Molecular Analysis of *Clostridium difficile* PCR Ribotype 027 Isolates from Eastern and Western Canada

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The prevalence and characteristics of PCR ribotype 027 strains of *Clostridium difficile* have come into question following recent outbreaks in Eastern Canada and elsewhere. In order to determine the distribution of this strain in other regions in Canada, we screened a bank of 1,419 isolates recovered from three different Canadian health regions between 2000 and 2004. Among isolates from a Montreal area hospital, PCR ribotype 027 strains represented 115/153 strains (75.2%) from 2003 to 2004, but ribotype 027 strains were absent in 2000 and 2001. In Calgary, by contrast, ribotype 027 rates have remained relatively stable over 4 years of surveillance, representing 51/685 (7.4%) hospital isolates and 62/373 (16.6%) strains from the community (P < 0.001). PCR ribotype 027 accounted for 8/135 (5.9%) hospital isolates in the Fraser Health Region in 2004. Repetitive extragenic palindromic PCR was used to subtype a random selection of 027 isolates from each region. All 10 of the isolates from Quebec were of a single subtype, which was also dominant among isolates from Alberta (8/10 isolates) and British Columbia (6/8 isolates). Comparative sequencing of the *tcdC* repressor gene confirmed the documented 18-bp deletion and identified a second, single-base-pair deletion at position 117. Both deletions were conserved across all three provinces and were identified in a United Kingdom reference strain. The presence of a frameshift in the early portion of the *tcdC* gene implies serious functional disruption and may contribute to the hypervirulence of the 027 phenotype. PCR ribotype 027 strains appear to be widely distributed, to predate the Montreal outbreak, and to have measurable community presence in Western Canada.

*Clostridium difficile* is the predominant microbial cause of nosocomial and antibiotic-associated diarrhea. The clinical features of infection are mediated by cellular exotoxins secreted into the colonic environment during bacterial growth. The majority of toxigenic strains produce two cellular cytotoxins, *TcdA* and *TcdB*, and a small percentage of pathogenic strains produce a truncated, nonfunctional *TcdA* with normal *TcdB*. The genes for these toxins lie within a 19.6-kb genomic pathogenicity locus (PaLoc), whose expression is closely regulated by a circuit involving *tcdC* and *tcdD*. A third toxin, the *C. difficile* binary toxin (CDT), which functions independently of PaLoc-associated regulatory elements (2, 8), has been identified in approximately 5% of clinical isolates.

Recent and dramatic outbreaks of PCR ribotype 027/NAP1 strains have been reported in Canada, the United States, the United Kingdom, and The Netherlands (10–12, 14, 24). In the laboratory, the PCR ribotype 027/NAP1 line has been shown to express high concentrations of *TcdA/B* during early-log-phase growth, whereas most other strains elaborate toxin in lower concentrations after achieving stationary phase (9). This difference in timing and potency is thought to account for the higher rates of complications and mortality associated with 027 outbreaks. PCR ribotype 027 strains also carry the gene for the CDT binary toxin, but at present, its role in disease severity and clinical outcomes remains unresolved (5, 13, 18, 24).

In the wake of these outbreaks, emphasis has been placed on the clinical epidemiology and virulence properties of PCR ribotype 027 strains, the implication being that the presence of an emergent and more pathogenic strain places the community at risk. Several centers in North America and Europe have identified PCR ribotype 027 strains among their current or historical isolates (14, 19, 21, 23), and despite its apparent widespread endemicity, the strain does not seem to have contributed unequivocally to the emergence of outbreaks.

This report describes the prevalence of PCR ribotype 027 isolates from three geographically distinct Canadian health regions and uses a combination of PCR-ribotyping and repetitive extragenic palindromic (REP) PCR to determine whether the isolates from each region represent truly identical organisms or closely related, but distinct, clinical analogs. A strain-specific point deletion in the *tcdC* toxin repressor gene is also described. This mutation appears to be well conserved in representative isolates from all three provinces as well as in clinical reference samples from abroad.

