

ORIGINAL ARTICLE

A Predominantly Clonal Multi-Institutional Outbreak of *Clostridium difficile*-Associated Diarrhea with High Morbidity and Mortality

Vivian G. Loo, M.D., Louise Poirier, M.D., Mark A. Miller, M.D., Matthew Oughton, M.D., Michael D. Libman, M.D., Sophie Michaud, M.D., M.P.H., Anne-Marie Bourgault, M.D., Tuyen Nguyen, M.D., Charles Frenette, M.D., Mirabelle Kelly, M.D., Anne Vibien, M.D., Paul Brassard, M.D., Susan Fenn, M.L.T., Ken Dewar, Ph.D., Thomas J. Hudson, M.D., Ruth Horn, M.D., Pierre René, M.D., Yury Monczak, Ph.D., and André Dascal, M.D.

ABSTRACT

BACKGROUND

In March 2003, several hospitals in Quebec, Canada, noted a marked increase in the incidence of *Clostridium difficile*-associated diarrhea.

METHODS

In 2004 we conducted a prospective study at 12 Quebec hospitals to determine the incidence of nosocomial *C. difficile*-associated diarrhea and its complications and a case-control study to identify risk factors for the disease. Isolates of *C. difficile* were typed by pulsed-field gel electrophoresis and analyzed for binary toxin genes and partial deletions in the toxin A and B repressor gene *tcdC*. Antimicrobial susceptibility was evaluated in a subgroup of isolates.

RESULTS

A total of 1703 patients with 1719 episodes of nosocomial *C. difficile*-associated diarrhea were identified. The incidence was 22.5 per 1000 admissions. The 30-day attributable mortality rate was 6.9 percent. Case patients were more likely than matched controls to have received fluoroquinolones (odds ratio, 3.9; 95 percent confidence interval, 2.3 to 6.6) or cephalosporins (odds ratio, 3.8; 95 percent confidence interval, 2.2 to 6.6). A predominant strain, resistant to fluoroquinolones, was found in 129 of 157 isolates (82.2 percent), and the binary toxin genes and partial deletions in the *tcdC* gene were present in 132 isolates (84.1 percent).

CONCLUSIONS

A strain of *C. difficile* that was resistant to fluoroquinolones and had binary toxin and a partial deletion of the *tcdC* gene was responsible for this outbreak of *C. difficile*-associated diarrhea. Exposure to fluoroquinolones or cephalosporins was a risk factor.

From McGill University Health Center (V.G.L., M.D.L., P.B., S.F., K.D., T.J.H., R.H., P.R.); McGill University (V.G.L., M.A.M., M.O., M.D.L., P.B., K.D., T.J.H., R.H., P.R., Y.M., A.D.); Hôpital Maisonneuve-Rosemont (L.P.); Université de Montréal (L.P., A.-M.B.); Sir Mortimer B. Davis-Jewish General Hospital (M.A.M., Y.M., A.D.); St. Mary's Hospital (M.D.L.); Centre Hospitalier Universitaire de Montréal Hôpital St. Luc (A.-M.B.); Hôpital Jean Talon (M.K.); McGill University and Genome Québec Innovation Center (K.D., T.J.H.) — all in Montreal; Cité de la Santé de Laval, Laval, Que., Canada (T.N.); Centre Hospitalier Universitaire de Sherbrooke (S.M.) and Université de Sherbrooke (S.M., C.F.) — both in Sherbrooke, Que., Canada; Hôpital Charles LeMoine, Longueuil, Que., Canada (C.F.); and Réseau Santé Richelieu-Yamaska, St. Hyacinthe, Que., Canada (A.V.). Address reprint requests to Dr. Loo at the Department of Microbiology, McGill University Health Center, 1650 Cedar Ave., Rm. D16.168, Montreal, QC H3G 1A4, Canada, or at vivian.loo@muhc.mcgill.ca.

N Engl J Med 2005;353:2442-9.
Copyright © 2005 Massachusetts Medical Society.

