Healthcare-Associated *Clostridium difficile* Infections and Strain Diversity in Pediatric Hospitals in the Canadian Nosocomial Infection Surveillance Program, 2007–2011

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Abstract. Children with healthcare-associated *Clostridium difficile* infection were identified. The incidence increased from 3.2/10,000 patient days in 2007 to 5.2/10,000 patient days in 2011 (*p* < 0.001). Of 169 isolates, the most common North American Pulsed-Field (NAP) types were NAP4 (*n* = 43; (25.4%), and NAP1 (*n* = 25; 14.8%) while 55 (32.6%) were non-assigned NAP types.

Key words. child; *Clostridium difficile*; healthcare-associated infection; strain typing.

INTRODUCTION

*Clostridium difficile* infection (CDI) in the pediatric age group was not frequently reported until early 2000, when analyses of large pediatric hospitalization databases revealed rates between 2.0 and 6.5 per 10,000 patient-days [1–3]. Strain genotyping in children has been limited to a few studies, but none has exclusively examined healthcare-associated (HA) CDI [4–6]. The objectives of this surveillance were to determine the incidence of HA CDI and determine the characteristics of the *C difficile* strains implicated.

METHODS

The Canadian Nosocomial Infection Surveillance Program is a collaboration between the Association of Medical Microbiology and Infectious Diseases of Canada and the Public Health Agency of Canada. Using the standard case definitions, prospective surveillance for HA CDI occurred year-long at the same 7 (2007–2009) or 8 (2010–2011) stand-alone pediatric hospitals (all tertiary or quaternary care and representing half of all the pediatric care centers in Canada). Each of these pediatric hospitals was from 1 of 6 provinces (Alberta-2, Manitoba-1, Ontario-2, Quebec-1, Nova Scotia-1, and Newfoundland-1). Children <12 months of age were excluded.

Case ascertainment of CDI was performed year-long from 2007 to 2011. Detailed case descriptions and stool specimens were submitted for 2-month periods for the first 2 years (March to April in 2007 and 2008) but because of low numbers was performed year-round in 2009.

The case definition for pediatric CDI was new documented diarrhea (at least 6 watery stools in the previous 36 hours, ≥3 unformed stools over 24 hours, or at least 8 unformed stools over 48 hours) accompanied by a positive *C difficile* toxin assay or pseudomembranous colitis in children 12 months to 18 years of age. The infection was considered healthcare-onset healthcare facility associated (HCFA) if symptoms occurred ≥72 hours after admission or community-onset HCFA if symptoms resulted in a
readmission to the same hospital within 4 weeks of a discharge. Patients admitted from long-term care institutions and inpatients on psychiatry units were excluded.

Infection-control professionals identified eligible patients by using daily laboratory reports of *C. difficile* toxin assay results, operating room records, and admission lists. Charts were then reviewed to determine inclusion in the study. Once a patient met the case definition, demographic data, admission and discharge dates, medical treatment notes, and outcome data were collected. Data on outcomes were collected at discharge; however, severe outcomes (intensive care unit [ICU] admission, colectomy, or death) were tracked until 30 days after the first positive *C. difficile* sample. Every severe outcome was assessed by the participating infection-prevention-and-control physician to determine if it was directly or indirectly attributable to CDI.

All available toxin A- and/or B-positive stool samples were frozen at −20°C. All except for 3 sites used an enzyme immunoassay for toxin detection throughout the study period. The other 3 sites implemented a 2-step methodology: glutamate dehydrogenase enzyme immunoassays were performed, and samples with a positive result underwent polymerase chain reaction (PCR) testing for the toxin B gene in June 2010, June 2011, and September 2011. Stool specimens were sent frozen to the National Microbiology Laboratory (NML), where *C. difficile* was isolated as previously described [7]. Multiplex PCR was used to detect the toxin A (*tcdA*), toxin B (*tcdB*), binary toxin (*cdtB*), negative regulator of toxin production (*tcdC*), and triose phosphate isomerase (*tpi*) gene targets, and pulsed-field gel electrophoresis was used to determine the North American pulsed-field (NAP) type [7]. Susceptibility testing was performed using Etest strips (bioMérieux, Marcy-L’Étoile, France), and the breakpoints used were determined by the Clinical Laboratory Standards Institute (according to standard M11-A7 [8]).

This surveillance project was observational and did not involve any alterations in patient care. In Canadian healthcare institutions, surveillance for HA infections is a routine component of quality assurance and patient care, and as such, informed consent of the patients was not required. The data were deidentified locally and transmitted nonnominally. Ethics approval may have been obtained if required locally at an individual site, but for surveillance, most sites required only institutional permission for these chart reviews.

