A Portrait of the Geographic Dissemination of the Clostridium difficile North American Pulsed-Field Type 1 Strain and the Epidemiology of C. difficile–Associated Disease in Québec

Bruno Hubert, Vivian G. Loo, Anne-Marie Bourgault, Louise Poirier, André Dascal, Élise Fortin, Marc Dionne, and Manon Lorange

Institut National de Santé Publique du Québec, Québec, and McGill University Health Centre, Centre Hospitalier de l’Université de Montréal Hôpital St. Luc, Hôpital Maisonneuve-Rosemont, Sir Mortimer B. Davis–Jewish General Hospital, and Laboratoire de Santé Publique du Québec, Institut National de Santé Publique du Québec, Montréal, Canada

Background. An increase in the incidence and severity of Clostridium difficile–associated disease in Québec and the United States has been associated with a hypervirulent strain referred to as North American pulsed-field type 1 (NAP1)/027.

Methods. In 2005, a prospective study was conducted in 88 Québec hospitals, and 478 consecutive nosocomial isolates of C. difficile were obtained. The isolates were subjected to pulsed-field gel electrophoresis (PFGE) typing, antimicrobial susceptibility testing, and detection of binary toxin genes and tcdC gene deletion. Data on patient age and occurrence of complications were collected.

Results. PFGE typing of 478 isolates of C. difficile yielded 61 PFGE profiles. Pulsovars A (57%), B (10%), and B1 (8%) were predominant. The PFGE profile of pulsovar A was identical to that of strain NAP1. It showed 67% relatedness with 15 other PFGE patterns, among which 11 had both binary toxin genes and a partial tcdC deletion but different antibiotic susceptibility profiles. Pulsovars B and B1 were identical to strain NAP2/ribotype 001. In hospitals showing a predominant clonal A or B-B1 PFGE pattern, incidence of C. difficile–associated disease was 2 and 1.3 times higher, respectively, than in hospitals without any predominant clonal PFGE pattern. Severe disease was twice as frequent among patients with strains possessing binary toxin genes and tcdC deletion than among patients with strains lacking these virulence factors.

Conclusions. This study helped to quantify the impact of strain NAP1 on the incidence and severity of C. difficile–associated disease in Québec in 2005. The identification of the geographic dissemination of this predominant strain may help to focus regional infection-control efforts.
**Table 1. MIC<sub>50</sub>s and MIC<sub>90</sub>s of 10 antibiotics for 477 Clostridium difficile isolates according to pulsovar.**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Pulsovar A (n = 274)</th>
<th>Pulsovar B-B1 (n = 86)</th>
<th>Other pulsovar (n = 117)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>1</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Meropenem</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>8</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>64</td>
<td>64</td>
<td>128</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>4</td>
<td>8</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>128</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>128</td>
<td>128</td>
<td>64</td>
</tr>
</tbody>
</table>

**NOTE.** Data are expressed in mg/mL.

6]. Because resistance to antimicrobials appears to be an important risk factor for colonization by *C. difficile* [7], study of susceptibility profiles stratified by genotype is needed.

A surveillance of CDAD, established in the province of Québec in August 2004, demonstrated a strong geographic variability in incidence of CDAD and case-fatality ratios [8], suggesting diversity in strain prevalence among hospitals. We conducted a prospective study to identify the genetic diversity of *C. difficile* isolates found in Québec, to measure the impact of the prevalence of the NAP1 strain on the incidence of CDAD, to compare disease severity in relation to patient age and strain characteristics, and to examine antibiotic susceptibility profiles with respect to pulsovars.

**METHODS**

**Microbiological analyses.** In February 2005, the 88 acute care hospitals participating in the provincial CDAD surveillance were requested to submit 15 consecutive stool samples from patients with nosocomial CDAD that were positive for toxin A and/or B. Samples were submitted by 58 hospitals (66%), which accounted for 92% of CDAD cases in Québec.

