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Impact of Strain Type on Detection of Toxigenic *Clostridium difficile*: Comparison of Molecular Diagnostic and Enzyme Immunoassay Approaches[▽]

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A multicenter clinical trial assessed the performance of the Cepheid Xpert *C. difficile* assay on stool specimens collected from patients suspected of having *Clostridium difficile* infection (CDI). A total of 2,296 unformed stool specimens, collected from seven study sites, were tested by Xpert *C. difficile* enrichment culture followed by cell culture cytotoxicity testing of the isolates (i.e., toxigenic culture with enrichment) and the study sites' standard *C. difficile* test methods. The methods included enzyme immunoassay (EIA), direct cytotoxin testing, and two- and three-step algorithms using glutamate dehydrogenase (GDH) screening followed by either EIA or EIA and an in-house PCR assay. All *C. difficile* strains were typed by PCR-ribotyping. Compared to results for toxigenic culture with enrichment, the sensitivity, specificity, and positive and negative predictive values of the Xpert assay were 93.5, 94.0, 73.0, and 98.8%, respectively. The overall sensitivity of the EIAs compared to that of enrichment culture was 60.0%, and the sensitivity of combined GDH algorithms was 72.9%; both were significantly lower than that of Xpert *C. difficile* ($P < 0.001$ and $P = 0.03$, respectively). The sensitivity of the EIA was significantly lower than that of the Xpert *C. difficile* assay for detection of ribotypes 002, 027, and 106 ($P < 0.0001$, $P < 0.0001$, and $P = 0.004$, respectively, Fisher's exact test), and the sensitivity of GDH algorithms for ribotypes other than 027 was lower than that for Xpert *C. difficile* ($P < 0.001$). The Xpert *C. difficile* assay is a simple, rapid, and accurate method for detection of toxigenic *C. difficile* in unformed stool specimens and is minimally affected by strain type compared to EIA and GDH-based methods.

Clostridium difficile continues to be a significant cause of infectious diarrheal disease among hospitalized patients, particularly in the United States and Europe (2, 11, 13). *C. difficile* isolates are capable of causing, in addition to diarrheal disease, serious syndromes, such as pseudomembranous colitis and toxic megacolon, which may result in death (11, 13). This organism is also responsible for increasing numbers of community-acquired infections (14, 16). Hypervirulent strains of *C. difficile* have emerged, including the J strain (9) and 027/NAP1/BI strain (20), the latter of which has been responsible for a series of hospital outbreaks around the world (10, 12, 20, 22). Recent studies indicate that several of the rapid enzyme immunoassay (EIA) methods used to diagnose *C. difficile* infection (CDI) are less sensitive than previously indicated (1, 29). The sensitivity of EIAs was often assessed initially using direct cytotoxin testing of stool samples, often in high-prevalence or outbreak settings (5). More-recent studies have compared the results of EIA methods against those of toxigenic culture, i.e., culturing *C. difficile* isolates from stool samples (often using broth enrichment) and testing the organism recovered in culture for cytotoxin production, which has higher sensitivity than direct cytotoxin testing (5, 25). The renewed use of toxigenic culture, particularly in North America, as the reference method has encouraged microbiologists to reassess diagnostic methods for CDI.

Another change in the diagnostic landscape has been the introduction of glutamate dehydrogenase (GDH) screening of stool specimens as part of two- or three-step algorithms in an attempt to enhance the sensitivity of *C. difficile* detection. Data from studies reported by Reller et al. (27) and Ticehurst et al. (36) supported the use of this approach, although a study by Gilligan raised concerns about using less-sensitive toxin EIAs for confirming GDH-positive assays (6).

More recently, PCR-based amplification methods for the detection of chromosomal genes encoding *C. difficile* toxin B (*tcdB*) or toxin regulatory genes (*tcdC*) directly in stool samples have been described (7, 15, 24, 29). Evaluations of several commercial amplification methods that target *tcdB* for detection of *C. difficile* in stool samples have been reported (8, 23,

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30, 31). Three commercial amplification methods have received Food and Drug Administration (FDA) clearance in the United States.

