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Clostridium difficile Infections in a Canadian Tertiary Care Hospital before and during a Regional Epidemic Associated with the BI/NAP1/027 Strain[▽]

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Since 2002, an epidemic of *Clostridium difficile* infections has occurred in southern Quebec, Canada. At Hôpital Maisonneuve-Rosemont, Montreal, Quebec, Canada, the incidence of *C. difficile* infections increased from 11/1,000 admissions (1999 to 2002) to 27/1,000 admissions (2003 to 2005). We compared the exposures and outcomes for patients infected with strains with different ribopatterns isolated before ($n = 55$) and during ($n = 175$) the epidemic, as well as the in vitro activities of antibiotics against those isolates. During the preepidemic period, 46 isolates (84%) were of ribotype 001, 1 was of ribotype 027, and 8 were of other ribopattern types. During the epidemic period, ribotype 027 strains accounted for 140 (80%) isolates; 26 (15%) were of ribotype 001, and 7 were of other ribopattern types. Ribotype 027 strains were highly resistant to fluoroquinolones (FQs) but were susceptible to clindamycin. A pattern of prior specific antibiotic exposure that selected for antibiotic-resistant ribotype *C. difficile* infections was observed for FQs (ribotype 027) and clindamycin (ribotype 001). The rate of mortality was higher among older patients, those with a high Charlson comorbidity index, and those with longer previous hospitalizations. By multivariate analysis, patients infected with ribotype 027 were twice as likely to die within 30 days of diagnosis than patients infected with other ribotypes (adjusted odds ratio, 2.06; 95% confidence interval, 1.00 to 4.22). The observations from this study support the notion that continued selective antibiotic pressure resulted in the superimposition of the hypertoxigenic ribotype 027 clone on top of the prior dominant ribotype 001 clone in a setting of preexisting high endemicity, thus leading to the high rates of morbidity and mortality seen in the Quebec outbreak. Stringent antibiotic stewardship measures, combined with aggressive infection control, are required to curtail the epidemic of *C. difficile* infections.

Clostridium difficile is a toxigenic, spore-forming anaerobic bacterium that is the primary cause of health care facility-associated diarrhea in North America (18). *C. difficile* infection results in a range of gastrointestinal syndromes, from mild gastrointestinal upset and antibiotic-associated diarrhea to pseudomembranous colitis, toxic megacolon, sepsis, and death. Beginning in 2002, the Montreal/Sherbrooke area of southern Quebec, Canada, experienced a sustained, multicenter epidemic of *C. difficile* infections that involved more than 30 hospitals. Between August 2004 and July 2007, more than 20,000 nosocomial cases were reported to the Quebec provincial surveillance program (Rodica Gilca, INSPQ, personal communication).

Clinical reports have implicated the emergence of a toxinotype III epidemic strain (18) which produces high levels of toxins A (TcdA) and B (TcdB) in vitro (36). This hypertoxigenicity appears to be associated with mutations to the *tcdC* regulatory gene, which normally inhibits the expression of *tcdA* and *tcdB* (19, 20, 36). The epidemic strain also produces the *C. difficile* binary toxin (CDT), whose impact on virulence remains unclear (31). By pulsed-field gel electrophoresis, the outbreak strain has been designated North American pulsovar 1 (NAP1) and also as restriction endonuclease analysis pattern BI or PCR ribotype 027 (22, 36). The BI/NAP1/027 strain has emerged in the United Kingdom, Belgium, The Netherlands, and France; it has also been implicated in hospital outbreaks throughout the United States (3, 11, 15–17, 22, 34).

Although several studies have explored the molecular epidemiology of outbreaks caused by the BI/NAP1/027 strain in Quebec and other areas (11, 15, 17, 18, 22, 34), an association between BI/NAP1/027 infection and increased disease severity compared to the disease severity previously associated with the common ribotype 001 strain has not previously been demonstrated. The objectives of the study described in this report were (i) to compare the clinical characteristics, risk factors, and outcomes of patients with *C. difficile* infections according to pathogen ribotype; (ii) to compare the in vitro activities of antibiotics against isolates with various ribopatterns; and (ii) to examine the associations between resistance to specific antibiotics, recent exposure to these antibiotics, and the occurrence of *C. difficile* infections.

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MATERIALS AND METHODS

***C. difficile* infection incidence rates.** Historical *C. difficile* infection incidence rates were obtained from MedEcho, a provincial database of diagnoses upon

hospital discharge. Although the vast majority of cases are likely to represent nosocomial *C. difficile* infections, these rates also include cases of community-acquired *C. difficile* infection severe enough to necessitate admission to a hospital. Since August 2004, these data are available through the Quebec provincial *C. difficile*-associated diarrhea surveillance program (13).