**MATERIALS AND METHODS**

(i) **Bacterial isolates.** Our laboratory has a catalog of clinical strains collected from Alberta, British Columbia, and Quebec between 2000 and 2005, including 1,058 nonduplicate isolates from the Calgary Health Region, 135 isolates from the Fraser Health Region, and 226 isolates from Montreal. Strains from the Calgary region were collected as part of a surveillance project on *C. difficile*-associated disease (CDAD) from 2001 to 2004 by routine, biannual screening of
all enzyme immunoassay-positive specimens from the region during 2-month sampling intervals, generally in the winter and late spring. Since 2000, all hospital cases of CDAD have been tracked in a regional infection control database. The Calgary Health Region represents a population base of approximately 1.2 million and is serviced by a single centralized diagnostic facility. This allows for the convenient recovery of isolates from both hospital and community sources. For the purposes of this study, specimens were classified according to hospital or community origin on the basis of laboratory accession coding without a formal review of recent admission history. In Calgary, TechLab A/B (TechLab, Blackburg, VA) or Cytocline A+B (Cambridge Biotech, Worcester MA) is used for the routine identification of C. difficile toxins in all clinical specimens, and the turnaround time for most assays is under 24 h.

Isolates from the Fraser Health Region were obtained during an investigation into an apparent region-wide increase in CDAD case rates during the fall of 2004. Strains from Montreal were provided by M. Lavenderie, from the Hospital Maisonneuve-Rosemont, which included consecutive hospital isolates from the winter months of 2000 to 2001 and 2003 to 2004 both prior to and during the Montreal PCR ribotype 027/NAP1 C. difficile epidemic. To our knowledge, no other hospital in Quebec retrieved strains in a consecutive manner in 2000 and 2001. Cell culture cytoxicity remains the predominant diagnostic approach for C. difficile at the Quebec and British Columbia sites. Calgary and Montreal are separated by a distance of ~4,100 km. Surrey, British Columbia (Fraser Health Region), is ~940 km to the southwest of Calgary.

Genomic DNA for all strains was extracted from 5-ml brain heart infusion cultures after 48 h of incubation by phenol-chloroform extraction. The identity of strains was confirmed using PCR probes for 16S genes and C. difficile toxin genes tcdA and tcdB. For both culture and PCR, Clostridium difficile ATCC 43255 (American Type Culture Collection, Manassas, VA) was used as a positive control organism, while a clinical isolate of Clostridium bifurcans was used as a negative control. All strains were assigned a molecular strain type using PCR-ribotyping as a primary typing method, as described below. For the purpose of this study, strains whose ribopattern matched the Montreal/Sherbrooke epidemic strain were flagged, and representative isolates from each geographic region were randomly selected for REP subtyping and detailed molecular analysis. This subset of 28 ribotype strains included 10 isolates from Alberta (A to J), 3 isolates from British Columbia (A to H), and 10 isolates from Quebec (A to J). A reference ribotype 027 reference strain was kindly provided by Jon Brazier of the Anaerobe Reference Unit Laboratory in Cardiff, Wales.

(ii) Molecular characterization. (a) PCR-ribotyping. PCR-ribotyping was conducted in 25-μl reaction volumes composed of 2.5 μl ThermPol buffer; 2.5 μl 2 mM deoxyribonucleoside triphosphates (New England Biolabs), 1 μl of both the forward and reverse primers (10 mM); 17 μl distilled, deionized water; and 2 μl of the genomic template (~100 ng). Primer sequences were described previously by Stubbs et al. and were used without modification (23). Samples were loaded into a Biometra T1 thermocycler (Montreal Biotech, Montreal, Quebec), and Taq DNA polymerase (2 units; New England Biolabs) was added to each sample immediately prior to cycling. The following time-temperature profile was used consistently for each sample: 1 cycle of 5 min at 94°C for initial denaturation; 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C; and a final extension cycle of 5 min at 72°C. A 2% (wt/vol) agarose gel was prepared using 0.5× Tris-borate-EDTA buffer. Samples were loaded onto the gel, and a 150-V (6.0-V/cm) current was applied across the gel for 3.5 h. The resulting electrophoretic patterns were visualized using ethidium bromide or SYBR green under UV light and digitally captured for further analysis.