C*LOSTRIDIUM DIFFICILE* IS THE LEADING cause of nosocomial infectious diarrhea.¹ The most important risk factor for *C. difficile*-associated diarrhea is prior antibiotic use.² Some patients remain asymptomatic after exposure to *C. difficile*, whereas illness ranging from mild diarrhea to fulminant colitis develops in others.² Only 1 to 5 percent of affected patients have severe disease, leading to colectomy, intensive care, or death.^{3,4}

The best-described *C. difficile* virulence factors are toxins A and B, encoded by the genes *tcdA* and *tcdB*, respectively.⁵ Together with two regulatory genes (*tcdC* and *tcdD*) and a porin gene (*tcdE*), they form the chromosomal pathogenicity locus.^{6,7} The expression of *tcdA* and *tcdB* is down-regulated by the *tcdC* gene. Polymorphisms or partial deletions of *tcdC* may lead to increased production of toxin A and toxin B.⁷ In addition, a separate binary toxin has been described in *C. difficile*.⁸ Two chromosomal genes (*cdtA* and *cdtB*), separate from the chromosomal pathogenicity locus, encode this toxin. The *cdtB* gene mediates cell-surface binding and intracellular translocation, whereas *cdtA* disrupts the assembly of the actin filament through ribosylation of adenosine diphosphate, causing cell death.⁹

Since March 2003, many hospitals in Montreal and its surrounding regions in Quebec have noted a rise in the incidence of *C. difficile*-associated diarrhea, with an accompanying increase in the proportion of cases having severe and fatal complications.¹⁰ We conducted a prospective study to evaluate the incidence, morbidity, and mortality of nosocomial *C. difficile*-associated diarrhea in 12 Quebec hospitals. We also performed a case-control study to identify risk factors in our patient population. We hypothesized that a common strain may have been linked to the nearly simultaneous outbreaks in multiple institutions and that this strain would demonstrate postulated virulence factors—specifically, the presence of binary toxin and partial deletions of the *tcdC* gene.

METHODS

PARTICIPATING HOSPITALS

From January 11 to June 26, 2004, surveillance for nosocomial *C. difficile*-associated diarrhea and its associated complications was implemented at 12 hospitals. The study was performed as part of the routine institutional management of outbreaks and approved by the director of professional services at each institution. Information was collected about

each hospital, including the type of health care facility, bed capacity, and age-specific admissions.

SURVEILLANCE DEFINITIONS

C. difficile-associated diarrhea was defined by the presence of diarrhea and a positive assay for *C. difficile* toxin A, toxin B, or both; by the sudden onset of diarrhea with no alternative explanation and a diagnosis of pseudomembranous colitis on the basis of endoscopy; or by histologic evidence of the condition. A case was considered nosocomial if symptoms started 72 hours or more after a patient was admitted or if *C. difficile*-associated diarrhea was diagnosed within one month after a previous admission. An episode was considered new if it occurred more than eight weeks after a previous diagnosis of *C. difficile*-associated diarrhea. Neonates and psychiatric inpatients were excluded.

PATIENTS' CHARACTERISTICS AND OUTCOME MEASURES

We collected data on each patient's age and sex, the ward in which *C. difficile*-associated diarrhea was acquired, the diagnosis-related group, and whether he or she had received antibiotics in the hospital within six weeks before the *C. difficile* diagnosis. The outcomes measured included the crude and attributable 30-day mortality rates and the rates of colectomy and disease requiring intensive care owing to *C. difficile*-associated diarrhea. For each death, two physicians judged independently whether *C. difficile*-associated diarrhea was an attributable cause, a contributing cause, or unrelated to the cause of death. It was deemed the attributable cause of death if the physician judged that the patient would not have died within 30 days in the absence of *C. difficile*-associated diarrhea. In the case of a disagreement, the two physicians reached a consensus. A case of *C. difficile*-associated diarrhea was classified as severe if the patient died within 30 days after the diagnosis attributable to this condition or if the patient required colectomy or intensive care as a result.

CASE-CONTROL STUDY

We used a computer-generated random sample of 15 percent of patients with *C. difficile*-associated diarrhea in the prospective study. We then selected one control patient per patient with *C. difficile*-associated diarrhea from a computer-generated list of patients who had been admitted and discharged from the same institution during the study period. Control patients were matched with case patients

by age (within five years), Charlson index, date of admission (within one month), ward, and length of time at risk for *C. difficile*-associated diarrhea. For case patients, the length of time at risk was defined as the number of days from admission to the development of *C. difficile*-associated diarrhea; for controls, it was defined as the number of days from admission to discharge. Controls had no known history of *C. difficile*-associated diarrhea. We also collected information on potential covariates such as the type of hospital (community or university-affiliated) and the use or nonuse of antibiotics, enteral feeding, chemotherapy, proton-pump inhibitors, and histamine H₂-blockers within six weeks before the diagnosis of *C. difficile*-associated diarrhea for the case patients and within six weeks before discharge for the controls.