Identification of isolates. During two distinct survey periods, *C. difficile* isolates were collected from consecutive cytotoxin-positive stool samples submitted to the clinical microbiology laboratory at Hôpital Maisonneuve-Rosemont, a 545-bed university-affiliated tertiary care hospital in Montreal, Quebec, Canada. Isolates from the preepidemic period (November 2000 to March 2001) were collected during the development and validation of an in-house PCR method for the identification of *C. difficile* (10). Isolates from the epidemic period (October 2003 to January 2004 and May 2004) were collected as part of the outbreak investigation. In both cases, inclusion criteria were (i) age ≥18 years and (ii) hospitalization at the time of the *C. difficile* infection episode or admission within 72 h of submission of a positive stool sample. We included patients experiencing their first episode of *C. difficile* infection as well as those with recurrent disease, which we defined as a new episode of diarrhea attributed to *C. difficile* between 14 and 60 days after a previous diagnosis of a *C. difficile* infection.

The stool samples were incubated overnight at 35°C in a cooked meat broth, and cytotoxicity assays were performed with Vero cells with a *C. difficile* toxin/antitoxin kit (TechLab, Blacksburg, VA). The broths from cytotoxin-positive stool samples were subcultured onto cycloserine-cefoxitin-fructose agar (Quelab Laboratories). *C. difficile* isolates were identified by their characteristic colony morphology and odor, and their identities were confirmed by latex agglutination (Serobact *C. difficile* latex slide agglutination test; Oxoid). The isolates were subcultured and frozen at −80°C in brain heart infusion glycerol broth medium until antibiotic susceptibility testing was performed.

Determination of antibiotic susceptibilities. MICs were determined by an agar dilution method on brucella 5% laked sheep blood agar, according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) (27). Control strains included *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, and *Clostridium difficile* ATCC 9689.

Molecular characterization and typing. Strains from each period were PCR-ribotyped and probed for four major *C. difficile* toxin genes (*tcdA*, *tcdB*, *cdtA*, and *cdtB*), as described previously (19). Briefly, genomic DNA was obtained from a pure bacterial culture by phenol-chloroform extraction. Following normalization, the DNA from each isolate was subjected to PCR-ribotyping and simplex PCR for toxin genes *tcdA*, *tcdB*, and *cdtA*/*cdtB*, as well as the macrolide-lincosamide-streptogramin B resistance element *ermB* and a 16S ribosomal control sequence. *C. difficile* strains ATCC 9689 and ATCC 43255 were included as controls. Each amplification run included a number of blind-coded and randomly selected repeat specimens.

Risk factors and outcomes. Patient records were reviewed to collect information on demographic characteristics, recent hospitalization (to classify cases as being community or nosocomially acquired), antibiotic exposure (within the previous 2 months), and other potential risk factors for *C. difficile* infection, including a history of *C. difficile* infection. We also collected diagnostic, therapeutic, and clinical data, including those required to calculate the Charlson comorbidity index, a measure of the overall burden of disease based on the presence of 19 chronic comorbidities (5).

C. difficile infection colonization pressure (CCP) (7, 8) was estimated only for patients who had their first episode of *C. difficile* infection. Data from prospective surveillance for nosocomial *C. difficile* infections by ward from April 2004 to March 2005 (earlier data were unavailable) and the number of days spent on those wards up to 60 days before the date of collection of the positive stool sample were used to estimate the CCP risk score. Patients who had not been admitted to the hospital or who had spent time only on a low-risk ward (a ward with a nosocomial *C. difficile* infection incidence rate of <10/1,000 admissions) were considered to have been exposed to a low CCP, those who spent 1 to 15 days on a medium-risk ward (a ward with a nosocomial *C. difficile* infection incidence rate of 10 to 45/1,000 admissions), or 1 to 5 days on a high-risk ward (a ward with a nosocomial *C. difficile* infection incidence rate of >45/1,000 admissions) were considered to have been exposed to a moderate CCP, while patients who spent >15 days on a medium-risk ward or >5 days on a high-risk ward were considered to have been exposed to a high CCP.

Reviewers were blinded to the ribotype identity and the in vitro susceptibilities of the isolates recovered from each case. The primary outcome was all-cause mortality occurring within 30 days of diagnosis of a *C. difficile* infection. The secondary outcome was a recurrence of *C. difficile* infection between 14 and 60 days after the diagnosis of the previous episode. Death was considered attributable to *C. difficile* infection if the physician judged that the patient would not have died within 30 days in the absence of *C. difficile* infection.

Antibiotic utilization. Antibiotic consumption was obtained from the pharmacy department and was expressed in defined daily doses (DDD) per 1,000 patient days (6, 37). Data were available from April 2000 to March 2007.

Statistical analyses. Data were analyzed with Stata (version 8.0) software (StataCorp, College Station, TX). Proportions were compared by the χ^2 test or, when the numbers were small, Fisher's exact test. Unconditional logistic regression was used for multivariate analysis. Models were built sequentially, starting with the variable most strongly associated with the outcome and continuing until no other variable reached significance. When the final model was reached, each variable was dropped in turn to assess its effect. Different models were compared by using the likelihood ratio test, with significance determined at a P value of 0.05.

