicity only the eight major patterns were used in this study (yielding an index of diversity of 78.25). Aside from the 027/NAP1/BI clone, the three methods showed the best congruence for strains that were 017/NAP9/CF. Knowing that PCR ribotype 017 isolates are typically PFGE type NAP9 and REA group CF may help identify these isolates in PFGE or REA databases and illuminate the epidemiology of toxin A-negative/toxin B-positive isolates. None of the other intersecting strain types, including 001/NAP2/J, 078/NAP7/BK, or 106/NAP11/DH were as tightly linked, and trying to predict a strain type based on one method (such as PFGE) using the other method (such as REA) may be problematic. This was reflected in the adjusted Rand coefficients for the pairwise comparisons of typing results, which ranged from 0.546 to 0.686, where values close to 1.0 indicate high congruence of results between methods. That said, the linkages of the three types provided here may still be useful as a starting point for examining the global spread of these common *C. difficile* lineages, recognizing, for example, that not all NAP11 isolates will be either PCR ribotype 106 or REA group DH.

Of the three methods used in this study, REA has the longest history as a molecular typing method for *C. difficile* (28) but is used primarily by a single laboratory in the United States and relies on visual interpretation of the data. This technique involves digesting total bacterial DNA with a frequent-cutting restriction endonuclease and separating the DNA fragments by agarose gel electrophoresis. REA was critical in defining a new epidemic strain of *C. difficile* (the J strain) in 1999 (19). It is interesting that of the 22 J group isolates in this study, only seven were both PCR ribotype 001 and NAP2, suggesting that REA typing tends to define a broader group of isolates than PFGE or PCR-ribotyping. This also seems to be true for REA groups BK, DH, G, and Y (Table 2). This may be consistent with the broader sampling of genomic information afforded by REA, in contrast to the more restricted location or number of chromosomal sites sampled by PCR-ribotyping and PFGE, respectively. However, as noted above, there appears to be a closer link between REA group CF strains, PCR ribotype 017, and NAP9, suggesting that this lineage is more clonal. Subtypes of the REA groups have also been described (e.g., BI6, BI8, and BI17) but those extended subtypes were not used in this study for strain comparisons as it made comparisons too complex. The discrepancies among REA, PFGE, and PCR-ribotyping have been noted previously (17, 22).

PCR-ribotyping is a more recent molecular typing technique that is playing a key role in defining the epidemiology of *C. difficile* infections in Europe, North America, and the Far East (24, 29, 40). There are now more than 200 PCR ribotypes described. Although this presumably provides higher levels of strain discrimination than PFGE, Martin et al. argue that if all PFGE patterns are considered and not just those patterns that are named, PFGE may provide a higher level of strain discrimination (32). Kuijper and colleagues established the link between the BI/NAP1 epidemic strain of *C. difficile* in North America and the PCR ribotype 027 in Europe in 2006, which helped advance our understanding of the epidemiology of this globally disseminated strain (27). Nonetheless, as noted by Killgore et al., not every ribotype 027 strain is REA group BI, nor is every NAP1 isolate ribotype 027 (22). However, of the three typing techniques, PCR-ribotyping is more readily implemented in a clinical laboratory.