The goal of this study was to assess the accuracy of the Cepheid Xpert *C. difficile* assay in a multilaboratory study using the results of toxigenic culture with broth enrichment (i.e., "enrichment toxigenic culture") as the reference method. The accuracy of the two commercial EIAs, direct cytotoxin testing, and two algorithms that incorporate GDH screening that were the standard-of-care methods at the study sites was also assessed. We also evaluated the sensitivity of EIA, GDH, and Xpert *C. difficile* assays for detecting multiple PCR ribotypes of *C. difficile*, including ribotype 027.

MATERIALS AND METHODS

Study population and sample collection. This prospective study was conducted at seven health care organizations, six in the United States and one in Canada, from November 2008 to January 2009. Eligible patients included those with suspected CDI for whom unformed stool specimens were ordered for *C. difficile* testing according to the institutions' standard practices. All specimens tested in this study represented excess, leftover specimens; therefore, informed consent was waived by the Institutional Review Board (IRB) at six of seven sites. The seventh site (in Canada) obtained written informed consent from all participating patients. Duplicate specimens from the same patient and from patients under 2 years of age were excluded.

Upon establishment of eligibility and following standard testing at the institution, a portion of the specimen was used for Xpert *C. difficile* testing. The remaining specimen was transferred into anaerobic tissue transport medium (ATTM) (Anaerobe Systems, Morgan Hill, CA) and shipped to a single central laboratory for reference culture and cell cytotoxicity testing on isolates.

Xpert methods. Xpert *C. difficile* assays were performed at each participating institution according to the manufacturer's instructions. On each day of study testing, one positive and one negative control prepared and provided by the sponsor were tested with the Xpert *C. difficile* assay prior to any patient samples. The controls consisted of one negative and two positives, one positive for toxin B gene sequence (*tcdB*) and the other for *C. difficile* 027/NAP1/BI; the positive controls were alternated daily.

Any Xpert *C. difficile* assays not yielding results on the first attempt were repeated once using new reagents and a new cartridge according to the manufacturer's instructions. If a result was not obtained upon retesting, the specimen was reported unresolved.

Culture methods. All cultures were performed at a single centralized reference laboratory that was blind to the test results obtained at the study sites. Toxigenic culture was initiated within 5 days of sample collection; all manipulations were conducted in an anaerobe chamber. Stool was inoculated to prereduced cycloserine-cefoxitin-fructose direct agar (CCFA-direct) and cycloserine-cefoxitin-mannitol broth with taurocholate lysozyme cysteine (CCMB-TAL) (both media were obtained from Anaerobe Systems). The CCFA-direct 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. After 24 h, the CCMB-TAL was subcultured to a second CCFA plate (CCFA-enriched).

Aerotolerance testing was performed from CCFA. The appearance of large Gram-positive rods on a Gram stain from an obligate anaerobe colony from CCFA that was susceptible to 5 μg of vancomycin was considered presumptive evidence of *C. difficile*. The presence of isocaproic, isovaleric, and isobutyric acids as end products of glucose fermentation by gas-liquid chromatography served as confirmation of identification. Positive *C. difficile* isolates (4 or 5 colonies) were subcultured to chopped-meat carbohydrate broth and incubated for 48 h, and the cell-free supernatant was used for a cell culture cytotoxin assay to detect *C. difficile* toxin B (using human fetal foreskin cells from Diagnostic Hybrids, Athens, OH). A *C. difficile* antitoxin was used to demonstrate the specificity of the tissue culture reaction by neutralizing the cytotoxin present in the supernatant.

If *C. difficile* was isolated from the CCFA-direct plate and the isolate was positive by a cell culture cytotoxicity assay, the specimen was classified as toxigenic *C. difficile* positive and the CCFA-enriched plate was not further analyzed. If no *C. difficile* was isolated from the CCFA-direct plate or if the isolate was negative by cell culture cytotoxicity assay, then the CCFA-enriched plate was analyzed. If the CCFA-enriched plate was positive for *C. difficile* and the isolate was positive by cell culture cytotoxicity assay, the specimen was classified as toxigenic *C. difficile* positive. The specimen was reported as negative if CCFA-enriched was negative for *C. difficile* or the isolate was negative by a cell culture cytotoxicity assay.

This procedure allowed assessment of the Xpert *C. difficile* assay performance relative to both direct culture and enrichment culture methods in which all negative direct cultures were subjected to a pre-enrichment step prior to plating. The culture results were not reported until all other site testing had been completed.