Isolation and identification were performed as previously described [2]. Of the 504 stool samples submitted, 478 (95%) had *C. difficile* strains isolated. Testing of *C. difficile* isolates for susceptibility to ciprofloxacin, gatifloxacin, levofloxacin, clarithromycin, clindamycin, ceftriaxone, meropenem, piperacillin-tazobactam, metronidazole, and vancomycin was performed according to the Clinical Laboratory Standards Institute agar dilution method for susceptibility testing of anaerobes [9]. Quality control was performed using *Eubacterium lentum* (ATCC 43055), *Bacteroides fragilis* (ATCC 25285), and *Bacteroides thetaiotaomicron* (ATCC 25285) isolates. For each antibiotic, distribution of MICs for all of the isolates was examined for clear-cut bimodal distributions. Epidemiological cutoff values to separate susceptible from resistant strains were determined by visual inspection of histograms [10].

PFGE of *C. difficile* isolates was performed according to the method described by Fawley and Wilcox [11]. The relatedness of the various isolates was determined according to the criteria of Tenover et al. [12] and with use of Molecular Analyst software (Bio-Rad). Genotypes were designated as follows: each letter represents a unique different strain (A–Z, followed by AA–KK), and a letter with a number indicates the number of band differences between the referent strain and the comparator strain. For example, pulsovar A1 has a 1-band difference from pulsovar A; pulsovar A1-1, similarly, has a 1-band difference from pulsovar A, but this band difference is not the same as that of pulsovar A1. The Dice coefficient was used to measure similarity between patterns. Clustering for dendrogram construction was based on the unweighted pair group method with arithmetic mean. The tolerance position was set at 1.25%.

Analyses for binary toxin genes (*cdtA* and *cdtB*) and partial *tcdC* deletions were performed as described elsewhere [2]. *C. difficile* (CIP 107932) was used as the positive control for binary toxin genes and an 18-bp *tcdC* deletion, and *C. difficile* (ATCC 43255) served as the negative control. A limited number of isolates of pulsovars A (8 isolates), B (10 isolates), and B1 (7 isolates) were tested, because characteristics of these genes were identical for all of the isolates within each of these pulsovars. Therefore, the binary toxin gene and *tcdC* deletion analyses were considered to be applicable to all of the isolates within each of these 3 pulsovars. All 117 isolates belonging to pulsovars other than A, B, and B1 were tested.

**Clinical data.** Clinical data were obtained from the provincial CDAD surveillance system. CDAD was defined by the presence of diarrhea and an assay result positive for either *C. difficile* toxin A or B or both; by the sudden onset of diarrhea with no alternative explanation and a diagnosis of pseudomembranous colitis made on the basis of endoscopic examination findings; or by histological evidence of the condition. A case was considered to be nosocomial if symptoms started ≥72 h after a patient was admitted to the hospital or if CDAD was diagnosed within 1 month after a previous hospital admission. Neonates and psychiatric inpatients were excluded from the study.

The following data were collected: patient age; community versus nosocomial acquisition; and complications occurring within 30 days of CDAD diagnosis, which included admission to the intensive care unit, colectomy, and whether death was associated with CDAD (attributable, contributive, or unrelated, according to the definitions indicated in the surveillance system [8]). A case of CDAD was classified as severe if it caused or contributed to the patient’s death within 30 days after CDAD.
Figure 1. Phylogenetic analysis, toxin characterization, and resistance to clindamycin, clarithromycin, and gatifloxacin of 61 PFGE profiles representing 477 Clostridium difficile isolates. For tcdC gene: –, no deletion; +, 18–base pair deletion; ++, >18–base pair deletion.

diagnosis or if the patient had a colectomy or required admission to the intensive care unit because of CDAD.

Statistical analyses. A “clonal predominance” was identified when at least 50% of the isolates in a given hospital belonged to the same pulsovar. This determination was possible only for 40 hospitals that submitted at least 6 isolates of nosocomial origin. Pulsovars B and B1 showed strongly related PFGE patterns and identical phenotypic characteristics and, therefore, were combined into 1 group and designated “B-B1.” However, pulsovars A and A1 were not combined because of differences in their antibiotic susceptibilities. To assess the relationship between incidence and clonal predominance, the incidence of nosocomial CDAD reported in the surveillance system from January through April 2005 was used. Hospitals were classified according to hospital size by number of beds, proportion of hospitalized patients aged \( \geq 65 \) years, and presence and type of clonal strain. Adjusted rate ratios for incidence were estimated by a Poisson regression.