DETECTION OF *C. DIFFICILE* TOXIN

Routine laboratory procedures were used at each institution to detect *C. difficile* toxin. Ten hospital laboratories used cell-culture cytotoxin assays to detect toxin B according to standard methods.¹¹ Two hospital laboratories used enzyme immunoassays according to the manufacturer's instructions: one used the Triage Micro *C. difficile* panel (Biosite) for the detection of glutamate dehydrogenase and toxin A in stool samples, and the other used the ColorPAC Toxin A kit (Becton Dickinson). For the Triage Micro *C. difficile* panel, samples were also tested by cell culture for toxin B if the glutamate dehydrogenase and toxin A results were discordant.

CULTURE OF *C. DIFFICILE*

Participating institutions were asked to submit 10 consecutive stool samples that were positive for toxin A or B from patients with nosocomial *C. difficile*-associated diarrhea. Samples were treated with alcohol, and the mixture was inoculated onto cefoxitin-cycloserine fructose agar plates (Oxoid Basingstoke).¹¹ After incubation at 35°C for 48 hours under anaerobic conditions, isolates were confirmed to be *C. difficile* on the basis of Gram's staining, typical odor, chartreuse fluorescence under ultraviolet light, and the presence of *C. difficile* antigen on Microscreen latex agglutination (Microgen Bioproducts). Isolates of *C. difficile* were frozen at -70°C in brain-heart infusion broth and 10 percent glycerol pending further characterization.

PULSED-FIELD GEL ELECTROPHORESIS

To determine whether the observed epidemiologic features were related to a clonal outbreak, pulsed-

field gel electrophoresis (PFGE) of *C. difficile* isolates was performed according to the method described by Fawley and Wilcox.¹² Gels were stained with ethidium bromide and photographed with the use of Image Master software (Bio-Rad Laboratories Canada). A molecular-weight marker and a reproducible *C. difficile* isolate were included in each gel migration. The relatedness of the various isolates was determined according to the criteria of Tenover et al.¹³

ANALYSES FOR BINARY TOXIN GENES AND PARTIAL DELETIONS OF THE *tcdC* GENE

The presence of binary toxin genes (*cdtA* and *cdtB*) and partial deletions of the *tcdC* gene was identified according to the methods of Gonçalves et al. and Cohen et al., respectively.^{14,15} The polymerase-chain-reaction (PCR) assays for *cdtA* and *cdtB* were performed separately, and the results were analyzed independently. Crude DNA was extracted from isolates of *C. difficile* by means of the InstaGene Matrix kit (BioRad) according to the manufacturer's instructions. We used *C. difficile* Collection de l'Institut Pasteur 107932 as a positive control for binary toxin genes and *C. difficile* American Type Culture Collection (ATCC) 43255, *C. spiroforme* ATCC 29900, and *Staphylococcus aureus* ATCC 25923 as negative controls for binary toxin genes. Amplified products underwent electrophoresis, were stained with ethidium bromide, and were photographed with the use of a Land camera (Polaroid). DNA sequencing of both strands of the PCR products was performed according to standard protocols, and the results were analyzed with the use of an ABI3700XL sequencer (Applied Biosystems).

SUSCEPTIBILITY TESTING

Testing of the *C. difficile* isolates for susceptibility to gatifloxacin, levofloxacin, moxifloxacin, ciprofloxacin, clindamycin, metronidazole, and vancomycin was performed with the use of the Etest (AB Biodisk), a 1.0 McFarland inoculum, brucella agar, and anaerobic conditions. As a means of quality control, appropriate ATCC strains of *Escherichia coli*, *S. aureus*, and *Bacteroides fragilis* were used for the tested antibiotics, according to the guidelines of the Clinical and Laboratory Standards Institute.¹⁶

STATISTICAL ANALYSIS

Epidemiologic and molecular data were collected and interpreted independently. A relational database was developed between patient identifiers and isolate identifiers. A Yates-corrected chi-square test was used for the analysis of proportions. If a cell

value was less than 5 in the two-by-two table, Fisher's exact test was used. All P values were two-sided. Conditional logistic regression was used in the case-control analysis to estimate the odds ratio of *C. difficile*-associated diarrhea associated with the use of specific classes of antibiotic. All analyses were adjusted for the concurrent use of other antimicrobial agents, as well as for all potential covariates. Analyses were performed with the use of SAS software (version 9.1, SAS Institute).

RESULTS

DESCRIPTION OF HOSPITALS

Of the 12 participating hospitals, 8 were in Montreal, 2 were in Sherbrooke, 1 was in Laval, and 1 was in St. Hyacinthe. There were eight university-affiliated centers and four community hospitals. The bed capacity ranged from 256 to 705. The number of admissions ranged from 5188 to 23,485 per year.

