Impact of the Type of Diagnostic Assay on Clostridium difficile Infection and Complication Rates in a Mandatory Reporting Program

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Background. Most Clostridium difficile infection (CDI) surveillance programs neither specify the diagnostic method to be used nor stratify rates accordingly. We assessed the difference in healthcare-associated CDI (HA-CDI) incidence and complication rates obtained by 2 validated diagnostic methods.

Methods. This was a prospective cohort study of patients for whom a C. difficile test was ordered between 1 August 2010 and 31 July 2011. All specimens were tested in parallel by a commercial polymerase chain reaction (PCR) assay targeting toxin B gene tcdB, and a 3-step algorithm detecting glutamate dehydrogenase and toxins A and B by enzyme immunoassay and cell culture cytotoxicity assay (EIA/CCA). CDI incidence rate ratios were calculated using univariate Poisson regression.

Results. A total of 1321 stool samples were tested during a period totaling 95,750 patient-days. Eighty-five HA-CDI cases were detected by PCR and 56 cases by EIA/CCA (P = .01). The overall incidence rate was 8.9 per 10,000 patient-days (95% confidence interval [CI], 7.1–10.9) by PCR and 5.8 per 10,000 patient-days (95% CI, 4.4–7.4) by EIA/CCA (P = .01). The incidence rate ratio comparing PCR and EIA/CCA was 1.52 (95% CI, 1.08–2.13; P = .015). Overall complication rate was 27% (23/85) when CDI was diagnosed by PCR and 39% (22/56) by EIA/CCA (P = .16). Cases detected by PCR only were less likely to develop a complication of CDI compared with cases detected by both PCR and EIA/CCA (3% vs 39%, respectively; P < .001).

Conclusions. Performing PCR instead of EIA/CCA is associated with a >50% increase in the CDI incidence rate. Standardization of diagnostic methods may be indicated to improve interhospital comparison.

Keywords. Clostridium difficile; surveillance; epidemiology; laboratory diagnosis; nosocomial infections.

Clostridium difficile infection (CDI) has been described worldwide and causes significant morbidity and mortality [1]. For more than a decade, several countries have reported an increasing incidence and severity due to the emergence of hypervirulent strains [2–4]. To better understand its epidemiology, detect outbreaks, and improve its control, the surveillance and reporting of CDI rates have been implemented in numerous jurisdictions [5–7]. To increase interinstitutional comparability, recommendations regarding optimal surveillance methods have been published [8, 9]. These guidelines provide standardized case definitions, as well as guidance regarding denominators and the expression of infection rates [8, 9]. Despite this attempt to formalize methodology, no clear guidance has been emitted regarding the type of laboratory test to diagnose CDI on stool samples [8–10]. Consequently, the choice of laboratory testing is usually left to the discretion of each institution [1, 8, 9, 11, 12], and incidence rates are usually not adjusted for the type of diagnostic test [1, 13, 14].
Various laboratory tests can be used to diagnose CDI, and institutions have a wide range of options [7, 10, 15–17]. However, these methods differ in terms of sensitivity, specificity, cost, and turnaround time [18], and considerable debate persists regarding the optimal method of detection [19]. Toxigenic culture and cell culture cytotoxicity assay (CCA) are the most sensitive methods, but are not widely used owing to slow turnaround times and technical requirements [7, 9, 20]. By contrast, enzyme immunoassay (EIA) tests for *C. difficile* toxins A and B (ToxA/B) have been widely adopted by clinical laboratories because of their short turnaround time and ease of use, but lack sensitivity [9, 21]. These tests are often combined with EIA testing for glutamate dehydrogenase (GDH), a more sensitive, but less specific, antigen present in both toxigenic and nontoxigenic *C. difficile* [17]. More recently, polymerase chain reaction (PCR) assays targeting the ToxA gene 

\[ tcdA \]

and/or the ToxB gene 

\[ tcdB \]

have been commercialized and appear to be rapid, sensitive, and specific [18, 22]. In addition to these 4 types of laboratory tests, some institutions have implemented multistep diagnostic algorithms that use EIA detection of GDH followed by CCA, toxigenic culture, or PCR to confirm toxigenicity [7, 10, 17].