The association of severe disease with patient age and strain characteristics was evaluated by logistic regression. Student’s \( t \) test and \( \chi^2 \) test were used when appropriate. All \( P \) values were 2-sided. Analyses were performed using Epi Info software, version 3.3.2 (Centers for Disease Control and Prevention) and SAS software, version 9.1 (SAS Institute).

RESULTS

Genotypes. PFGE typing performed on 478 C. difficile isolates revealed 61 different PFGE patterns. One isolate was untypeable. Pulsovars A (274 isolates; 57%), B (49 isolates; 10%), and B1 (37 isolates; 8%) were predominant. The presence of cdtA and cdtB genes was evaluated independently, and results were concordant for the presence of both genes. These genes were present in 309 isolates (65%). A partial tcdC deletion was observed in 311 isolates (65%). An 18-bp tcdC deletion was found in 304 isolates (64%), and 7 isolates (4 pulsovars) had a deletion of a more significant size. Overall, 304 isolates (64%) had both a partial tcdC deletion and binary toxin genes. Twelve isolates had only 1 of these 2 characteristics, and 162 isolates (34%) had neither characteristic.

Antibiotic susceptibility. Vancomycin, metronidazole, meropenem, and piperacillin-tazobactam MICs were low for all pulsovars (table 1). In contrast to pulsovars A, B, and B1, the other pulsovars had lower ceftiraxone, clarithromycin, ciprofloxacin, gatifloxacin, and levofloxacin MIC\(_{50}\)s. Pulsovars B and B1 differed from other pulsovars by having higher clindamycin MIC\(_{50}\)s.

The distribution of MICs for all of the isolates showed clear-cut bimodal distributions, allowing definition of epidemiological cutoff values for clindamycin (MIC \( \geq 32 \) mg/L), clarithromycin (MIC \( \geq 2 \) mg/L), gatifloxacin (MIC \( \geq 16 \) mg/L),
levofloxacin (MIC ≥32 mg/L), and ciprofloxacin (MIC ≥64 mg/L). Seventy-nine percent of all isolates were resistant to all 3 fluoroquinolones, 19% were susceptible, and only 1% of the isolates had a discordant susceptibility classification for the 3 tested fluoroquinolones.

**Pulsovar lineages.** On the basis of the presence of the binary toxin genes, partial \( \text{tcdC} \) deletion, and the susceptibility profile to clindamycin, 4 major lineages of pulsovars (I–IV) could be identified (figure 1). The majority of the pulsovars (303 of 304) with both the binary toxin genes and a partial \( \text{tcdC} \) deletion clustered into 2 lineages (I and II); 91% of the strains resistant to clindamycin (MIC ≥32 mg/L) were distributed within 2 other lineages (III and IV).

Lineage I included 16 pulsovars showing 67% relatedness. Pulsovar A accounted for 90% of the isolates of this lineage. All but 4 pulsovars possessed both the binary toxin gene and a partial \( \text{tcdC} \) deletion. The antibiotic susceptibility profile particular to pulsovar A was similar to that of pulsovars A2 and A3, to 2 of 7 isolates of pulsovar A1, and to 2 of 3 isolates of pulsovar A4. Lineage II was unique, with its 2 pulsovars pos-

---

**Figure 2.** Geographic distribution of 40 hospitals indicating clonal predominance. **Black dot,** clonal predominance of pulsovar A; **gray star,** clonal predominance of pulsovar B-B1; **gray dot,** clonal predominance of pulsovar W; **white dot,** nonclonal predominance.

---

### Table 2. Nosocomial incidence of *Clostridium difficile*-associated diarrhea (CDAD) per 10,000 person-days (January–March 2005) and crude and adjusted rate ratios (Poisson regression) according to hospital characteristics.