PATIENT POPULATION

A total of 1703 patients had 1719 episodes that met the case definition of nosocomial *C. difficile*-associated diarrhea. The patients' characteristics and their use of antibiotics in the hospital within the six weeks before the diagnosis are shown in Table 1. The most common classes of antibiotic administered were cephalosporins and fluoroquinolones. Data on antibiotic use were available for only 1512 patients (88.8 percent).

INCIDENCE AND OUTCOME MEASURES

The overall mean incidence of *C. difficile*-associated diarrhea was 22.5 per 1000 admissions (range, 10.2 to 39.9) during the study period. The incidence increased with age (Table 2). A total of 422 patients died within 30 days after the diagnosis of *C. difficile*-associated diarrhea, for a crude mortality rate of 24.8 percent. Among these 422 patients, *C. difficile*-associated diarrhea was the attributable cause of death in 117 of the 1703 patients (6.9 percent), contributed to but was not the attributable cause of death in another 127 (7.5 percent), and was unrelated to the cause of death in 178 (10.5 percent). The attributable mortality rate increased with age (Table 2). Because of *C. difficile*-associated diarrhea, 110 patients (6.5 percent) required intensive care and 33 patients (1.9 percent) required colectomy.

CASE-CONTROL STUDY

A total of 237 case patients were matched to 237 controls from 10 of the 12 institutions. Table 3 com-

Table 1. Characteristics of 1703 Patients with *Clostridium difficile*-Associated Diarrhea.

Characteristic	Value
Median age — yr	76
Sex — no. (%)	
Female	882 (51.8)
Male	821 (48.2)
Diagnosis-related group — no. (%)	
Cardiovascular	403 (23.7)
Gastrointestinal	326 (19.1)
Respiratory	267 (15.7)
Bone and joint	162 (9.5)
Genitourinary	138 (8.1)
Neurologic	83 (4.9)
Miscellaneous	324 (19.0)
Antibiotic use during hospitalization within 6 wk before diagnosis of <i>C. difficile</i> -associated diarrhea — no. (%)*	
Ciprofloxacin	575 (38.0)
Other fluoroquinolones (gatifloxacin, levofloxacin, moxifloxacin)	358 (23.7)
Clindamycin	87 (5.8)
Cephalosporins	
First-generation	388 (25.7)
Second-generation	200 (13.2)
Third-generation	244 (16.1)
Fourth-generation	13 (0.9)
Carbapenems	91 (6.0)
Penicillins	177 (11.7)
Penicillins combined with β -lactamase inhibitor	272 (18.0)
No antibiotics	42 (2.8)

* The category included data on 1512 patients.

Table 2. Age-Specific Incidence and Mortality Attributed to *Clostridium difficile*-Associated Diarrhea.

Age	No. of Cases	No. of Cases/ 1000 Admissions*	Attributable 30-Day Mortality Rate
yr			%†
<40	76	3.5	2.6
41–50	85	11.2	1.2
51–60	191	20.0	3.2
61–70	272	24.4	5.1
71–80	523	38.3	6.2
81–90	458	54.5	10.2
>90	114	74.4	14.0

* Values are based on 1719 episodes of nosocomial *C. difficile*-associated diarrhea.

† Values are based on data from 1703 patients with nosocomial *C. difficile*-associated diarrhea.

compares the demographic and clinical variables in the two groups. The two groups were similar with respect to age, sex, ward, and Charlson index. The median time at risk for *C. difficile*-associated diarrhea was 13 days among case patients and 16 days among controls ($P=0.02$). Case patients were more likely than controls to have been exposed to antibiotics (79.3 percent vs. 59.3 percent, $P<0.001$) and enteral feeding (18.6 percent vs. 11.8 percent, $P=0.04$). Matched logistic-regression analysis of case patients and controls revealed that exposure to cephalosporins (odds ratio, 3.8; 95 percent confidence interval, 2.2 to 6.6) and exposure to fluoroquinolones (odds ratio, 3.9; 95 percent confidence interval, 2.3 to 6.6) were significant independent risk factors for *C. difficile*-associated diarrhea (Table 4). Exposure to other classes of antibiotics,

proton-pump inhibitors, enteral feeding, histamine H_2 -blockers, or chemotherapy was not significantly associated with the development of *C. difficile*-associated diarrhea (Table 4). To examine the risk associated with specific types of fluoroquinolones and cephalosporins, specific adjusted odds ratios were calculated (Table 4). Ciprofloxacin, gatifloxacin or moxifloxacin, and first-, second-, and third-generation cephalosporins were all independently associated with the development of *C. difficile*-associated diarrhea.