The rates for HA CDI were calculated using patient-days (excluding children <12 months old) as denominators with 95% confidence intervals (CIs). Differences between the categorical variables were analyzed by the χ² or Fisher’s exact test, as appropriate. All statistical tests were performed at a significance level of .05 and were 2 sided. Statistical analyses were performed using SAS version 9.2 (SAS Institute, Inc., Cary, NC).

**RESULTS**

A total of 472 children (≥1 to ≤18 years old) with HA CDI were reported during the 5-year surveillance period. The overall HA CDI incidence rate was 4.3 per 10 000 patient-days. The rate per 10 000 patient-days increased from 3.2 (95% CI, 2.38–3.92) in 2007 to 5.2 (95% CI, 4.32–6.13) in 2011 (p < .001). The 5-year average rate for the central region of Canada (Ontario and Quebec) was consistently higher (5.19 per 10 000 patient-days) than those of the eastern (1.58 per 10 000 patient-days) and western (3.2 per 10 000 patient-days) regions.

Of the 472 reported patients with HA CDI, 319 (67.6%) had detailed case reports. Of the 319 patients, 22 (6.9%) were between ≥1 and <2 years old, 197 (61.8%) were between 2 and <12 years old, and 100 (31.3%) were between ≥12 and ≤18 years old; 169/319 (52.7%) were male. There were 70 (21.9%) patients with CDI identified in hematology/oncology units, 71 (22.3%) in medical units, 62 (19.4%) in surgical units, 20 (6.3%) in combined medical/surgical units, 24 (7.5%) in bone marrow transplant units, 20 (6.3%) in ICUs, and 27 (8.5%) in other/unknown units, and 25 (7.8%) patients had community-onset HCFA CDI. The median length of stay before symptom onset was 9 days (interquartile range, 4–28 days). The rate increases over time were not significantly different between the units.

Overall, 8 (2.5%) children were in an ICU at the onset of their CDI. Of 311 children who were not in an ICU at diagnosis, 4 (1.3%) were admitted to an ICU for management of their CDI. Two children (0.6%) (both aged 2 to <12 years) required a colectomy. Of the 319 children, 5 (1.6%) died of causes not attributable to CDI, and none died as a result of CDI.

In total, 180 fecal specimens were submitted for analysis to the NML. There were no differences in age, sex, or ward location between the patients for whom stool samples were submitted and those for whom no sample was sent. From these samples, 169 (93.9%) *C. difficile* isolates were recovered and underwent typing with pulsed-field gel electrophoresis (Table 1). The proportion of different NAP types did not vary according to year, age of patient, or region of the country. In addition, there were no statistically significant differences in median ages of the children with NAP1 and those with any other NAP type or between children with NAP4 and those with any other NAP type. We did not observe any differences in the median numbers of days of hospitalization before the onset of symptoms.
Susceptibility testing revealed that resistance to moxifloxacin was present in 13 (52%) NAP1, 7 (58.9%) NAP2, and 2 (4.7%) NAP4 strains and in 3 (5.5%) of the nonassigned NAP strains, and the remaining isolates were susceptible to moxifloxacin. Of the moxifloxacin-susceptible NAP1 isolates identified, all but 1 were tcdA and tcdB positive, and all of them contained the 18-bp deletion in tcdC, indicative of a typical NAP1 genotype. One isolate was tcdB negative according to PCR but contained the other NAP1 genotypic characteristics. Clindamycin resistance was present in 9 (36%) NAP1, 7 (53.9%) NAP2, 20 (46.5%) NAP4, 2 (25%) NAP10, and 1 (11.1%) NAP11 strain and in 26 (47.3%) of the nonassigned NAP strains. All of the strains were susceptible to rifampin.

CONCLUSIONS

These data provide rates of HA CDI in Canadian pediatric hospitals over time and highlight the increasing incidence of HA CDI in these institutions over a 5-year period. The overall rates are slightly lower than those seen in US studies in which discharge-coding data from large pediatric hospital administrative databases were used; however, the US studies likely included patients who were hospitalized with community-acquired CDI and infants <1 year of age, in whom toxin-producing strains may colonize the gut [1, 3]. Our rates are higher than the nosocomial rate of CDI (2.3 per 10 000 patient-days) in general hospitals in Finland in 2010 and in New Zealand where the rate of CDI in hospitalized children in 2012 was reported as 2.0 per 10 000 patient-days [9, 10]. Although it is possible that the implementation of PCR-based toxin detection at 3 of our sites in the last 6 to 18 months contributed to the increased rates in 2011, the rates were already increasing as of 2008. The increases in healthcare-associated Clostridium difficile infection (HA-CID) rates were found in all patient areas, and there were no significant differences in the rates over time between these units, which suggests that specific patient subgroups or environments do not explain the changes in rates over time.