In conjunction with incidence rates, many surveillance programs also monitor the rate of complications, such as death or colectomy, to detect any change in the virulence of *C. difficile*. However, the complication rate could be influenced by the type of laboratory test used. For example, institutions that use a more sensitive method could report a greater number of less severe cases, which could subsequently lead to a decrease in the proportion of cases with complications, but this hypothesis has not been investigated so far. Considering that mandatory and public reporting of CDI rates may place institutions under pressure to report the lowest rates as possible and that international guidelines do not recommend any specific laboratory approach for CDI surveillance, there is a need to assess the extent to which these rates may vary depending on the diagnostic strategy.

A mandatory and public province-wide surveillance of CDI was introduced in Quebec, Canada (population 8 million) in August 2004 by the Quebec National Institute of Public Health (INSPQ) [23, 24]. As of 1 October 2011, all 95 acute care facilities admitting >1000 patients per year have the obligation to participate in this surveillance program. Incidence rates are subject to government-imposed targets, which are risk stratified according to the number of beds, the proportion of hospitalized patients aged ≥65 years, and academic status [25]. Institutions report CDI incidence rates computed as the number of cases divided by the number of hospital-days (ie, incidence density) per 4-week period. CDI rates are publicly reported in annual [25] and quarterly [26, 27] reports. In compliance with international guidelines [8, 10], the type of laboratory diagnostic approach is left to the discretion of each participating center.

In the present study, we compared the performance of 2 different diagnostic approaches on CDI incidence and complication rates in a single institution over a 12-month period: a 3-step algorithm based on detection of GDH by EIA followed by detection of ToxA/B by EIA or CCA, and a 1-step approach based on the detection of ToxB gene by PCR. We investigated also whether cases detected by PCR differed in terms of complication rates compared with those detected by both PCR and the 3-step algorithm.

**METHODS**

**Study Design and Setting**

We performed a prospective observational cohort study of all patients admitted to the Quebec University Institute of Cardiology and Pneumology (IUCPQ), Quebec City, Canada, between 1 August 2010 and 31 July 2011, to compare incidence rates of healthcare-associated CDI (HA-CDI) obtained using 2 different diagnostic strategies. The IUCPQ is a 350-bed primary and tertiary teaching healthcare facility admitting 13 000 patients annually. The study was approved by the institutional review board of the IUCPQ.

**Definitions**

CDI was defined by the INSPQ as (1) a clinical history of documented diarrhea (≥3 loose or liquid stools in <24 hours and symptoms lasting ≥24 hours) without other known etiology combined with a positive test result for toxin-producing *C. difficile*, or (2) a clinical diagnosis based on histopathology or direct visualization of pseudomembranes by colonoscopy. A case was considered to be HA-CDI if symptoms appeared ≥72 hours after admission and up to 4 weeks following discharge. A community-acquired CDI case was defined as a patient who developed symptoms within 72 hours of admission and who had not been hospitalized in the previous 4 weeks. Recurrence was defined as a relapse of symptoms <8 weeks after the end of the previous treatment. For the purpose of this study, only HA-CDI cases were included. In accordance with surveillance policy, the following complications are reported if they occur within 30 days of diagnosis of HA-CDI: 30-day all-cause mortality; colectomy; admission to the intensive care unit (ICU); and hospital readmission for CDI. Complications were collected prospectively by trained infection control nurses according to standardized surveillance definitions [28]. The records of all patients with a positive sample for *C. difficile* were reviewed by trained infection control professionals to assess whether they met the case definition. Relevant information was collected using a structured data collection form.