Strain typing methods. Strain typing was performed by PCR-ribotyping as described by Stubbs et al. (32) and modified by Svenungsson et al. (34).

Data analysis. Sample size was calculated using the binomial distribution with the lower confidence interval as the acceptance criteria. Two-sided confidence intervals were used in the analysis to be conservative, with respect to statistical power for both sensitivity and specificity for *C. difficile*, relative to reference culture. The sample size was determined to be a minimum of 241 culture-positive specimens, which was achieved in the clinical trial. Performance characteristics were calculated for the Xpert *C. difficile* assay relative to direct and enrichment culture. A sample was considered toxigenic-culture positive for *C. difficile* if the presence of cytotoxin B was detected from the isolate in the cell culture assay.

Site-specific *C. difficile* testing. In addition to Xpert *C. difficile* and toxigenic culture testing, results from the individual study sites' own specific testing procedures for *C. difficile* were collected for each eligible patient. Of the seven study sites, four utilized a toxin A/B EIA for routine *C. difficile* testing (two used Premier toxins A and B [Meridian Bioscience, Inc.] and two used *C. difficile* Tox A/B II [Techlab, Blacksburg, VA]); one utilized a cell culture cytotoxin assay; one utilized a GDH test (C. Diff Chek-60 [Techlab]) with a toxin A/B EIA of GDH-positive samples (ProSpecT *Clostridium difficile* toxin A/B microplate assay [Remel, Lenexa, KS]); and one utilized a GDH test (C. Diff Quik Chek; Wampole, Princeton, NJ), with an EIA of GDH-positive samples (Tox A/B Quik Chek [Wampole]) and in-house PCR targeting *tcdB* for GDH-positive, EIA-negative samples. The site-specific results were compared to those for toxigenic culture with enrichment for each of the seven sites.

Reproducibility. Reproducibility was assessed using panels of seven specimens consisting of cultured material in a simulated matrix with various concentrations of toxigenic *C. difficile*. Each specimen was tested once per day for 10 days by two different operators at three separate testing sites (7 specimens × one time/day × 10 days × 3 sites × 2 operators/site) for a total of 20 runs of each sample per site. The operators at each site included one with prior PCR testing experience and one with no prior PCR testing experience.

RESULTS

Homogeneity of Xpert assay results. A total of 2,296 eligible patient samples, collected at seven different investigational sites, were tested by Xpert *C. difficile* assay, anaerobic culture with enrichment broth, and the sites' standard-of-care testing procedures for *C. difficile*. Fisher's exact test analyses showed homogeneity of the Xpert *C. difficile* assay results for sensitivity and specificity across all seven study sites ($P = 0.8526$ and $P = 0.9708$ for sensitivity and specificity, respectively), five test lots, four age categories, gender, and whether or not antimicrobial therapy was administered prior to sample collection (all P values for sensitivity and specificity for the last four categories were >0.05). Positive predictive value (PPV) and negative predictive value (NPV) data were also homogeneous (data not shown). This indicates that the Xpert *C. difficile* test results were consistent across all seven study sites and could be pooled for analysis. Compared to direct toxigenic culture results (without enrichment), the sensitivity, specificity, PPV, and NPV of the Xpert *C. difficile* assay were 98.8%, 90.8%, 56.6%, and 99.8%, respectively (Table 1). When enrichment broth was used to enhance the sensitivity of the toxigenic culture method, an additional 90 cultures were positive, an increase of 26.6%. Compared to enrichment culture, the sensitivity, specificity, PPV, and NPV of the Xpert *C. difficile* assay changed to 93.5%, 94.0%, 73.0%, and 98.8%, respectively (Table 1). This suggests that some stool samples contained concentrations of *C. difficile*

TABLE 1. Performance of the Xpert *C. difficile* assay compared to those of direct and enrichment cultures[a]

Xpert *C. difficile tcdB* assay result	Direct culture (no. of samples)			Enrichment culture (no. of samples)		
	Positive	Negative	Total	Positive	Negative	Total
Positive	245	188	433	316	117	433
Negative	3	1,860	1,863	22	1,841	1,863
Total	248	2,048	2,296	338	1,958	2,296