In this sample size of 169 isolates, there was a diversity of NAP types, with the most abundant being NAP4 (25%) and NAP1 (15%) strains. The study of HA-CDI isolates from New Zealand found 21 different PCR ribotypes among 32 isolates from children [10]. In contrast, one third of the isolates from adult patients with HA CDI in Canada (2004–2005) were from NAP1 strains, and another third were from NAP2 strains, whereas NAP4 represented only 6.1% of the strains [7]. Another cohort study of pediatric CDI identified the NAP1 type in 19% of isolates [4]. Although the number of NAP1 isolates in this cohort was small, only half of them were resistant to moxifloxacin compared with 83% of isolates from adults with HA CDI in Canada in 2005 [7]. Whether the lower prevalence of quinolone use in children has prevented the selection of moxifloxacin-resistant strains is not yet known [11, 12].

A limitations of this study is the potential for other nonparticipating sites in Canada to have either higher or lower rates of CDI but to not be included in this surveillance study.

It is possible that the shift to outpatient care for children with less-severe conditions who historically have been treated in the hospital, coupled with increased chronic care for children with medically complicated conditions, plays a role in the increasing rates of CDI. Additional characterization of these strains and determination of the sources of C difficile in this population should be done to further

### Table 1. Strain Type According to Year

<table>
<thead>
<tr>
<th>Strain Type</th>
<th>2007 (n = 13)</th>
<th>2008 (n = 9)</th>
<th>2009 (n = 34)</th>
<th>2010 (n = 53)</th>
<th>2011 (n = 60)</th>
<th>Overall (n = 169)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAP1</td>
<td>2 (15.4)</td>
<td>0 (0.0)</td>
<td>8 (23.5)</td>
<td>7 (13.2)</td>
<td>8 (13.3)</td>
<td>25 (14.8)</td>
</tr>
<tr>
<td>NAP2</td>
<td>3 (23.1)</td>
<td>0 (0.0)</td>
<td>2 (5.9)</td>
<td>3 (5.7)</td>
<td>5 (8.3)</td>
<td>13 (7.7)</td>
</tr>
<tr>
<td>NAP3</td>
<td>1 (7.7)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>NAP4</td>
<td>4 (30.7)</td>
<td>2 (22.2)</td>
<td>9 (26.5)</td>
<td>11 (20.7)</td>
<td>17 (28.3)</td>
<td>43 (25.4)</td>
</tr>
<tr>
<td>NAP5</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>NAP6</td>
<td>0 (0.0)</td>
<td>1 (11.1)</td>
<td>0 (0.0)</td>
<td>3 (5.7)</td>
<td>4 (6.7)</td>
<td>8 (4.7)</td>
</tr>
<tr>
<td>NAP7</td>
<td>0 (0.0)</td>
<td>1 (11.1)</td>
<td>2 (5.9)</td>
<td>0 (0.0)</td>
<td>1 (1.7)</td>
<td>4 (2.4)</td>
</tr>
<tr>
<td>NAP8</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (2.9)</td>
<td>0 (0.0)</td>
<td>1 (1.7)</td>
<td>2 (1.2)</td>
</tr>
<tr>
<td>NAP9</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>NAP10</td>
<td>0 (0.0)</td>
<td>1 (11.1)</td>
<td>1 (2.9)</td>
<td>0 (0.0)</td>
<td>6 (10.0)</td>
<td>10 (5.7)</td>
</tr>
<tr>
<td>NAP11</td>
<td>0 (0.0)</td>
<td>1 (11.1)</td>
<td>1 (2.9)</td>
<td>4 (7.5)</td>
<td>3 (5.0)</td>
<td>9 (5.3)</td>
</tr>
<tr>
<td>NAP12</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (1.7)</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>Other, not assigned</td>
<td>3 (23.1)</td>
<td>3 (33.4)</td>
<td>10 (29.5)</td>
<td>25 (47.2)</td>
<td>14 (23.3)</td>
<td>55 (32.6)</td>
</tr>
</tbody>
</table>

Abbreviations: NAPn, North American pulsed field type.

aData are no. (%) of isolates.

bTwo months of sampling only.
inform risk-management strategies for hospitalized children.

Acknowledgments
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