**Laboratory Diagnostic Algorithms**

During the study period, all specimens submitted to the laboratory for *C. difficile* were prospectively tested in parallel using the
2 different diagnostic algorithms (Figure 1): a 1-step approach based on PCR targeting the ToxB gene tcdB (BD GeneOhm Cdiff, Franklin Lakes, New Jersey), and a 3-step algorithm (subsequently referred to as EIA/CCA). The first 2 steps of EIA/CCA consisted in the detection of GDH antigen (Diff Chek-60, Techlab, Blacksburg, Virginia) and ToxA/B (ToxA/B QuikCheck, Techlab) by EIA. Samples yielding a positive result for GDH and ToxA/B were considered positive for the presence of C. difficile. Samples with a positive result for GDH but a negative result for ToxA/B were immediately retested in a subsequent step by CCA. This assay uses a Vero cell line to detect the presence of a cytopathic effect neutralized by C. difficile antitoxin (Bartels Immunodiagnostic Supplies, Bellevue, Washington). A sample was considered positive if a cytopathic effect was observed within 72 hours with neutralization by the antitoxin. According to institutional policy, only loose or watery stools were tested for C. difficile. Samples with a positive result for GDH but a negative result for ToxA/B were immediately retested in a subsequent step by CCA. This assay uses a Vero cell line to detect the presence of a cytopathic effect neutralized by C. difficile antitoxin (Bartels Immunodiagnostic Supplies, Bellevue, Washington). A sample was considered positive if a cytopathic effect was observed within 72 hours with neutralization by the antitoxin. According to institutional policy, only loose or watery stools were tested for C. difficile (consistency of 6 or 7 on the Bristol stool form scale). During the study, physicians and the infection control team had only access to the results of the PCR assay and were blinded to the result of the EIA/CCA algorithm.

### Infection Control Issues

Patients were placed under contact precautions on the basis of PCR result. During the entire duration of the study, the indications to perform a C. difficile assay on stool samples were left to the discretion of the treating physician and isolation precautions remained unaltered. More specifically, patients presenting diarrhea were placed under contact precautions pending confirmation of the diagnosis [29].

### Statistical Analysis

Monthly incidence rates of HA-CDI were computed according to each diagnostic strategy by expressing the number of HA-CDI per 10,000 patient-days. We compared the absolute number of cases diagnosed by PCR and EIA/CCA, the number of periods with incidence rates above the government-imposed target, and the number of cases with complicated CDI using the chi-squared test and Fisher exact test. Incidence rate ratio (IRR) by PCR compared with EIA/CCA was estimated with 95% confidence intervals (CIs) using Poisson regression. Significance was
Based on $\alpha < .05$ and all hypothesis tests were 2-sided. All analyses were performed using SAS, version 9.2.

**RESULTS**

Between 1 August 2010 and 31 July 2011 (a period totaling 95,750 patient-days), all 1321 stool samples from 888 patients sent to the laboratory for *C. difficile* detection were tested in parallel by PCR and EIA/CCA. Of these, 224 (17.0%) were positive for the *tcdB* gene by PCR and 162 (12.3%) were positive by EIA/CCA (Table 1 and Figure 1). Overall, PCR yielded a positive result more often than EIA/CCA (absolute difference, 62 cases, $P < .001$). 158 samples were positive by both PCR and the 3-step algorithm. Sixty-six samples were positive by PCR, but not by EIA/CCA. Of these, 43 (65%) were GDH-positive, but ToxA/B and CCA negative. Four samples were positive by EIA/CCA, but not by PCR. However, none of these samples was related to HA-CDI. In addition, 11 samples (0.8%) had an undetermined result by PCR despite retesting, all of which tested negative by EIA/CCA. Ninety-two cases were excluded because they did not fulfill the time frame requirements for HA-CDI. Thirty-three cases were also excluded because they were recurrences, and 10 cases were excluded because of the presence of an alternate cause for the symptoms, such as laxative use, viral gastroenteritis, or gastrointestinal hemorrhage. Finally, 8 cases were excluded because they did not meet the standardized definition for diarrhea. Hence, 85 cases fulfilled the case definition for HA-CDI and were eligible for the comparison of HA-CDI rates by PCR and EIA/CCA.