[a] Compared to *C. difficile* direct culture results, the Xpert *C. difficile* assay had the following results: sensitivity, 98.8%; specificity, 90.8%; positive predictive value (PPV), 56.6%; negative predictive value (NPV), 99.8%; prevalence, 10.8%. Compared to *C. difficile* enrichment culture, the Xpert *C. difficile* assay had the following results: sensitivity, 93.5%; specificity, 94.0%; PPV, 73.0%; NPV, 98.8%; prevalence, 14.7%.

organisms below the limit of detection of the Xpert *C. difficile* test. The limit of detection of the Xpert *C. difficile* test was assessed in-house using seven different *C. difficile* isolates of five different toxinotypes (i.e., 0, III, V, VIII, and XII); results ranged from 41 to 460 CFU/swab (data not shown). The overall prevalence of *C. difficile* in the study based on the results of enrichment toxigenic culture was 14.7%. Reproducibility studies showed that operators with no prior PCR experience performed the test equally as well as those with PCR experience. A total of 96.3% of the specimens analyzed yielded results on the first attempt. Only 0.4% of the specimen results were not resolved on the second attempt. Approximately 1.7% of samples yielded results that were likely due to inhibition.

Homogeneity of other test results. The sensitivity and specificity results for the seven site-specific methods were not homogeneous, i.e., gave statistically different results for sensitivity and specificity ($P = 0.0055$ and $P = 0.0052$, respectively). The outlier for sensitivity was a single site, site 6, which used the Premier toxin A and B EIA method (sensitivity data of the six remaining sites were homogenous when results from site 6 were excluded; $P = 0.2831$). For specificity, site 1, which also used Premier toxins A and B, was the only outlier (the specificity data of the six remaining sites were homogeneous when results from site 1 were excluded; $P = 0.2950$). The EIA method performed at sites 3 and 4 (i.e., TechLab *C. difficile* Tox A/B II) gave data that were consistent with each other and the other four sites. The reasons for the aberrant results at sites 1 and 6 are unknown. A comparison of each individual site's results against the results of toxigenic culture with enrichment is presented in Table 2.

The sensitivities of the EIA results from sites 1, 3, and 4 and from site 6 independently were significantly lower than that of the Xpert *C. difficile* assay compared to that of the method of toxigenic culture with enrichment reference (for both, $P < 0.001$) (Table 3). The Xpert *C. difficile* assay also was more sensitive than the two GDH algorithms (sites 2 and 5) ($P = 0.03$). There were relatively few positive cytotoxin results ($n = 11$) for statistical comparison to Xpert *C. difficile* results. Although the sensitivity of the direct cytotoxin method at site 7 was only 54.5%, compared with Xpert *C. difficile* sensitivity of 90.9%, this was not statistically significant (Table 3). The specificity data from all sites (except site 1) were uniformly high, and the PPVs were similar and statistically homogeneous ($P = 0.609$). The NPVs were not homogeneous due to site 6 ($P = 0.003$); however, when site 6 data were removed, homogeneity was achieved ($P = 0.616$) and the NPVs were uniformly high.

Effect of strain type on detection. We compared the PCR-ribotypes of isolates from samples that were positive by EIAs, the GDH algorithms, and the Xpert *C. difficile* assay. The EIA results were statistically less sensitive than the Xpert *C. difficile* assay for ribotypes 002, 027, and 106 (15.4%, 78.4%, and 18.8% sensitivity compared to 100%, 100%, and 75%, respectively; $P < 0.0001$, $P < 0.0001$, and $P = 0.004$, Fisher's exact test) (Table 4). The 33 culture-positive isolates from site 6, which were distributed across 12 PCR-ribotypes, were included in this analysis to maximize the number of ribotype comparisons between the Xpert *C. difficile* assays and EIAs.