<table>
<thead>
<tr>
<th>Hospital characteristic</th>
<th>No. of hospitals (( n = 39 ))</th>
<th>No. of CDAD cases (( n = 2012 ))</th>
<th>No. of CDAD cases per 10,000 person-days(^a)</th>
<th>Crude rate ratio (95% CI)</th>
<th>Adjusted rate ratio(^b) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clonal predominance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonclonal(^c)</td>
<td>6</td>
<td>143</td>
<td>9.0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Clonal B-B1</td>
<td>8</td>
<td>297</td>
<td>13.6</td>
<td>1.5 (1.2–1.8)</td>
<td>1.3 (1.1–1.6)</td>
</tr>
<tr>
<td>Clonal A</td>
<td>25</td>
<td>1572</td>
<td>21.2</td>
<td>2.4 (2.0–2.8)</td>
<td>2.0 (1.7–2.4)</td>
</tr>
<tr>
<td><strong>Proportion of patients with age ≥65 years</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35%</td>
<td>13</td>
<td>497</td>
<td>13.2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>≥35%</td>
<td>26</td>
<td>1515</td>
<td>20.4</td>
<td>1.5 (1.4–1.7)</td>
<td>1.3 (1.1–1.4)</td>
</tr>
<tr>
<td><strong>No. of beds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;250 beds</td>
<td>15</td>
<td>501</td>
<td>16.9</td>
<td>1</td>
<td>...</td>
</tr>
<tr>
<td>≥250 beds</td>
<td>24</td>
<td>1511</td>
<td>18.4</td>
<td>1.1 (0.99–1.2)</td>
<td>...</td>
</tr>
</tbody>
</table>

\(^a\) Mean no. of CDAD cases per 10,000 person-days, 18.0.

\(^b\) Adjusted for clonal predominance and proportion of elderly.

\(^c\) One hospital with a clonal predominance of pulsovar W was excluded from the analysis.
Table 3. Risk for deaths attributable to *Clostridium difficile*-associated diarrhea (CDAD) or in which CDAD was contributive and for severe CDAD disease according to patient age and presence of binary toxin genes and partial *tcdC* deletion.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of patients</th>
<th>CDAD-attributable death</th>
<th>Severe CDAD disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%) OR (95% CI)</td>
<td>No. (%) OR (95% CI)</td>
<td></td>
</tr>
<tr>
<td>Patient age group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;75 years</td>
<td>213</td>
<td>6 (2.8) 1</td>
<td>11 (5.2) 1.0</td>
</tr>
<tr>
<td>≥75 years</td>
<td>256</td>
<td>27 (10.5) 3.9 (1.6–9.7)</td>
<td>31 (12.1) 2.4 (1.2–4.9)</td>
</tr>
<tr>
<td>Binary toxin/tcdC deletion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>172</td>
<td>8 (4.7) 1</td>
<td>9 (5.2) 1.0</td>
</tr>
<tr>
<td>Present</td>
<td>297</td>
<td>25 (8.4) 1.7 (0.7–3.9)</td>
<td>33 (11.1) 2.1 (0.98–4.6)</td>
</tr>
</tbody>
</table>

NOTE. Variables listed in the table are mutually adjusted in a logistic regression.

sessing a binary toxin and a *tcdC* deletion of >18 bp. Lineage III included 5 pulsosvars showing 81% similarity. Pulsosvars B and B1 accounted for 96% of the isolates of this lineage. Lineage IV is also unique in its high clindamycin and clarithromycin MICs. Apart from these 4 lineages, the 68 remaining isolates (14%) belonged to 34 different pulsosvars. Those isolates were characterized by their low MICs, and only 3 pulsosvars had the binary toxin genes and/or a *tcdC* deletion.