RESULTS

***C. difficile* infection incidence.** At Hôpital Maisonneuve-Rosemont, the historical incidence of *C. difficile* infection was 10.9/1,000 admissions (range, 4.1 to 20.4/1,000 admissions from January 1999 to March 2003), with marked seasonality (Fig. 1a). From April 2003 to March 2005, the incidence of *C. difficile* infections increased sharply to a monthly average of 27.1/1,000 admissions (range, 16.9 to 40.6/1,000 admissions). From April 2005 to March 2007, the incidence of *C. difficile* infections averaged 17.9/1,000 admissions (range, 10.2 to 29.5/1,000 admissions).

Ribotypes. A total of 230 isolates (55 in the 2000 and 2001 preepidemic period and 175 in the 2003 and 2004 epidemic period) were recovered (Fig. 1b). Two principal ribopatterns were identified and were annotated as ribotype 001 (*tcdA*, *tcdB*, and *ermB* positive and CDT negative) and ribotype 027 (*tcdA* and *tcdB* positive, *ermB* negative, and CDT positive). During the preepidemic period, 46 isolates (84%) were of ribotype 001, 1 was of ribotype 027, 2 were of type R0002, and 2 were of type R0003. The remaining isolates from this period included single isolates each of types R0014, R00017, R0018, and R0019. In the epidemic period, by contrast, ribotype 027 strains accounted for 140 (80%) of the isolates recovered, and only 26 (15%) were of ribotype 001. A number of minor strains were also detected, but they were overshadowed by the predominant endemic/epidemic strains. These were designated ribotypes R0002 ($n = 4$), R0003 ($n = 1$), R0006 ($n = 1$), and R0028 ($n = 1$). Two strains had patterns that were new to our database and were considered distinct.

Patient characteristics. The demographic and clinical characteristics of the patients according to the ribotypes of the infecting *C. difficile* strains are shown in Table 1. The characteristics of patients infected with ribotypes 001 and 027 were similar. Both ribotype 001 and ribotype 027 strains were isolated more frequently from older patients (median ages, 76.5 and 75 years, respectively) than strains of other ribotypes (median age, 64 years) ($P = 0.02$). Compared to the patients infected with ribotype 001 or 027 strains, patients infected with strains of other ribotypes were more likely to have a concomitant diagnosis of lymphoma or leukemia ($P = 0.001$) and to have received chemotherapy within the past 2 months ($P < 0.001$). The median Charlson comorbidity index score and the cumulative patient hospitalization days within the preceding 6 months were similar in all three ribotype groups. The estimated CCP was significantly lower for patients infected with ribotype 027 strains than for patients infected with ribotype 001 strains ($P = 0.001$) and was similar to the CCP of patients infected with strains of other ribotypes.