C. DIFFICILE ISOLATES

Nine hospitals submitted stool samples that yielded 157 *C. difficile* isolates for PFGE, binary toxin analyses, and *tcdC* analyses. Sixty-seven isolates (42.7 percent) came from one institution. Three institutions submitted isolates outside the defined study period.

PULSED-FIELD GEL ELECTROPHORESIS

All 157 isolates were typeable by PFGE, and 129 (82.2 percent) had an identical PFGE pattern, or "pulsovar," displaying eight bands ranging from 90 to 360 kb, with the rest of the genomic DNA unresolved at more than 500 kb (Fig. 1 in the Supplementary Appendix, available with the full text of this article at www.nejm.org). Of the 28 other isolates, 12 additional pulsovars were observed.

PCR ANALYSES FOR BINARY TOXIN GENES AND PARTIAL DELETIONS OF THE *tcdC* GENE

On amplification of *C. difficile* 16s ribosomal DNA, all 157 isolates had the expected amplicon of 270 bp, indicating successful DNA extraction and the absence of PCR inhibition. Of the 157 isolates, 132 (84.1 percent) produced the expected amplicon of 370 bp with the use of the *cdtA* primer set (data not shown). The same 132 isolates generated the expected amplicon of 510 bp with the use of the *cdtB* primer set (Fig. 2A of the Supplementary Appendix). Furthermore, all 132 isolates possessing the binary toxin genes also had a partial deletion of the *tcdC* gene (Fig. 2B of the Supplementary Appendix). Of these, 129 (97.7 percent) belonged to the predominant pulsovar and the remaining 3 (2.3 percent) belonged to three unrelated pulsovars (Fig. 1 of the Supplementary Appendix). Sequence analysis showed that the predominant strain had an 18-bp deletion in the *tcdC* gene. For the three unrelated pulsovars with a partial deletion of the *tcdC* gene, two strains had a 39-bp deletion and the other had

Table 3. Characteristics of Case Patients and Control Patients.*

Characteristic	Case Patients (N=237)	Controls (N=237)	P Value
Age — yr			0.48
Median	75	75	
Interquartile range	66–82	66–82	
Male sex — no. (%)	115 (48.5)	126 (53.2)	0.3
Charlson index†	2.6±1.9	2.6±2.0	0.66
Ward			0.82
Medicine	133 (56.1)	142 (59.9)	
Surgery	78 (32.9)	70 (29.5)	
Geriatrics	17 (7.2)	15 (6.3)	
Oncology	9 (3.8)	10 (4.2)	
Community hospital — no. (%)	68 (28.7)	67 (28.3)	0.9
Days at risk for <i>C. difficile</i> -associated diarrhea			0.02
Median	13	16	
Interquartile range	6–25	8–29	
No. of antibiotics received	1.9±1.1	1.3±1.3	<0.001
Any exposure to antibiotics — no. (%)	188 (79.3)	141 (59.5)	<0.001
Cephalosporins	115 (48.5)	65 (27.4)	<0.001
Clindamycin	19 (8.0)	6 (2.5)	0.007
Fluoroquinolones	128 (54.0)	75 (31.6)	<0.001
Chemotherapy — no. (%)	17 (7.2)	13 (5.5)	0.45
Proton-pump inhibitors — no. (%)	112 (47.3)	111 (46.8)	0.92
Histamine H_2 -blockers — no. (%)	47 (19.8)	47 (19.8)	1.0
Enteral feeding — no. (%)	44 (18.6)	28 (11.8)	0.04

* Plus-minus values are means ±SD.

† Scores for the Charlson index can range from 0 to 37, with higher scores indicating more coexisting conditions.

an 18-bp deletion (Fig. 2C of the Supplementary Appendix).

ASSOCIATION WITH PULSOVARS, BINARY TOXIN GENES, AND PARTIAL DELETIONS OF THE *tcdC* GENE

Severe *C. difficile*-associated diarrhea was observed in 20 of 129 patients with the predominant pulsovar (15.5 percent), as compared with 2 of 28 patients with other pulsovars (7.1 percent, $P=0.37$). However, severe *C. difficile*-associated diarrhea was observed in 22 of 132 patients with isolates that had both binary toxin genes and a partial deletion of the *tcdC* gene (16.7 percent), as compared with 0 of 25 patients with isolates that had neither binary toxin genes nor a partial deletion of the *tcdC* gene ($P=0.03$). The institution that submitted the majority of *C. difficile* isolates did not differ significantly from the other institutions in terms of its attributable mortality rate ($P=0.71$), its colectomy rate ($P=1.0$), or its patients' need for intensive care ($P=0.88$).