(b) REP-PCR. REP-PCR was conducted using the same basic reaction volumes and composition as described above for PCR-ribotyping. Heterogeneous primers were generated as described previously by Rahmati et al., but thermocycler conditions were modified from previously published reports (16, 20). The modified profile is as follows: an initial denaturation cycle of 2 min at 95°C and 3 min at 94°C, followed by 35 cycles of 30 s at 92°C, 1 min at 40°C, and 1 min at 65°C, with a final extension cycle of 8 min at 65°C. Although the methodology for the use of REP-PCR for the analysis of C. difficile has not been standardized, and has not been previously applied to PCR ribotype 027 strains. The sensitivity and specificity of previously published methods have yet to be definitively assessed, and the potential impact of protocol modifications was duly considered. Regardless of the target organism, most REP-PCR protocols include an extended elongation step (often 8 to 10 min) to account for heterogeneous primer binding. In our hands, the previously published elongation time of 8 min per cycle was found to result in incoherible and nonspecific smearing. As the extension time for each cycle was gradually reduced, the banding patterns for each strain began to resolve into reproducible patterns of 4 to 12 major bands, with many minor bands. The modified protocol was assessed for reproducibility and sensitivity using 96 blind-coded samples of known and varied ribotypes. The modified assay demonstrated 100% reproducibility between triplicate runs and excellent correlation with the known ribotype and strain features of each isolate and offered a run time improvement of over 4 h. As described above, samples were electrophoretically separated using a 2% (wt/vol) agarose gel at 6.0 V/cm in 0.5× Tris-borate-EDTA buffer for 3.0 h. Banding patterns were visualized using ethidium bromide or SYBR green under UV light and digitally captured for further analysis.

(c) PCR for specific virulence markers. All strains were PCR probed for the virulence markers tcdA, tcdB, cdtA, and cdtB and the macrocide-lincomamide-streptogramin B resistance marker ermm (6, 8, 15). Thermocycler and reaction conditions for each marker were conducted in accordance with the correspondingly published methods.

(d) Molecular typing. PCR-ribotype and REP-PCR fingerprints were imported into BioNumerics 4.01 software (Applied-Maths, Pl. Worth, Tex.), normalized, and visually compared. For phylogenetic estimation, a comparison matrix was constructed using Dice/unweighted-pair group method using average linkages clustering with 1% positional tolerance.

(iii) tcdC sequencing. Amplification and sequencing of the tcdC-negative toxin regulator gene was accomplished in representative strains using primers described previously by Spigaglia and Maistrantion (22). The isolates selected for tcdC sequencing included Alberta 027 A (April 2001), Alberta 027 G (July 2004), BC 027 A, BC 027 C, Quebec 027 A, Quebec 027 F, an 027 reference strain from J. Brazier, and ATCC 43255 (VPI 10463) as a control organism. Gel-purified amplicons were submitted to the University of Calgary DNA Core Facility for sequencing. The resulting sequences were manually assessed for reading errors using their respective complementary strand and aligned using T-Coffee 3.18 (Linux 2.6.x) under default parameters. Tools from the Emboss 3.0.0 application suite were used for all other sequence manipulations and the prediction of open reading frames. Alignments were prepared for publication using GeneDoc 2.6.

RESULTS

(i) Bacterial strains. From our strain type library, PCR ribotype 027 was found to represent 10.7% (113/1,058) of all isolates obtained from the Calgary Health Region between 2001 and 2004. In British Columbia, it represented 5.9% (8/135) of isolates from a single point prevalence study in the Fraser Health Region in late 2004. In Quebec, it represented 75.2% (115/153) of isolates from the Montreal area that were
obtained during the height of the outbreak in late 2003 and early 2004 but none of the 69 strains from 2000 to 2001. All identified PCR ribotype 027 strains were found to carry the binary toxin gene (cdtA/B) and the C. difficile toxin genes tcdA and tcdB.

(ii) Molecular epidemiology of type 027 in Southern Alberta. PCR ribotype 027 strains have been circulating in Southern Alberta since at least early 2001 (Fig. 1). The relative frequency of ribotype 027 strains among recovered clinical isolates has remained relatively consistent across all sampling intervals, suggesting that this ribotype strain has attained a stable endemic presence in our local population. When isolates from 2001 to 2004 were stratified according to community or hospital origin, the proportion of PCR ribotype 027 strains among community isolates was significantly higher than that among hospital isolates in 3 out of the 4 years (Table 1). For the remaining year, the proportion of PCR ribotype 027 strains still remained slightly higher among community isolates, but the difference was not significant.