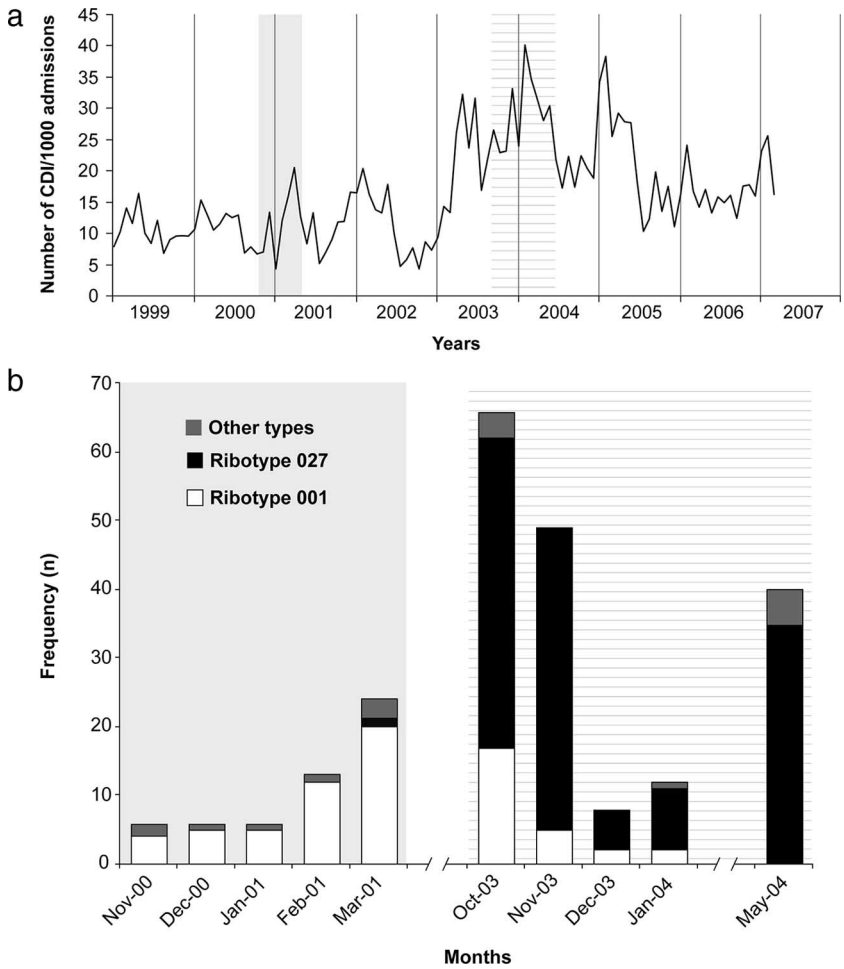

FIG. 1. (a) Monthly rates of admissions at Hôpital Maisonneuve-Rosemont for a first episode of *C. difficile* infection, January 1999 to March 2007 (source, MedEcho, Institut National de Santé Publique du Québec). (b) Ribotype frequencies of isolates during the two study periods (55 patients in 2000 and 2001 and 175 patients in 2003 and 2004).

Among the 171 (74%) patients experiencing their first episode of *C. difficile* infection, 97% had taken at least one antibiotic before this first episode (Table 2). Patients infected with a ribotype 027 strain were more likely to have received a fluoroquinolone (FQ) than those infected with a ribotype 001 strain ($P = 0.005$) or strains of any of the other ribotypes ($P = 0.05$). In a multivariate analysis, the association between FQ exposure and ribotype (ribotype 027 versus ribotype 001) remained significant after adjustment for age, the estimated CCP, and exposure to all other antibiotics listed in Table 2 (adjusted odds ratio [AOR], 2.99; 95% confidence interval [CI], 1.29 to 6.94; $P = 0.01$). When the multivariate analysis was restricted to the epidemic period, the association remained significant (69/99 [70%] patients infected with a ribotype 027 strain had received a quinolone, whereas 26/56 [46%] patients infected with a ribotype 001 strain had received a quinolone [AOR, 4.19; 95% CI, 1.23 to 14.24; $P = 0.02$]). By contrast, only 4% of the patients infected with a ribotype 027 strain had received clindamycin within the preceding 2 months, whereas 29% of the patients infected with a ribotype 001 strain had received clindamycin within the preceding 2 months ($P < 0.001$). This association also remained significant after adjustment for age, the estimated CCP, and other previous antibiotic exposure (AOR, 0.08; 95% CI, 0.02 to 0.30; $P < 0.001$) and also when the analysis was restricted to data from the epidemic period (4/99 [4%] patients infected with a ribotype 027 strain had received clindamycin, whereas 16/56 [29%] patients infected with a ribotype 001 strain had received clindamycin [AOR, 0.17; 95% CI, 0.03 to 0.94; $P = 0.04$]). In this multivariate model, the only other variable that remained significantly associated with ribotype was CCP: 46% of the patients infected with a ribotype 027 strain were exposed to a high CCP, whereas 70% of the patients infected with a ribotype 001 strain were exposed to a high CCP [AOR, 0.12; 95% CI, 0.03 to 0.51; $P = 0.004$]).

Antibiotic susceptibilities. The in vitro activities of antibiotics against *C. difficile* isolates, according to ribotype, are shown in Table 3. While all isolates remained susceptible to ≤2 mg/liter of metronidazole (CLSI breakpoint, ≥32 mg/liter), 52% of ribotype 027 isolates and 10% of ribotype 001 isolates had MICs >1 mg/liter ($P < 0.001$). A similar pattern was observed with tinidazole. Prior exposure to metronidazole did not account for higher metronidazole MICs. Among the 83 isolates recovered from patients who had received oral or intravenous

TABLE 1. Characteristics of patients by infecting *C. difficile* ribotype

Characteristic[a]	No. (%) of patients infected with *C. difficile* of:		
	Ribotype 027 (n = 141)	Ribotype 001 (n = 72)	Other ribotypes (n = 17)
Sex			
Male	69 (49)	33 (46)	9 (53)
Female	72 (51)	39 (54)	8 (47)
Age (yr)			
20–64	37 (26)	17 (24)	9 (53)
65–74	30 (21)	13 (18)	4 (24)
≥75	74 (52)	42 (58)	4 (24)
Comorbidities			
COPD	56 (40)	29 (40)	4 (24)
Steroids	52 (37)	27 (38)	3 (18)
Leukemia or lymphoma	5 (4)	4 (6)	4 (24)
Chemotherapy, last 2 mo	4 (3)	3 (4)	4 (24)
Renal transplant	5 (4)	3 (4)	1 (6)
Stem cell transplant	2 (2)	1 (1)	1 (6)
HIV infection	0	0	1 (6)
IBD	5 (4)	1 (1)	2 (12)
Charlson comorbidity index			
0	14 (10)	6 (8)	3 (18)
1–3	83 (59)	43 (60)	9 (53)
4–6	30 (21)	19 (26)	4 (24)
7 or more	14 (10)	4 (6)	1 (6)
Previous episodes of *C. difficile* infection			
None	101 (72)	56 (78)	14 (82)
1	21 (15)	9 (13)	3 (18)
2	18 (13)	6 (8)	0
3 or more	1 (1)	1 (1)	0
No. of hospitalization days in previous 6 mo			
0–30	89 (63)	46 (64)	11 (65)
31–60	36 (26)	20 (28)	6 (36)
61–183	16 (11)	6 (8)	0
Nosocomial acquisition	131 (93)	71 (99)	16 (94)
CCP[b]			
Low	29/101 (29)	3/56 (5)	3/14 (21)
Moderate	26/101 (26)	14/56 (25)	7/14 (50)
High	46/101 (46)	39/56 (70)	4/14 (29)