The combined results for the two GDH algorithms are in Table 5. While the Xpert *C. difficile* and GDH algorithm results showed equivalent sensitivities for ribotype 027 strains, the Xpert *C. difficile* assay was significantly more sensitive than GDH for detecting strains of ribotypes other than 027. The lower sensitivity of the two GDH algorithms could not be attributed to any specific ribotype, as the isolates that were not

TABLE 2. Comparison of performance results for Xpert *C. difficile*, EIA, and two GDH algorithms compared to toxigenic culture with enrichment by site[a]

Site no.	Site assay	*n*	Sensitivity (%)		Specificity (%)		PPV (%)		NPV (%)	
			Xpert	Site	Xpert	Site	Xpert	Site	Xpert	Site
1[b,c]	Toxin A/B EIA	1,023	94.1	67.5	93.7	92.0	74.6	62.6	98.8	93.5
2[d]	GDH-EIA	268	91.4	74.3	93.6	94.8	68.1	68.4	98.6	96.1
3[e]	Toxin A/B EIA	293	92.3	53.8	94.5	97.6	72.0	77.8	98.8	93.2
4[f]	Toxin A/B EIA	312	91.4	54.3	94.2	95.7	66.7	61.3	98.9	94.3
5[g]	GDH-EIA-PCR	114	92.3	61.5	96.0	94.1	75.0	57.1	99.0	95.0
6[b]	Toxin A/B EIA	173	97.0	33.3	93.6	93.6	78.0	55.0	99.2	85.6
7[h]	Cytotoxin	110	90.9	54.5	94.9	98.0	66.7	75.0	98.9	95.1

[a] PPV, positive predictive value; NPV, negative predictive value.
[b] Premier toxin A and B.
[c] Three specimens were excluded from analysis since strain typing information was not available.
[d] GDH test (C. Diff Chek-60) with toxin A/B EIA (ProSpecT *Clostridium difficile* toxin A/B microplate assay) of GDH-positive samples.
[e] Tech Lab *C. difficile* toxin A/B.
[f] Tech Lab *C. difficile* toxin A/B microtiter plate assay.
[g] GDH test (C. Diff Quik Chek), with EIA of GDH-positive samples (Tox A/B Quik Chek) and in-house PCR targeting *tcdB* for GDH-positive, EIA-negative samples.
[h] Cell culture cytotoxin assay.

TABLE 3. Sensitivity of Xpert C. *difficile* assay and other detection methods compared to the results of toxigenic culture with enrichment

Site(s)	*n*	Method(s)	Xpert sensitivity (%)	Other method sensitivity (%)	Difference in sensitivity (%)	Confidence interval (difference in sensitivity)	*P* value[a]
1, 3, 4	243	Toxin A/B EIA	93.4	63.4	30.0	23.2–36.9	<0.001
6	33	Toxin A/B EIA	97.0	33.3	63.7	46.5–80.8	<0.001
2, 5	48	GDH-EIA, GDH-EIA-PCR	91.7	72.9	18.8	3.95–33.6	0.03
7	11	Direct cytotoxin	90.9	54.5	36.4	2.39–70.3	0.149

[a] Fisher's exact test.

detected by the GDH algorithms were of multiple PCR-ribotypes. Specifically, five isolates were ribotype 001, 002, 014, 020, or 104, and the remaining six isolates yielded undesignated ribotyping patterns. Of the isolates with undesignated PCR-ribotype patterns, two were type NAP4 by pulsed-field gel electrophoresis (PFGE) and both were also group Y by restriction endonuclease analysis (REA) typing. The others did not yield designated REA or PFGE patterns.

DISCUSSION

Rapid detection of CDI cases using sensitive laboratory methods is critical to ensuring optimal therapy of the individual patient and initiation of timely infection control measures to interrupt transmission of *C. difficile* to other patients (21, 25). Recently, the sensitivities of several widely used EIAs have been reported to be surprisingly low, raising new questions about the best method for CDI detection (1, 5). The use of toxigenic culture with enrichment broth for *C. difficile* detection as the reference method in recent studies instead of direct cytotoxin assays of stool specimens is largely responsible for the lower sensitivities reported for the EIAs (5, 25). Although toxigenic culture is arguably the best test indicator of infectious risk and the need for infection control interventions, it is slow and laborious, often requiring 72 to 96 h to complete (5, 6), and is unlikely to be adopted by many clinical laboratories as their standard method of *C. difficile* testing.