ANTIMICROBIAL SUSCEPTIBILITY

We assessed the antimicrobial susceptibility of 47 isolates belonging to the predominant pulsovar and 12 isolates representing the other observed PFGE patterns. All isolates were susceptible to metronidazole and vancomycin, with minimal 90 percent inhibitory concentrations of 0.5 and 1.0 μg per milliliter, respectively. All isolates of the predominant pulsovar were resistant to ciprofloxacin, moxifloxacin, gatifloxacin, and levofloxacin (minimal inhibitory concentrations of at least 32 μg per milliliter) and susceptible to clindamycin. Of the remaining 12 isolates with other PFGE patterns, all were resistant to ciprofloxacin, 4 (33.3 percent) were resistant to moxifloxacin and gatifloxacin, and 6 (50.0 percent) were resistant to levofloxacin and clindamycin. Among the three isolates that belonged to the nonpredominant pulsovar and had binary toxin genes and a partial deletion of the *tcdC* gene, all three were resistant to ciprofloxacin, but only one was resistant to moxifloxacin, gatifloxacin, and levofloxacin.

DISCUSSION

We describe a simultaneous outbreak of severe *C. difficile*-associated diarrhea with high morbidity and mortality at multiple institutions. In a 1997 survey of 18 Canadian institutions, the mean inci-

dence of *C. difficile*-associated diarrhea was 6 per 1000 admissions, and 1.5 percent of affected patients died as a direct or indirect result of this complication.^{17,18} In our study, the overall incidence was approximately four times that described in the Canadian survey.¹⁷ This increase was unlikely to be due to a reporting artifact, because laboratory and surveillance methods had not changed among the participating institutions. We found that the age-specific incidence of *C. difficile*-associated diarrhea increased markedly after the age of 50 years and the attributable mortality rate increased after the age of 60 years. This is consistent with a Swedish study that showed an age-related increase in the incidence of positive assays for *C. difficile* toxin.¹⁹

The PFGE results indicate that a single predominant strain was circulating among the participating Quebec institutions. This strain had the same patterns on PFGE and restriction-endonuclease analyses as an epidemic strain recently found in the United States and Europe.^{20,21} This strain may have been imported into Quebec or may have arisen as a result of a mutation of a previously circulating strain.

Table 4. Multivariate Model of the Risk of *Clostridium difficile*-Associated Diarrhea According to the Use of Antibiotics among Case Patients, as Compared with Matched Controls, January 11 through June 26, 2004.*

Antibiotic	Odds Ratio	95% Confidence Interval
Any cephalosporin	3.8	2.2–6.6
First-generation	2.4	1.2–4.6
Second-generation	6.0	2.1–17.5
Third-generation	3.0	1.4–6.8
Any fluoroquinolones	3.9	2.3–6.6
Ciprofloxacin	3.1	1.8–5.4
Gatifloxacin or moxifloxacin	3.4	1.5–7.7
Levofloxacin	0.6	0.2–1.9
Clindamycin	1.6	0.5–4.8
Aminoglycosides	0.7	0.3–1.9
Macrolides	1.3	0.6–2.9
Intravenous vancomycin	1.3	0.5–3.1
Penicillins combined with β -lactamase inhibitor	1.2	0.7–2.3
Penicillins	0.7	0.3–2.9
Carbapenems	1.4	0.3–6.3

* Values were adjusted for the use of all other antibiotics, age, sex, number of days at risk for *C. difficile*-associated diarrhea, the Charlson index, and the use of chemotherapy, proton-pump inhibitors, histamine H₂-blockers, and enteral feeding.

Reports have suggested that *C. difficile*-associated diarrhea is evolving into a more severe disease, but the link between the organism's potential virulence factors and disease severity has not been clearly established.^{10,22-24} The contribution of the binary toxin to virulence is not well defined. The attributable mortality rate of 6.9 percent in our study is higher than the rate of 0.5 to 5.5 percent reported in other studies and may reflect increased virulence of the predominant strain.^{3,4,18,25} Our study of isolates demonstrated that the presence of binary toxin genes is closely associated with partial deletions in the *tdc* gene and that severe *C. difficile*-associated diarrhea is significantly associated with these two putative virulence factors. In addition, this genotype has been associated with levels of toxins A and B that are 16 to 23 times than those found in control strains.²⁰ The presence of these two factors may act synergistically to result in severe *C. difficile*-associated diarrhea.