PFGE is a third molecular technique used in this study for typing isolates of *C. difficile*. Initial attempts to type *C. difficile* by PFGE were largely unsuccessful due to degradation of DNA in the gels (23). The PFGE method was later optimized by incorporating thiourea into the gels and was used in conjunction with REA in early epidemiologic studies to define the most recent epidemic strain of *C. difficile*, designated BI/NAP1/027 (33). While the BI/NAP1/027 epidemic strain is classified as toxinotype III (38), the multicenter typing study reported by Killgore and colleagues showed that PFGE classified two toxinotype IX isolates as NAP1 (22). These two isolates did not contain the classic deletion in *tcdC* (a single base deletion resulting in a frameshift at position 117), indicative of the BI/NAP1/027 epidemic strain. Thus, as a typing method, PFGE is known to include within the NAP1 designation some strains of *C. difficile* that do not have the characteristics of the 027/NAP1/BI epidemic strain. However, only two NAP1 strains that did not contain the characteristic *tcdC* deletion of the epidemic 027/NAP1/BI strain were seen in our study. Thus, like REA, some NAP strain designations appear to encompass more isolate types than are recognized by the other typing methods. Martin and colleagues have made this same observation (32). For a laboratory wishing to initiate typing of *C. difficile* isolates, the Simpson's index of diversity scores and Wallace coefficients from our data set indicate that PCR-ribotyping should be the primary method of strain differentiation. Adding PFGE results provided better strain discrimination than adding REA typing as a second method (Wallace coefficient of 0.902 versus 0.834) and is likely to be easier to implement in the laboratory.

The Xpert *C. difficile* test provided a presumptive identification of a single strain, 027/NAP1/BI, in parallel with the detection of the *tcdB* gene for *C. difficile* identification, which may be of value particularly for infection control activities in hospitals (2, 15). The ability to identify rapidly a cluster of patients in a hospital that is infected with the 027/NAP1/BI strain could be the key to implementing infection control efforts to halt transmission in the hospital, including restriction of fluoroquinolones (15, 20, 35). The 027/NAP1/BI isolates have the potential to produce high levels of spores and require enhanced environmental cleaning efforts for eradication (1, 35). In addition, the 027/NAP1/BI strain may cause more severe disease in patients than other ribotypes (11, 14, 31, 36), and patients infected with this strain may disseminate the strain more broadly in health care settings due to the enhanced volume of stool produced and subsequent environmental con-

tamination. However, therapeutic decisions should be based on disease severity and not on organism strain type (9) as not all *C. difficile* infections caused by PCR ribotype 027 isolates are severe (8, 21).

Overall, the Xpert *C. difficile* results for presumptive identification of 027/BI/NAP1 agreed well with the results of PCR-ribotyping, REA, and PFGE. There were only four instances out of 96 results where the positive presumptive identification of an isolate by the Xpert *C. difficile* test was not type 027, NAP1, or BI. Of the techniques tested in this study, only the Xpert *C. difficile* assay could be run directly on stool samples and provide presumptive evidence of type 027/NAP1/BI in 45 min. Confirmation of the strain type would require culturing of the stool sample to recover the *C. difficile* isolate and then sending the isolate to a reference laboratory for typing using one of the three methods discussed above. There are relatively limited options available for strain typing *C. difficile* isolates in the United States since few state public health laboratories provide this service. However, testing the isolate for moxifloxacin resistance by the Etest method could be used to support the identification of the isolate as 027/NAP1/BI (33). The unique combination of the three genetic markers (*tcdB*, *cdt*, and the *tcdC* deletion at nucleotide 117) make the presumptive identification of the 027/NAP1/BI strain feasible. Similar loci are not readily available for identification of other *C. difficile* strains.

In conclusion, PCR-ribotyping, REA, and PFGE are all useful for typing *C. difficile* isolates although they each provide different patterns of strain clustering. One must be cautious about predicting a strain type based on any single method (i.e., not all NAP1 strains can be assumed to be ribotype 027 or REA group BI). Using more than a single method increases strain differentiation and may better illuminate the changing epidemiology of *C. difficile* strains, but few laboratories perform more than a single typing method. The Xpert *C. difficile* assay was the only method that reported strain data directly from specimens and yielded the information at the same time that the toxin B gene data were available. Rapid identification of the 027/BI/NAP1 strain type may be useful for tracking in-hospital or between-hospital outbreaks of *C. difficile* in real time, which may be of particular value for improving infection control interventions in conjunction with antimicrobial stewardship programs to decrease the duration of *C. difficile* outbreaks (12, 20, 35, 44).

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