Correlation of patient data from all 113 PCR ribotype 027 strains in our collection identified 24 strains that were nosocomially acquired (Table 2). Two patients suffered transient pseudomembranous colitis, which subsequently resolved. No serious CDAD-related clinical endpoints, such as colectomy, toxic megacolon, or death, were noted. Ultimately, all 24 patients either were discharged (n = 22) or died (n = 2) due to unrelated factors (Table 2). Among antibiotic exposures, none of the 24 patients had received clindamycin prior to the onset of symptoms, but 10/24 (42%) were exposed to fluoroquinolones. The remaining 89 patients did not access the hospital system and are presumed to have had illness that resolved in the community. Community patients were significantly younger than hospital patients. Comparable patient data were available for 490 isolates of different (e.g., non-027) PCR ribotypes. Of these, 53 (11%) patients had been exposed to oral or intravenous clindamycin in the 6 weeks preceding diagnosis, and 165 (34%) had exposure to fluoroquinolones (not significant).

(iii) REP-PCR, PCR-ribotyping, and specific probes. All 10 of the isolates from Quebec presented identical REP subtypes. While the epidemic subtype was predominant among the isolates from both Alberta (8/10) and British Columbia (6/8), isolates H and I from Alberta and A and F from British Columbia each resolved differently. Regardless of subtype, all isolates were positive for the binary toxin (CDT) gene (cdtA/B) and both of the primary C. difficile toxin genes (tcdA and tcdB). All strains were also negative for ermB, a common marker for transposon-mediated macrolide-lincosamide-streptogramin B resistance in C. difficile (Fig. 2).

From the dendrogram (Fig. 2), both isolates in the outlying Albertan cluster (H and I) were obtained from a later sampling interval during 2004. Differences in REP subtype banding are subtle, however, at least among the PCR ribotype 027 strains, and it is possible that minor genomic changes are not effectively captured by this subtyping methodology.

(iv) Sequencing of the tcdC toxin repressor gene. The tcdC gene was sequenced from eight isolates, including two isolates from each province, an ATCC control organism (ATCC 43255 [VPI 10463]), and a PCR ribotype 027 reference strain from the United Kingdom. In addition to the expected 18-bp dele-

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### Table 1. Ribotype 027 isolates from hospital and community sources in the Calgary Health Region from 2001 to 2004

<table>
<thead>
<tr>
<th>Yr</th>
<th>Community origin</th>
<th>Hospital origin</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean age of patients (SD)</td>
<td>No. (%) of type 027 isolates</td>
<td>Total no. of isolates</td>
</tr>
<tr>
<td>2001</td>
<td>50.9 (24.7)</td>
<td>11 (16.9)</td>
<td>65</td>
</tr>
<tr>
<td>2002</td>
<td>46.1 (25.5)</td>
<td>18 (12.9)</td>
<td>140</td>
</tr>
<tr>
<td>2003</td>
<td>46.4 (25.3)</td>
<td>18 (20.9)</td>
<td>86</td>
</tr>
<tr>
<td>2004</td>
<td>51.7 (23.7)</td>
<td>15 (18.3)</td>
<td>82</td>
</tr>
</tbody>
</table>

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### Table 2. Demographics and CDAD-related morbidity/mortality among hospitalized patients with the PCR ribotype 027 strain (Calgary, Montreal, and Surrey)

<table>
<thead>
<tr>
<th>Health region</th>
<th>Area of acquisition</th>
<th>No. of patients</th>
<th>Age of patients (SD)</th>
<th>No. (%) of patients with major CDAD-related endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PMC&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Calgary (Alberta)</td>
<td>Hospital</td>
<td>24</td>
<td>63.8 (19.1)</td>
<td>2 (8.3)</td>
</tr>
<tr>
<td>Montreal (Quebec)</td>
<td>Community</td>
<td>89</td>
<td>47.8 (21.7)</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Surrey (British Columbia)</td>
<td>Hospital</td>
<td>115</td>
<td>68.7 (16.5)</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

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<sup>a</sup> Ages are given in years.

<sup>b</sup> All strains were negative for ermB, a common marker for transposon-mediated macrolide-lincosamide-streptogramin B resistance in C. difficile.