**Incidence and disease severity according to strain characteristics.** Among the 40 hospitals for which at least 6 isolates were genotyped, 33 (83%) had at least 1 strain of pulsosvar A, and 25 (63%) had pulsosvar A as their predominant strain; 20 hospitals (50%) had at least 1 strain of pulsosvar B or B1, and 8 (20%) had pulsosvar B-B1 as their predominant strain. One hospital had a clonal predominance of pulsosvar W (this strain was not found in any other Québec hospital). The likelihood for a particular strain to cause a clonal predominance was 1.9 (95% CI, 1.1–3.4) times higher for pulsosvar A than for pulsosvar B-B1. Clonal predominance of pulsosvar A was prevalent among hospitals located in Montréal and its surrounding regions (found in 24 of 29 hospitals) (figure 2). Clonal predominance of pulsosvar B-B1 was found in 7 of 8 hospitals in the area of Québec City (located ~200 km northeast of Montréal). During the 4-month study, the nosocomial incidence of CDAD was higher in hospitals with a clonal predominance of pulsosvar A or pulsosvar B-B1 than in hospitals without any predominant strain (table 2).

The median age of patients with nosocomial CDAD was 76 years. The proportion of strains of pulsosvar A increased with patient age, from 51% among persons aged <75 years to 62% for those aged ≥75 years (P < .05). A total of 68 patients died within 30 days after the diagnosis of CDAD, for a crude mortality rate of 14.5%. CDAD was the attributable cause of death for 12 (2.6%) of the 469 patients, contributed to death for another 21 (4.5%), and was unrelated in 35 cases (7.5%). Because of CDAD, 15 patients (3%) had to be transferred to the intensive care unit, and 5 patients (1%) had a colectomy. Patient age was the major risk factor for death and for severe disease. Severe disease was 2.3 times more frequent (95% CI, 1.1–4.9 times more frequent) when both binary toxin genes and a partial *tcdC* deletion were present. When adjusted for patient age group, this relation remained marginally significant (P = .054) (table 3).

**DISCUSSION**

This study is the first to document the geographic dissemination of the NAP1 strain in Québec and the epidemiological impact of this strain in a large territory. Strains of pulsosvar A and A1 have PFGE patterns identical to that of the current (NAP1a) and historical (NAP1b) strains, respectively, described in Montréal, the United States, and the United Kingdom. Similar to the current NAP1a strains, strains of pulsosvar A are resistant to gatifloxacin; 5 of the 7 strains belonging to pulsosvar A1 are susceptible to gatifloxacin, as were the historical NAP1b strains. However, the genetic diversity (67% relatedness) of the toxin gene–variant strains found in Québec appears to be more significant than that of NAP1 strains in the United States (>80% related). It is difficult, from a single survey, to distinguish between preexisting variant strains of pulsosvar A, such as pulsosvar A1 (NAP1b), and more recent variant strains that could have emerged during the epidemic. In the same way, the identification of other toxin gene–variant pulsosvars with the same antimicrobial susceptibility profile as pulsosvar A raises the possibility that other factors, such as an increased sporulation capacity [13], may be responsible for the clonal emergence of NAP1a. The epidemic potential of pulsosvar A is important, because it demonstrates a greater ability than pulsosvar B for becoming predominant in hospitals and for being responsible for a higher incidence of CDAD.

Pulsosvars B and B1 are similar to NAP2 strains (ribotype 001 or restriction endonuclease analysis group J) that were
previously described in outbreaks in the United States [14] and were dominant in the United Kingdom [15]. Pulsosvar B-B1 strains are responsible for an incidence of CDAD intermediate between that caused by pulsosvar A strains and strains of other PFGE types. The other pulsosvars, with few exceptions, have low MICs, lack the binary toxin gene, and do not have a partial tcdC deletion.

Antibiotics—clindamycin, cephalosporins, and fluoroquinolones—are regarded as risk factors for C. difficile colonization because they can disrupt the normal intestinal microflora barrier, thus permitting C. difficile to establish itself. It seems reasonable to believe that C. difficile strains that are resistant to a given antibiotic could have a greater potential for colonization during treatment with that same antibiotic within a given patient [7]. This potential was demonstrated for clindamycin [14, 16] and suggested for fluoroquinolones [17]. To describe the antimicrobial susceptibility of different C. difficile genotypes, most studies have used MIC clinical break points. These values are defined to predict success or failure of antimicrobial therapy; they do not adequately describe the phenotypic consequence of resistance mechanisms and may not be suitable to identify risk factors for intestinal colonization by C. difficile. For those reasons, we adopted the concept of epidemiological cutoff values of MICs, recently introduced to differentiate microbiological resistance from clinical resistance [10]. This method was particularly suitable for clindamycin and gatifloxacin.