[a] COPD, chronic obstructive pulmonary disease; HIV, human immunodeficiency virus; IBD, inflammatory bowel disease.
[b] CCP was estimated only for patients with their first episode of *C. difficile* infection.

TABLE 2. Antibiotics received in the previous 2 months by patients with first episode of *C. difficile* infection, by ribotype

Antibiotic received in the previous 2 mo	No. (%) of patients infected with:		
	Ribotype 027 (n = 99[a])	Ribotype 001 (n = 56)	Other ribotypes (n = 14)
Any antibiotic	95 (96)	55 (98)	14 (100)
Metronidazole	20 (20)	6 (11)	3 (21)
Intravenous vancomycin	16 (16)	10 (18)	4 (29)
Cephalosporins (any)	69 (50)	49 (68)	7 (50)
Narrow spectrum	22 (22)	9 (16)	4 (29)
Expanded spectrum	3 (3)	12 (21)[b]	1 (7)
Broad spectrum	33 (33)	26 (46)	4 (29)
Clindamycin	4 (4)	16 (29)[b]	1 (7)
Macrolides	27 (27)	22 (39)	1 (7)
FQs (any)	69 (70)	26 (46)[c]	6 (43)[c]
Ciprofloxacin	56 (57)	23 (41)[c]	5 (36)
Gatifloxacin	16 (16)	5 (9)	1 (7)

[a] Data on previous antibiotic use were available for 99/101 patients with their first episode of infection with a *C. difficile* ribotype 027 strain.
[b] $P \leq 0.001$ compared to patients infected with ribotype 027.
[c] $P \leq 0.05$ compared to patients infected with ribotype 027.

metronidazole within the previous 2 months, 13% had an MIC of ≤0.25 mg/liter, 51% had an MIC of 0.5 mg/liter, and 36% had an MIC of 1 to 2 mg/liter. This was similar to the corresponding proportions of 16%, 50%, and 34% for isolates recovered from 145 patients who had not received metronidazole.

Vancomycin MICs were 1 dilution lower for ribotype 027 isolates than for ribotype 001 isolates (except for two ribotype 027 isolates that showed an MIC of 4 mg/liter), and ramoplanin appeared to be two- to fourfold more active than vancomycin. Ciprofloxacin and gatifloxacin were both poorly active against the ribotype 027 strains, which showed the highest MICs. Azithromycin was generally inactive, regardless of the ribotype. While 32% of the patients infected with isolates exhibiting azithromycin MICs of >64 mg/liter had received a macrolide within the previous 2 months, none of the 19 patients infected with a strain with an azithromycin MIC of ≤64 mg/liter reported such exposure ($P = 0.004$). Ribotype 027 isolates had lower clindamycin MICs than ribotype 001 isolates (MIC_{50}s, 4 and >64 mg/liter, respectively). All ribotype 027 strains were susceptible to clindamycin and were negative for the *ermB* gene, whereas all ribotype 001 strains were clindamycin resistant; 88% of those strains were positive for the *ermB* gene.

Treatment. Among the patients with their first episode of *C. difficile* infection, 4/45 (9%) in 2000 and 2001 and 14/124 (11%) in 2003 and 2004 received no specific treatment, other than the cessation of treatment with the predisposing agent. Among those who were treated in 2000 and 2001, 98% initially received oral metronidazole monotherapy and one patient received vancomycin. The corresponding values for 2003 and 2004 were 88% and 5%, with 7% of patients receiving a combination of oral vancomycin and intravenous metronidazole on the first day of therapy. Among the 40 patients who initially received metronidazole monotherapy for their first episode of *C. difficile* infection in 2000 and 2001, 3 (8%) were eventually switched to vancomycin due to a perceived failure, whereas 29/97 (30%) were eventually switched to vancomycin in 2003 and 2004 ($P = 0.005$). During the latter period, the probability of a switch from metronidazole to vancomycin was not significantly higher for patients infected with a ribotype 027 strain (25/75; 33%) than for those infected with a ribotype 001 strain (3/18; 17%) or a strain of another ribotype (1/4; 25%).