**Comparison of Reportable CDI Rates**

Over a 12-month period, 85 cases of HA-CDI were detected by PCR compared with 56 cases by EIA/CCA ($P = .01$). The incidence density per administrative period varied from 2.9 to 18.7 per 10,000 patient-days by PCR and from 0 to 16.1 by EIA/CCA algorithm (Figure 2). The overall incidence density was 8.9 per 10,000 patient-days (95% CI, 7.1–10.9) by PCR and 5.8 per 10,000 patient-days (95% CI, 4.4–7.4) by EIA/CCA (absolute difference, 3.1 cases per 10,000 patient-days, $P = .01$). Incidence rates were above the government-imposed target of 9.0 per 10,000 patient-days in 7 of 13 (54%) surveillance periods by PCR, and in 4 of 13 (31%) surveillance periods by EIA/CCA ($P = .4$). There was a wide variation in the magnitude of discordance between the 2 diagnostic methods depending on the surveillance period, ranging from no discordance to an absolute difference of 6.7 cases per 10,000 patient-days (incidence by EIA/CCA and PCR, 3.3 and 10.0, respectively). The IRR comparing PCR and EIA/CCA was 1.52 (95% CI, 1.08–2.13; $P = .015$).

**Complications of CDI Infection**

Overall, $\geq 1$ complication occurred in 27% (23/85) of patients. In total, 11 patients died within 30 days of diagnosis, 1 patient...
underwent a colectomy, and 11 patients were readmitted for CDI. The overall complication rate was 27% (23/85) when PCR was used to diagnose CDI compared with 39% (22/56) when diagnosed by EIA/CCA ($P = .16$). Cases detected by PCR, but not by EIA/CCA, were less prone to present a complication of CDI compared with cases detected by both PCR and EIA/CCA (3% vs 39%, respectively; $P < .001$) (Table 2).

### Table 2. Frequency of Complications Associated With Clostridium difficile Infection as Detected by Polymerase Chain Reaction (PCR) Only and by Both PCR and Enzyme Immunoassay/Cell Culture Cytotoxicity Assay Algorithm, University Institute of Cardiology and Pneumology, Quebec, Canada, August 2010–July 2011

<table>
<thead>
<tr>
<th>Complications</th>
<th>CDI Cases Detected by PCR, but Not by EIA/CCA (n = 29)</th>
<th>CDI Cases Detected by Both PCR and EIA/CCA (n = 56)</th>
<th>$P$ Value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-d mortality (%)</td>
<td>1 (3)</td>
<td>10 (18)</td>
<td>.09</td>
</tr>
<tr>
<td>Colectomy (%)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Admission to ICU (%)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Readmission for CDI (%)</td>
<td>0 (0)</td>
<td>11 (20)</td>
<td>.01</td>
</tr>
<tr>
<td>Occurrence of $\geq 1$ complication (%)</td>
<td>1 (3)</td>
<td>22 (39)$^b$</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations: CDI, Clostridium difficile infection; EIA/CCA, detection of glutamate dehydrogenase antigen and toxins A and B by enzyme immunoassay and cell culture cytotoxicity assay; ICU, intensive care unit; PCR, detection of toxin B gene tcdB by polymerase chain reaction.

$^a$ By Fisher exact test.

$^b$ One patient with colectomy was admitted to the ICU.

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**DISCUSSION**

CDI surveillance is increasingly popular among policymakers in many developed countries including the United States, United Kingdom, Germany, and Belgium [5, 6]. To ensure optimal interfacility comparison, international guidelines state that CDI rates must be adjusted to take into account potential differences in risk factors such as hospital size and the proportion of hospitalized elderly patients [1, 23, 30]. However, the type of laboratory test to diagnose CDI is usually not taken into account in surveillance programs [1, 14].

This study demonstrates that laboratory methods can have a significant impact on CDI rates. Indeed, performing PCR instead of EIA/CCA to diagnose CDI led to a 50% increase in incidence rates. This difference is highly significant from an epidemiological perspective as it can theoretically raise an institution’s incidence rate above government-imposed limits. For this reason, our study supports the notion that diagnostic methods should be standardized to allow a fair comparison across institutions. Without taking this critical variable into consideration, surveillance programs and policymakers may unwittingly incite healthcare institutions to choose a less sensitive method to obtain better rates. Such “gaming” may be a particular concern in areas where rates are publicly reported [5, 31].