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<sup>c</sup> No contact with the regional hospital system.
tion at positions 330 to 347 of the gene, a second, as-yet-undocumented, single-base-pair deletion was detected at position 117. This mutation appears to be conserved across all seven PCR ribotype 027 strains, including the United Kingdom reference strain. Both mutations were absent from the ATCC control strain. Unlike the 18-bp deletion downstream, the mutation at position 117 represents a frameshift early in the repressor gene sequence and, as such, has more profound implications for the integrity of the final tcdC transcript (Fig. 3). Both mutations appeared to be well conserved, regardless of the geographic origin or REP subtype of the organism.

**DISCUSSION**

REP-PCR has recently been put forth as a useful corollary or subtyping methodology for PCR ribotype 001 strains, offering improved strain type resolution over PCR-ribotyping alone (16, 20). While PCR-ribotyping and REP-PCR are both based on the differential amplification of bacterial genomic sequences, their coverage and basis for discrimination are fundamentally different. PCR-ribotyping exploits interstrain differences in the intergenic spacer region between the highly conserved 16S and 23S ribosomal genes. Since the ribosomal
gene cassette occurs with variable frequency and spacing throughout the genome, a strain-specific pattern is obtained (3, 21). REP-PCR, on the other hand, uses heterogenous primers to differentiate between strains based on subtle differences in the number and position of common extragenic palindromic repeat sequences that are scattered throughout the bacterial genome. The combination of these two PCR-based molecular typing methods may offer improved resolution over ribotyping alone, and the combination is both rapid and inexpensive relative to PFGE typing. Furthermore, because some C. difficile strains are highly resistant to PFGE, PCR-based approaches may offer more consistency and speed for routine strain typing and diagnostics, although the relative sensitivity and specificity of paired typing methods have yet to be definitively assessed, particularly in light of modified PFGE protocols for recalcitrant organisms (1, 7).

REP-PCR subtyping of PCR ribotype 027 strains from Alberta, British Columbia, and Quebec illustrates the broad geographic range of the 027 clonal line. The Montreal/Sherbrooke outbreak appears to be the result of a single REP subtype, and while the identical strain was predominant in both Alberta and British Columbia, the composition of circulating PCR ribotype 027 strains in both western regions appears to be more heterogenous. The resolution of PCR ribotype 027 REP subtypes remains more subtle than differences reported among type 001 strains (20). Despite the endemicity of the PCR ribotype 027 clone in the Calgary population, no outbreaks have been noted. The failure of this strain to emerge as an outbreak strain could be accounted for by the predominant community prevalence, the younger age of patients in the community, and regional differences in hospital infection control practices (in Calgary, the bed-to-infection control practitioner ratio is 1:140) and/or antibiotic utilization. Nosocomial case rates of CDAD across Canada from 2004 to 2005 during a 6-month national survey demonstrated that Western Canada had the lowest rates of disease (on average, 4/1,000 admissions [2/1,000 admissions in Calgary hospitals]), amounting to approximately one-half of the rate of disease in Ontario hospitals and one-quarter of the rate seen in Quebec area hospitals (Shirley Paton, [Public Health Agency of Canada], personal communication). Several recent epidemiologic studies of hospitalized patients have emphasized the importance of fluoroquinolones and proton pump inhibitors in the emergence and course of hospital-associated PCR ribotype 027/NAP1 epidemics (5, 18). The contribution of strain differences to the emergence of outbreaks is unclear at present.

In the Calgary Health Region, the majority of patients who are infected with a PCR ribotype 027 strain of C. difficile (89/113 patients) are believed to have acquired the infection in the community and are significantly younger than CDAD patients in area hospitals (P < 0.01) or in other jurisdictions where hospitalized patients are more frequently affected by outbreaks of the 027 strain (17). We hypothesize that these differences in age distribution between hospital and community patient cohorts may contribute to the apparent epidemiologic disparity of C. difficile infections across Canada and that the diminished impact of the PCR ribotype 027 strain in our region is likely a function of age and health status. To date, the penetration of the PCR ribotype 027 strain into local hospitals and long-term care facilities has been limited, but the outbreak potential of this strain and other hypervirulent strains should not be discounted, especially if they become established in nursing homes and/or the inpatient populations. Factors that may mediate the emergence of 027 strains in hospitals might include (i) the existing composition and dynamics of circulating C. difficile strain types in both the hospital and community settings; (ii) the condition of the hospital physical plant and level of support from infection control and housekeeping; (iii) regional differences in patient demographics, case mix (i.e., the categories of cases with respect to type and severity of disease), and admission/discharge practices; and (iv) differences in hospital antimicrobial usage and the allocation of agents that affect gastrointestinal motility or function. In a context such as this, where PCR ribotype 027/NAP1 has yet to establish itself as a major hospital clone, proactive attention to infection control measures, facility management, and antibiotic formulary controls may be useful in attenuating or preventing the ingress of hypervirulent strains into the hospital setting.