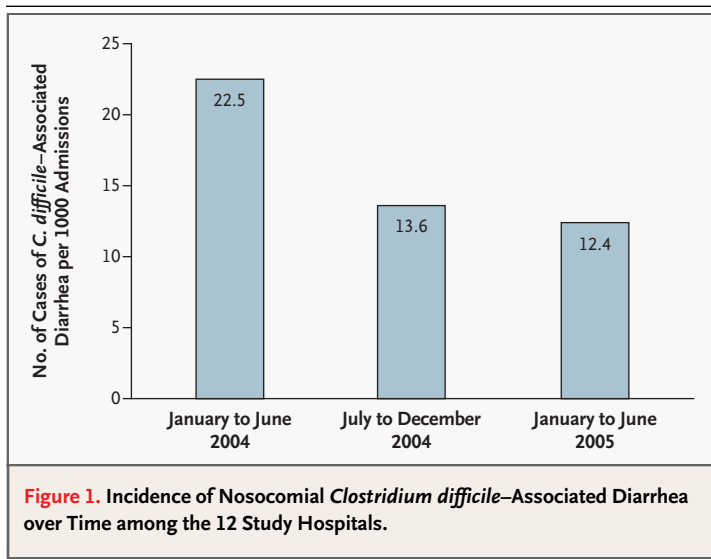
Exposure to antibiotics is the chief precipitant of *C. difficile*-associated diarrhea. Fluoroquinolones have been associated with an increased risk of *C. difficile*-associated diarrhea, which was corroborated in our study.^{24,26-28} Use of the newer fluoroquinolones among our patients may have promoted the outbreak of this fluoroquinolone-resistant strain, similar to the epidemic of a clindamycin-resistant strain among patients who received clindamycin.²⁹ Our predominant strain was susceptible to clindamycin, and clindamycin was not an independent risk factor for *C. difficile*-associated diarrhea in our study.

Transmission of this predominant strain among hospitals could have occurred as the result of transfers of colonized or infected patients or, perhaps, from colonized health care workers who worked at multiple institutions. In these institutions, the majority of rooms have multiple beds, with shared toilets, facilitating transmission within hospitals. It has been demonstrated that patients housed in single rooms have a lower incidence of *C. difficile*-associated diarrhea than patients accommodated in double rooms.³⁰

Our study has a number of limitations. The outcomes were measured 30 days after the first diagnosis of *C. difficile*-associated diarrhea, which would result in underestimates of the rates of attributable mortality, colectomy, and intensive care related to the condition. We assessed neither the severity of illness nor the presence of coexisting conditions at admission in our population. The severity of illness has been shown to be an important predictor of mortality for a variety of conditions and is also a predictor of the acquisition of *C. difficile*.^{31,32} In addition, we recorded the use of antibiotics in hospitalized patients within six weeks before the diagnosis of *C. difficile*-associated diarrhea but did not track the use of antibiotics before this period or before hospitalization. Finally, we did not have isolates available from all patients. However, the isolates were consecutively collected at each institution, making it unlikely that they represented a biased sample. Although isolates were overrepresented from one institution, the rate of severe *C. difficile*-associated diarrhea at this institution was not significantly different from the rates at the other institutions.

Coincident with the recognition of the multi-institutional outbreak of *C. difficile*-associated diarrhea in June 2004, major infection-control measures were implemented to curb the spread of *C. difficile*. The incidence of *C. difficile*-associated diarrhea in the study institutions subsequently decreased to 12.4 per 1000 admissions (Fig. 1).

In summary, we have identified a predominant strain of *C. difficile* associated with high rates of severe disease in a number of hospitals in Quebec. Resistance to fluoroquinolones may have selected for the spread of this organism, and the presence of binary toxin genes and a partial deletion of the *tdc* regulatory gene may confer increased virulence, leading to the observed high rates of morbidity and mortality. This outbreak illustrates that known pathogens can change their behavior and emerge as new threats.



Dr. Brassard is supported by the Canadian Institutes of Health Research.

Dr. Libman reports having received lecture fees from Bayer HealthCare and Sanofi-Aventis; and Dr. Miller, lecture fees from ActivBiotics and Genzyme and consulting fees from ActivBiotics and the U.S. Food and Drug Administration.

We are indebted to all the infection-control practitioners at the participating institutions for performing the surveillance for nosocomial *C. difficile*-associated diarrhea; to the quality-management and medical-records personnel for providing the statistics on admis-

sions and diagnosis-related groups; to the pharmacy department for providing information on medication use; to Dr. Xiaolan Zhang and Ms. Milena Crosato for technical assistance; to Mr. Andrei Brennan and Drs. Daniela Di Iorio, Annie-Claude Labbé, Louise Dion, and Isabelle Alarie for reviewing several patients' charts; to Mr. Raun De Souza for assistance in the preparation of the manuscript; to Dr. Marcel Behr for reviewing the manuscript; to Dr. Anthony Harris for advice on the methods used for the case-control study; and to Genome Québec and Genome Canada for their support of the sequencing activities.