TABLE 3. MIC ranges, MIC_{50}s, and MIC_{90}s, by ribotype

Ribotype	MIC (mg/liter)																				
	Ciprofloxacin			Gatifloxacin			Clindamycin			Metronidazole			Ramoplanin			Tinidazole			Vancomycin		
	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%
027 (n = 141)	8–>128	>128	>128	1–>32	>32	>32	0.06–8	4	8	0.12–2	1	1	0.06–0.25	0.12	0.25	0.06–2	1	1	0.5–4	0.5	2
001 (n = 72)	8–64	64	64	2–32	32	32	64–>64	>64	>64	0.12–1	0.5	0.5	0.06–0.25	0.25	0.25	0.06–2	0.5	0.5	0.06–2	1	1
Other (n = 17)	8–>128	16	>128	1–>32	2	>32	1–>64	4	8	0.06–2	0.25	0.5	0.06–0.25	0.25	0.25	0.06–8	0.25	0.5	0.5–2	1	1

Outcomes. Whereas the overall 30-day rates of mortality were not different among the patients between 2000–2001 (22%; 12/55) and 2003–2004 (25%; 43/175), those infected with a ribotype 027 strain were more than twice as likely to die within 30 days (Table 4). The rates of mortality were higher among older patients, those with a high Charlson comorbidity index score, and those with longer prior hospitalizations. A high level of leukocytosis ($\geq 20 \times 10^9$/liter) and acute renal failure were also strongly correlated with mortality. The association between infection with a ribotype 027 strain and death remained significant (AOR, 2.06, 95% CI, 1.00 to 4.22) after adjustment for age, Charlson comorbidity index score, and the duration of prior hospitalization. The peak levels of leukocytosis and creatinine were not fitted into this model, as these variables were clearly on the causal pathway between the more proximal variables and death. The association was stronger and more significant when the analysis was restricted to the 2003-2004 outbreak period: 41/140 (29%) patients infected with a ribotype 027 strain died within 30 days, whereas 2/35 (6%) patients infected with a ribotype 001 strain or a strain of another ribotype died within 30 days (AOR, 7.53; 95% CI, 1.60 to 35.52; $P = 0.01$). Death was attributed to *C. difficile* infection in 26/41 (63%) patients infected with a ribotype 027 strain and 2/12 (17%) patients infected with a ribotype 001 strain ($P = 0.004$).

The proportion of patients experiencing at least one recurrence of *C. difficile* infection did not differ between those infected with a ribotype 027 strain (31%), a ribotype 001 strain (26%), and strains of other ribotypes (25%) ($P = 0.20$). The risk of a recurrence was not associated with age or the treatment received.

Antibiotic consumption. From April 2000 to March 2007 (Fig. 2), the rate of use of narrow-spectrum cephalosporins remained stable (mean, 51 DDD/1,000 patient days), while the rates of use of extended- and broad-spectrum cephalosporins decreased from 119 to 54 DDD/1,000 patient days (mean, 76 DDD/1,000 patient days). The rate of clindamycin use decreased from 27 to 16 DDD/1,000 patient days (mean, 22 DDD/1,000 patient days). The rate of macrolides use decreased from 62 to 26 DDD/1,000 patient days (mean, 44 DDD/1,000 patient days). In contrast, the overall rate of FQ use increased from 94 to 151 DDD/1,000 patient days (mean, 121 DDD/1,000 patient days). This increase was attributable to the introduction of respiratory quinolones (gatifloxacin was introduced on the formulary in November 2001 but was replaced by moxifloxacin in June 2005), since the rate of ciprofloxacin use remained unchanged from 2000 to 2007, with a mean of 87 DDD/1,000 patient days.

DISCUSSION

The emergence of a BI/NAP1/027 strain in Canada and the United States coincided with an increase in the incidence of nosocomial *C. difficile* infections and in the rates of mortality associated with *C. difficile* infections. In Quebec, the number of cases of mortality from *C. difficile* infection listed as the main cause of death on the death certificate increased from 85 in 2000 to 691 in 2004 (14). It was estimated that 14 to 17% of patients with a nosocomial *C. difficile* infection died as a direct or an indirect consequence of the infection (18, 30). In the United States, the number of hospital discharges for which *C. difficile* infection was listed as any diagnosis increased from 82,000 in 1996 to 178,000 in 2003 and >250,000 in 2005 (21, 23); and on a population basis, the rates of mortality from *C. difficile* infection increased from $5.7/10^6$ population in 1999 to $23.7/10^6$ population in 2004 (32). Among patients hospitalized in the United States, the rates of mortality attributed to *C. difficile* infection increased from 20.3 per 100,000 discharges in 1993 to 50.2 per 100,000 discharges in 2003; during the same interval, the case-fatality ratio increased modestly, from 7.8 to 9.3% (33).