Recent reports have emphasized disease severity and hypervirulence of the outbreak strain during the Quebec outbreak (18, 24). While this subset of strains in the Calgary Health region was not associated with lethality, the strain library accounts for approximately one-third of all cases in the region during the survey period. Patients outside of the surveillance period could have developed severe disease. Disruption to regulatory sequences of tcdC in the C. difficile PaLoc with attendant overproduction of toxins could account for the increased virulence. Studies of polymorphisms in this region have generally reinforced the importance of tcdC sequence integrity in toxigenic suppression. Spigaglia and Mastrantonio previously hypothesized that disruptions to the gene may contribute to natural variations in toxin expression between clinical isolates (22). Multiple alignment of tcdC repressor sequences from PCR ribotype 027 isolates from all three provinces revealed an 18-bp deletion at positions 330 to 347, as described previously in the literature (24). This deletion corresponds to an in-frame contraction of the final tcdC transcript by six residues; however, the impact of this change on repressor function and bacterial toxin kinetics remains unknown (22). In the PCR ribotype 027 strain, however, an additional, undocumented, single-base-pair deletion was detected in the tcdC sequence at position 117. This single-base-pair deletion (Δ117) was present in all seven isolates of PCR ribotype 027 that were sequenced, including the reference strain sent to us from the United Kingdom. This consistency suggests that the Δ117 mutation, like the 18-bp deletion, was established early in the development of the PCR ribotype 027 clonal line. The broad geographic range of PCR ribotype 027 and its variants is testament to its evolutionary success as a genetic feature of this clinical pathogen.

Unlike the 18-bp deletion, Δ117 represents a frameshift and premature stop in the early portion of the gene, and the downstream effects on the functional capacity of any resulting tcdC transcripts would constitute a major disruption of tcdC function (Fig. 3). From transcriptional studies of PaLoc genes in “normal” toxin-producing strains, it has been demonstrated that tcdC is heavily transcribed in the early phases of bacterial growth, tapering gradually as the bacteria approach stationary phase (4, 9). Since tcdC is a negative regulator of PaLoc gene expression, this transcriptional profile underscores its impor-
tance in throttling toxin production in an ecologic background that is conducive to exponential growth. The wild-type TcdC regulator protein is 231 residues in length, but in PCR ribotype 027, the compound deletions result in a 65-residue N-terminal fragment, with a considerable portion (34%) of the distal peptide transcribed out of frame. In light of these mutations, it seems logical that the regulation of toxin expression could be severely impeded, resulting in a hypervirulent phenotype. Studies of PCR ribotype 027 toxin kinetics have demonstrated that unlike many other toxigenic variants, PCR ribotype 027 begins toxin production almost immediately upon log-phase growth, a phenotypic behavior that could be explained by a dysfunctional tcdC regulatory circuit (24).

We conclude that REP-PCR subtyping of PCR ribotype 027 strains from Alberta and British Columbia confirms that the 027 epidemic clone line, with variations, is endemic to Western Canada and clonal in one of the Quebec hospitals during the recent outbreak. Sequence analysis revealed a series of disruptive mutations in the tcdC toxin repressor gene that were unique to the PCR ribotype 027 strain, but it remains unclear if differences in the overall in vivo toxin expression are due solely to these changes. Differences in the regional epidemiology of PCR ribotype 027 strains suggest a role for contextual and environmental conditions in the development of hospital-associated outbreaks. Effective infection control and antibiotic formulary policy may help to prevent the establishment of this clonal line in health care facilities.

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