Two alternative approaches to enhancing the sensitivity of detecting toxigenic *C. difficile*-positive stool specimens are PCR-based assays for *tcdB* or *tcdC* and GDH screening algorithms. Commercial GDH assays detect an enzyme present in the cell wall of *C. difficile* isolates, including both toxigenic and nontoxigenic strains (17, 18), while current commercial PCR assays target a single chromosomal gene, *tcdB*, which is essential for virulence (19).

At present, there are three FDA-cleared PCR assays available in the United States and several additional amplification tests available elsewhere around the world for detection of *tcdB*. The sensitivities of the commercial PCR assays, compared to toxigenic culture results, range from 83.6% to 94.4% (23, 30, 31). In our multicenter study, the overall sensitivity of the Xpert *C. difficile* PCR assay for *tcdB* was statistically greater than those of the two commercial EIAs and the two GDH screening algorithms, compared to that of toxigenic culture with enrichment. The Xpert *C. difficile* results were comparable across all seven study sites, indicating that the assay is robust even among users not specifically trained in PCR testing methods. All *C. difficile* detection methods (except those from site 1) showed comparably high levels of specificity.

With regard to GDH screening, the presumed high sensitivity but lower specificity of GDH assays prompted both Reller et al. (27) and Ticehurst et al. (36) to propose a two-step algorithm approach using GDH as a screening assay, followed by a more specific confirmation assay. This would allow laboratories to screen out the majority of negative specimens, given the perceived high sensitivity of the GDH assay. However, the choice of confirmation assays has been a source of considerable debate. Gilligan evaluated two EIAs as confirmation assays and noted that neither test was sufficiently sensitive to be used for confirmation (6). Instead, he advocated use of the slower but more sensitive direct cytotoxin testing of the stool sample for confirming GDH-positive samples (6). An alternative approach is to use a PCR method to confirm GDH-positive samples. This approach has been advocated recently by Doing et al. (4), Quinn et al. (26), and Swindells et al. (35). These studies have focused primarily on those specimens that

TABLE 4. Sensitivity of Xpert *C. difficile* versus enzyme immunoassays for specific PCR ribotypes[a]

Ribotype	*n*	Xpert *C. difficile*			Enzyme immunoassay			*P* value[b]
		No. positive	No. negative	Sensitivity (%)	No. positive	No. negative	Sensitivity (%)	
001	8	8	0	100	6	2	75.0	0.46
002	13	13	0	100	2	11	15.4	<0.0001
017	11	10	1	90.9	7	4	63.6	0.311
027	74	74	0	100	58	16	78.4	<0.0001
053	12	12	0	100	8	4	66.7	0.093
078	11	9	2	81.8	7	4	63.6	0.635
104	6	5	1	83.3	3	3	50.0	0.545
106	16	12	4	75.0	3	13	18.8	0.004
Other ribotypes	124	115	9	92.7	71	53	57.3	<0.0005

[a] Includes data from site 6 to maximize the number of ribotype comparisons but is limited only to those isolates for which a known PCR ribotype could be established.
[b] Fisher's exact test.

TABLE 5. Sensitivity of Xpert *C. difficile* versus GDH algorithms for ribotype 027 and non-027 isolates[a]

Ribotype	*n*	Sensitivity (%)		*P* value[b]
		Xpert *C. difficile*	GDH algorithms	
027	11	90.9	90.9	1.0
Non-027	36	91.7	69.4	0.001

[a] Excludes one isolate for which PCR-ribotyping data were not available.
[b] Fisher's exact test.

are GDH positive but EIA negative, due to the low sensitivity of the EIA component of the assays. In these reports, GDH sensitivity ranged from 97% to 100%. The high sensitivity of the GDH assays noted in the above studies is in contrast to those of several others that have reported sensitivities ranging from 76% to 87.7% when GDH results were compared to toxigenic culture (5, 23, 25). The recent comprehensive study of *C. difficile* detection methods by Eastwood et al. (5), which reported the sensitivity of a commonly used GDH assay as 87.6% compared to that of toxigenic culture, is consistent with the report of Larson et al., which evaluated a direct in-house PCR assay on stool samples (targeting *tcdB*) and a three-step algorithm that included a GDH screening assay, EIA confirmation, and PCR testing of GDH-positive, EIA-negative specimens (15). These investigators retested GDH-negative stool samples by PCR and identified four additional positive samples. All four patients had subsequent stool samples positive both by PCR and toxigenic culture, and a review of the patients' charts revealed that three of four patients had been treated for CDI. By retesting GDH-negative samples by PCR, the authors noted that the sensitivity of the GDH algorithm dropped from 97.1% to 83.8% and the NPV dropped from 99.7% to 97.9%.