The increase in the rates of mortality from *C. difficile* infection observed concurrently with the emergence of the BI/NAP1/027 strain may reflect the higher incidence (possibly due to the more effective transmission of this strain within hospital environments), the aging population, or the higher intrinsic severity of infection with BI/NAP1/027 compared with the severity of infection with other strains. A recent study conducted by Hubert et al. in the province of Quebec (12) did not show a significant association between outbreak strains that were binary toxin positive and from which *tcdC* was deleted and the attributable or contributive rate of mortality as a result of *C. difficile* infection (AOR, 1.7; 95% CI, 0.7 to 3.9). Severe disease (death within 30 days after a diagnosis of *C. difficile* infection, a requirement for colectomy, and/or admission to intensive care because of *C. difficile* infection) was more frequent among patients infected with the outbreak strain, but this association did not remain significant after adjustment for age (AOR, 2.1; 95% CI, 0.98 to 4.6). To our knowledge, this is the first study documenting a higher case-fatality ratio specifically in association with BI/NAP1/027 infection, after adjustment for confounding factors such as age and the burden of chronic comorbidities. This is in line with in vitro measurements of toxin production, which showed that the levels of production of toxins A and B by BI/NAP1/027 were 16- and 23-fold higher, respectively, than the levels of production by other strains (36). Strains were not recovered in the preepidemic period from

TABLE 4. Risk factors for 30-day mortality among patients with *Clostridium difficile* infection

Characteristic	No of patients who died within 30 days/total no. of patients (%)	Odds ratio (95% CI)	
		Unadjusted	Adjusted[a]
Infecting ribotype			
001 or other types	14/89 (16)	1.00	1.00
027	41/141 (29)	2.20 (1.12–4.32)[b]	2.06 (1.00–4.22)[b]
Age (yr)			
20–64	8/63 (13)	1.00	1.0
65–74	14/47 (30)	2.92 (1.11–7.69)[b]	3.61 (1.24–10.51)[b]
≥75	33/120 (28)	2.61 (1.12–6.06)[b]	3.18 (1.26–8.02)[b]
Sex			
Female	26/119 (22)	1.00	
Male	29/111 (26)	1.27 (0.69–2.32)	
Charlson comorbidity index			
0	1/23 (4)	1.00	1.00
1–3	30/135 (22)	6.29 (0.81–48.57)	5.39 (0.66–43.64)
4–6	14/53 (26)	7.90 (0.70–47.57)	6.56 (0.77–56.04)
7 or more	10/19 (53)	24.44 (2.72–219.93)[b]	22.15 (2.27–216.01)[b]
Immunosuppression[c]			
No	50/200 (25)	1.00	
Yes	5/30 (17)	0.60 (0.22–1.65)	
No. of prior episodes of *C. difficile* infection			
None	36/168 (21)	1.00	
One or more	18/59 (31)	1.61 (0.83–3.13)	
No. of hospitalization days in previous 6 mo			
0–30	26/146 (18)	1.00	1.00
31–60	20/62 (32)	2.20 (1.11–4.34)[b]	1.91 (0.91–4.02)
61–183	9/22 (41)	3.20 (1.24–8.26)[b]	4.10 (1.46–11.52)[b]
Peak creatinine level[d]/baseline creatinine level			
<1.5	28/168 (17)	1.0	
≥1.5	22/39 (56)	6.47 (3.05–13.72)[e]	
Peak white cell count[d] (10^9/liter)			
<20	30/163 (18)	1.0	
20.0–49.9	19/42 (45)	3.66 (1.77–7.56)[e]	
≥50	5/8 (63)	7.39 (1.67–32.63)[b]	

[a] Odds ratios were adjusted for infecting ribotype, age, Charlson's comorbidity index, and number of previous hospitalization days.
[b] $P \leq 0.05$.
[c] Immunosuppression was from leukemia or lymphoma, chemotherapy within the past 2 months, renal or stem cell transplantation, human immunodeficiency virus infection, or any combination thereof.
[d] The peak level is the highest value within 7 days of diagnosis of the current episode.
[e] $P < 0.001$.

patients who were part of the initial reports of the Quebec outbreak (18, 29, 30).

The MICs of metronidazole were comparable to those published by others (2, 24). The observation that the MIC_{50}s and MIC_{90}s of metronidazole were 1 dilution higher for ribotype 027 strains than for ribotype 001 strains would seem to be inconsequential, as they were less than the CLSI susceptibility breakpoint (27) and could be within the margin of error for susceptibility tests. However, tinidazole also showed the same trend, and the same observation was made in the study of Hubert et al. (12). Since the fecal concentrations of metronidazole are low, especially when colitis has subsided (4), even a modest upward drift of MICs might become clinically relevant.