Increases of approximately 50% in CDI rates following the introduction of PCR-based testing has been reported in 2 before-and-after studies [32, 33]. However, these studies were limited to 6-month periods and could not take into account the seasonality of CDI rates [34]. By comparing PCR to EIA/CCA in parallel over a 12-month period, the present study shows that there is a wide monthly variation in the magnitude of the
discordance between the 2 diagnostic approaches. The reason behind this variation is unclear and deserves further study. The apparent clustering of discordant cases could be due to random variation. Alternatively, it could be linked to factors external to the laboratory, such as antibiotic prescription rates [35] or the activity of respiratory viruses and norovirus [34].

Considerable uncertainty persists regarding how to handle discordant cases and as to whether cases detected by PCR, but not by EIA and CCA, are clinically less severe. This study shows that complications rates are 30% lower when CDI cases are diagnosed by PCR rather than by EIA/CCA, although this difference did not quite reach statistical significance owing to the small sample size. This trend is at least in part due to the fact that patients with cases detected by PCR, but not by EIA/CCA, are much less likely to die from any cause within 30 days or be readmitted for CDI. This finding is in line with a recent retrospective case-control study that shows that cases detected by PCR, but not by EIA, are less likely to have high-level leukocytosis (>15 × 10⁹/L) than cases detected by both PCR and EIA [33]. This suggests that the increase in sensitivity is biased toward detecting less severe cases, which are less likely to present a complicated course. Alternatively, the decrease in complication rate could be due to earlier detection and treatment of CDI. As a third hypothesis, the lower complication rate could be due to the detection of C. difficile carriers who develop diarrhea for another unrelated reason. Clearly, further studies will be required to better understand the causes behind our findings.

However, our results are in striking contrast with those of another recently published study that detected no difference between PCR and EIA in the clinical presentation [36]. This apparent discrepancy is probably related to case definition: the latter study excluded PCR-positive but GDH-negative samples, whereas our study included both GDH-positive and GDH-negative samples.

This study presents some limitations. As toxigenic culture was not performed, it is not possible to determine whether cases detected by PCR, but not by EIA/CCA, represent true or false-positive cases in the presence of compatible symptoms. Nevertheless, performing toxigenic culture would not invalidate the main finding regarding the discrepancy in incidence rates between PCR and EIA/CCA. Furthermore, the PCR used in this study compares favorably with toxigenic culture, with sensitivity and specificity of 85%–95%. [37] In addition, our study was performed in a single center and caution should be exercised in extrapolating these data to other settings. PCR-based assays can detect CDI at an earlier stage [38]. It is possible that in an institution using a less sensitive diagnostic method such as EIA, repeat testing would have detected additional cases, albeit with a short delay. It was not possible to control for this potential confounder as it would be unethical to withhold disclosing the PCR result and delay the treatment of PCR-positive, EIA-negative patients for the sole purpose of this study. Furthermore, repeat testing is usually not recommended as the incremental yield of repeat testing is negligible [20, 39]. In addition, as our surveillance program does not collect information on management, it was not possible to compare therapy between the 2 patient populations. A before-and-after study has shown that the type of diagnostic test can impact on antimicrobial prescribing practices [40]. However, because treating physicians in our study were blinded to the result of EIA/CCA, any potential variation in treatment would be attributable to a difference in disease presentation, rather than the diagnostic test result.

This study shows that institutions should expect an increase in CDI rates when introducing a more sensitive laboratory test for CDI. Whether this increase is short-lived (ie, ≤1 year) or permanent remains to be determined. In theory, an increase in case finding could be offset by the use of better infection control strategies, such as the correct use of contact precautions and the use of chlorine-based disinfectants for patients with CDI who would have been missed by EIA/CCA. This could ultimately lead to a decrease in CDI rates.

In conclusion, this study shows that incidence and complication rates may be greatly influenced by the type of laboratory test used to diagnose CDI and that cases detected by PCR, but not by EIA/CCA, are less likely to present a complication within 30 days of diagnosis. There is a need to develop new strategies to address this issue and improve interhospital comparison of incidence and complication rates.

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