The 2 main genetic mechanisms of resistance identified in C. difficile are, for fluoroquinolones, mutations in the gene gyrA coding for the DNA gyrase [18, 19] and, for macrolide and clindamycin, ermA genes. However, NAP1 strains were found to be ermB negative [20]. Other mechanisms that could explain macrolide resistance have been suggested, such as efflux systems, inactivating enzyme, or mutations in the 23S rRNA [19].

Several studies have confirmed that fluoroquinolone exposure was a risk factor for CDAD by NAP1 strains [2, 21, 22] but not by NAP2 strains [14]. Fluoroquinolone resistance was observed in both NAP1 and NAP2 strains, and the 1-dilution difference in gatifloxacin MIC at between these 2 genotypes is too small to be conclusive. However, more studies on the genetic mechanisms of fluoroquinolone resistance of these strains are needed.

Our study confirms that the presence of binary toxin genes is closely associated with a partial tcdC deletion, which makes it difficult to study their independent effect on disease severity. The importance of the 18-bp tcdC deletion and of the binary toxin as virulence factors in C. difficile remains unclear [23, 24]. Moreover, these 2 characteristics may not be solely responsible for an increased virulence, and their presence may only reflect the plasticity of the C. difficile genome [25], with associated mutations in the tcdA and tcdB genes [26]. The analysis of the impact on mortality suffered from a lack of statistical power related to a lower observed rate of attributable and contributive deaths to CDAD than originally expected. However, using the severe disease definition, including both mortality and clinical complications, was more effective in highlighting strain virulence. To our knowledge, this is the first study demonstrating that the risk of developing severe CDAD is twice as high with a toxin gene–variant strain as it is with a non-NAP1 strain. In addition, increase in patient age [1, 27] may also have affected the increase in mortality observed during the epidemic period [28].

There are several limitations to this study. The clinical data were obtained from the surveillance system and, therefore, comprised limited clinical information and a greater heterogeneity in coding, particularly with respect to cause of death. Hospitals of small size or with a low incidence of CDAD did not have enough cases of CDAD to be represented in the study, probably resulting in an overestimation of the proportion of hospitals in clonal predominance. Last, this study was carried out after the diffusion of guidelines on the control and management of CDAD in Québec. These measures resulted in a 40% decrease in incidence, mainly in hospitals with the highest incidence [2, 29]. This would result in minimizing the differences in incidence between clonal and nonclonal settings.

In conclusion, before the outbreak, microbiology laboratories in Québec did not culture for C. difficile because of costs and technical challenges. However, culture of stools for C. difficile is crucial for the understanding of the epidemiology and pathogenesis of CDAD. Culture for the bacterium allows for typing to determine if there is a clonal predominance, antibiotic susceptibility testing to determine if there are trends in resistance patterns, and analysis of virulence factors [30]. This study demonstrates that incidence and case severity of CDAD is related to strain prevalence in the province of Québec. PFGE can help to identify hospitals with NAP1 strains and, thus, target provincial efforts for infection control. However, improved rapid-identification methods are needed. As well, in-depth studies on genetic mechanisms of resistance among NAP1 and NAP2 strains and hypersporulation studies are required to better understand the epidemiology of CDAD.

Acknowledgments

We thank Claire Bélieveau and François Lamothe, for strain isolation and antimicrobial susceptibility testing; Susan Fenn, for performing PFGE; Milena Crousso and Yury Monczak, for characterizing gene toxins; Isabelle Rocher, for collecting clinical data; Rodica Gilca, for statistical advice; Marie Gourdeau and Jean Joly, for their help in supporting the survey; and hospital laboratories and infection control teams, for providing specimens and clinical data.

Financial support. Ministère de la Santé et des Services Sociaux du Québec.
References