The study of Larson et al. illustrates the dilemma of many clinical microbiology laboratories: specifically, are the increased sensitivity and speed of direct PCR testing worth the added expense of the assay? In their cost analysis, Larson et al. concluded that the increased cost of either direct PCR testing or the three-step algorithm was justified by the earlier detection of CDI cases, which would prevent additional cases of nosocomial CDI and shorten the length of stay for patients with CDI. In fact, the costs of both PCR approaches were similar on a "case detected" basis. Thus, the tradeoff was the lower cost and longer turnaround time of the three-step approach versus the higher sensitivity (97.5% versus 83.8%) of direct PCR testing (15). A similar situation was recently reported in a study by Novak-Weekley et al., who noted that the Xpert *C. difficile* assay reported the highest sensitivity and NPV in the least amount of time (usually less than 1 h) among the various testing methods examined, but at added expense to the laboratory (23). Novak-Weekley et al. also noted that the sensitivity of GDH algorithms was lower than that for direct testing of stool using Xpert *C. difficile* in their study (83.1% versus 94.4%, respectively).

One novel aspect of our study was the levels of sensitivity of the Xpert *C. difficile* PCR assay that were significantly higher than those of EIA methods, specifically for ribotypes 002, 027, and 106. The Xpert PCR assay was also significantly more sensitive than the two algorithms that incorporated GDH screening, but only for ribotypes other than 027. Ribotype 027 strains represented 28.6% of the 350 isolates recovered in the clinical trial among the seven study centers (range, 11 to 54%, including 34 isolates from specimens that were ultimately excluded from the clinical trial data set for noncompliance with the study protocol; data not shown), while ribotype 106 represented 4.9% of isolates and ribotype 002 represented 5.1% of isolates. Together, these three ribotypes represent over a third of isolates recovered at the seven centers and likely explain levels of EIA sensitivity lower than those of toxigenic culture in many studies. Hospital-to-hospital variation of *C. difficile* strain types may also explain, at least in part, the variable sensitivity of GDH assays in several recent studies. The overall sensitivity of the Xpert *C. difficile* assay was minimally affected by ribotype.

Unfortunately, ribotyping data are rarely reported for *C. difficile* isolates from the United States. Thus, assessing the broader significance of the decreased ability of EIA and GDH to detect some *C. difficile*-positive specimens is difficult. However, ribotype 027 corresponds to pulsed-field gel electrophoresis (PFGE) type NAP1, which represented 19 of 92 (20.6%) community-associated *C. difficile* strains in a recent Centers for Disease Control and Prevention (CDC) survey (16), while ribotype 106, which includes NAP11 (F. C. Tenover, unpublished data), represented 6 of 92 (6.5%) *C. difficile* strains. Ribotype 002 does not currently have a corresponding NAP type, so its prevalence could not be assessed from the CDC survey. A recent United Kingdom survey by Sundram et al. (33) demonstrated that among 97 recent cases of *C. difficile*, ribotype 027 represented 45% of isolates, 106 accounted for 39% of isolates, and 10% were ribotype 001. Thus, ribotype 106 may well have the potential to cause an epidemic of CDI in the United States. Such an epidemic could go largely unrecognized if EIAs remain the major mode of testing for CDI, given the particularly poor performance of EIA for detection of this strain.

In summary, CDI continues to spread worldwide. PCR has been proposed by Peterson and Robicsek as the most sensitive and specific method for detecting *C. difficile* in clinical specimens (25), while Schmidt and Gilligan have referred to PCR-based testing as "the next logical step in the evolution of *C. difficile* diagnostics" (28). Our data suggest that the Xpert *C. difficile* assay has both the high sensitivity and high NPV necessary to give clinicians confidence in the laboratory's *C. difficile* testing results, obviating the need for submission of multiple stool samples, as frequently observed in laboratories using EIA methods (3, 25). The assay has received a "moderate complexity" classification by the FDA, which makes it accessible to both large and small laboratories.

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