Although exposure to nearly all antimicrobial classes can trigger the onset of *C. difficile* infection (28), our data support the linkage of prior specific antimicrobial exposure, the development of (or the selection for) specific-antibiotic-resistant clones of *C. difficile*, and the development of *C. difficile* infection caused by the selected antibiotic-resistant ribotype. The BI/NAP1/027 isolates were highly resistant to FQs, as reported by others (9, 12, 18, 22, 26, 29), and were strongly associated with prior patient FQ exposure. An association between the BI/NAP1/027 clone and FQ use has consistently been reported in the literature (18, 26, 29). In a mouse model of gut colonization, FQs (gatifloxacin and moxifloxacin and, to a lesser extent, ciprofloxacin and levofloxacin) inhibited the growth of

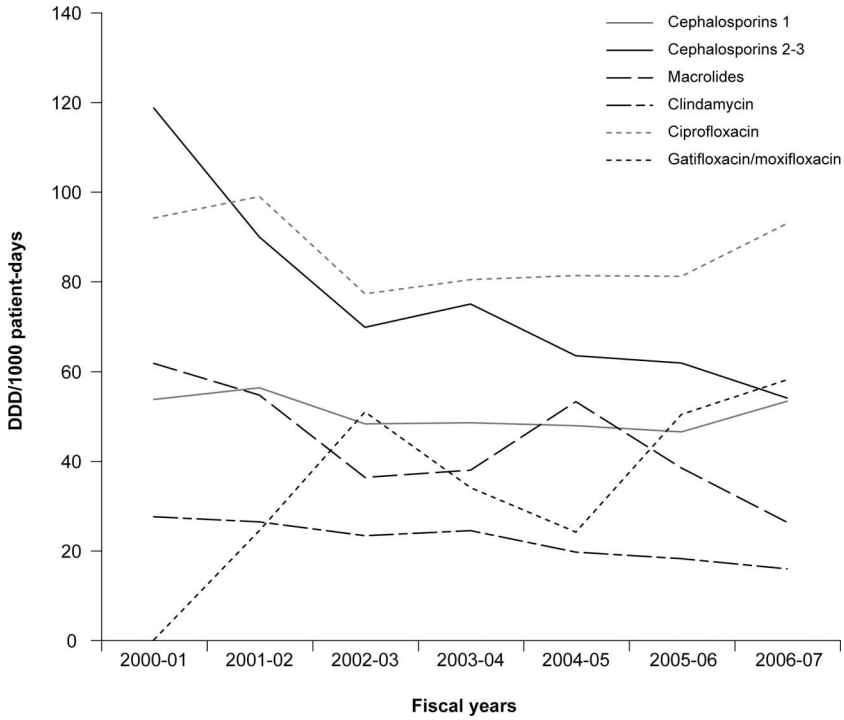

FIG. 2. Antibiotic consumption at Hôpital Maisonneuve-Rosemont (April 2000 to March 2007). Cephalosporins 1, cephalexin, cefadroxil, and cefazolin; Cephalosporins 2-3, cefuroxime, cefoxitin, ceftriaxone, and ceftazidime; Macrolides, erythromycin, clarithromycin, and azithromycin. Gatifloxacin was introduced on the formulary in November 2001 and was replaced by moxifloxacin in June 2005.

FQ-susceptible *C. difficile* strains in the cecum and promoted the overgrowth of FQ-resistant epidemic strains (1). Historical isolates of BI/NAP1 were susceptible to FQs (22). The acquisition of resistance to FQs presumably facilitated its transmission within the hospital environment, eventually promoting its emergence as an epidemic strain. The reasons for the differences in the propensities of various FQs to induce *C. difficile* infection remain to be elucidated. The activities of the antimicrobials against the pathogen and normal flora components, colonic antimicrobial concentrations, and the presence of sources of resistance determinants are only a few of the factors at play. Interestingly, in a parallel fashion, clindamycin and macrolide exposure was also shown to select for infection with *C. difficile* isolates resistant to the respective antimicrobials. The observed differences in estimated CCP risk scores suggest that ribotype 027 strains may be more transmissible than ribotype 001 strains, and this should be further investigated in prospective studies.

This study also shows that strain and susceptibility profiles are important for the identification of control measures. Historically, the restriction of clindamycin use has been particularly effective in the setting of high levels of clindamycin use and the presence of clindamycin-resistant *C. difficile* strains. However, in centers where BI/NAP1/027 isolates occupy a predominant place in the local repertoire of *C. difficile* strains, measures to reduce the use of FQs (35), combined with the use of comprehensive infection control measures (25), are required. At Hôpital Maisonneuve-Rosemont, the winter peak incidence decreased from 40 (2004) to 25 (2007) per 1,000 admissions, remaining higher than the mean preepidemic winter peak rates of ≈18 (1999 to 2002) per 1,000 admissions (Fig. 1a). Our results suggest that, in addition to vigorous environmental and infection control efforts, the effective control of *C. difficile* infections requires focused antimicrobial stewardship based on detailed epidemiological and microbiological information on the current local distribution of *C. difficile* ribotypes.

The outbreak described here involved the superimposition of the new epidemic clone on top of the prior existing and dominant ribotype 001 clone. Such an event resulted in extremely high rates of *C. difficile* infection. Infection with ribotype 027 strains was found to double the likelihood of 30-day mortality. The observed associations between the in vitro resistance of *C. difficile* to specific classes of antibiotics, previous patient exposure to those antibiotics, and the development of *C. difficile* infection caused by a resistant ribotype illustrate the complex nature of the evolving *C. difficile* infection epidemic.

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