REFERENCES

- Barbut F, Corthier G, Charpak Y, et al. Prevalence and pathogenicity of *Clostridium difficile* in hospitalized patients: a French multicenter study. *Arch Intern Med* 1996; 156:1449-54.
- Johnson S, Gerding DN. *Clostridium difficile*-associated diarrhea. *Clin Infect Dis* 1998; 26:1027-36.
- Jobe BA, Grasley A, Deveney KE, Deveney CW, Sheppard BC. *Clostridium difficile* colitis: an increasing hospital-acquired illness. *Am J Surg* 1995;169:480-3.
- Rubin MS, Bodenstien LE, Kent KC. Severe *Clostridium difficile* colitis. *Dis Colon Rectum* 1995;38:350-4.
- Poxton IR, McCoubrey J, Blair G. The pathogenicity of *Clostridium difficile*. *Clin Microbiol Infect* 2001;7:421-7.
- Braun V, Hundsberger T, Leukel P, Sauerborn M, von Eichel-Streiber C. Definition of the single integration site of the pathogenicity locus in *Clostridium difficile*. *Gene* 1996;181:29-38.
- Spigaglia P, Mastrantonio P. Molecular analysis of the pathogenicity locus and polymorphism in the putative negative regulator of toxin production (TcdC) among *Clostridium difficile* clinical isolates. *J Clin Microbiol* 2002;40:3470-5.
- Popoff MR, Rubin EJ, Gill DM, Boquet P. Actin-specific ADP-ribosyltransferase produced by a *Clostridium difficile* strain. *Infect Immun* 1988;56:2299-306.
- Barth H, Aktories K, Popoff MR, Stiles BG. Binary bacterial toxins: biochemistry, biology, and applications of common *Clostridium* and *Bacillus* proteins. *Microbiol Mol Biol Rev* 2004;68:373-402.
- Pépin J, Valiquette L, Alary ME, et al. *Clostridium difficile*-associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. *CMAJ* 2004; 171:466-72.
- Allen SD, Emery CL, Lyster DM. *Clostridium*. In: Murray PR, Baron EJ, Tenover JC, Tenover FC, eds. *Manual of clinical microbiology*. 8th ed. Vol. 1. Washington, D.C.: American Society of Microbiology Press, 2003:835-56.
- Fawley WN, Wilcox MH. Pulsed-field gel electrophoresis can yield DNA fingerprints of degradation-susceptible *Clostridium difficile* strains. *J Clin Microbiol* 2002;40: 3546-7.
- Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995;33:2233-9.
- Gonçalves C, Decr D, Barbut F, Burghoffer B, Petit JC. Prevalence and characterization of a binary toxin (actin-specific ADP-ribosyltransferase) from *Clostridium difficile*. *J Clin Microbiol* 2004;42:1933-9.
- Cohen SH, Tang YJ, Silva J Jr. Analysis of the pathogenicity locus in *Clostridium difficile* strains. *J Infect Dis* 2000;181:659-63.
- Clinical and Laboratory Standards Institute (formerly National Committee for Laboratory Standards). Methods for antimicrobial susceptibility testing of anaerobic bacteria. 6th ed. Wayne, Pa.: National Committee for Clinical and Laboratory Standards, 2004. Approved standard document M11-A6.
- Hyland M, Ofner-Agostini M, Miller M, Paton S, Gourdeau M, Ishak M. Nosocomial *Clostridium difficile*-associated diarrhea in Canada: results of the Canadian Nosocomial Infection Surveillance Program (CNISP) 1997 N-CDAD Prevalence Surveillance Project. *Can J Infect Dis* 2001;12:81-8.
- Miller MA, Hyland M, Ofner-Agostini M, Gourdeau M, Ishak M. Morbidity, mortality, and healthcare burden of nosocomial *Clostridium difficile*-associated diarrhea in Canadian hospitals. *Infect Control Hosp Epidemiol* 2002;23:137-40.
- Karlström O, Fryklund B, Tullus K, Burman LG. A prospective nationwide study of *Clostridium difficile*-associated diarrhea in Sweden. *Clin Infect Dis* 1998;26:141-5.
- Warny M, Pépin J, Fang A, et al. Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. *Lancet* 2005;366:1079-84.
- McDonald LC, Killgore GE, Thompson A, et al. An epidemic, toxin gene-variant strain of *Clostridium difficile*. *N Engl J Med* 2005;353:2433-41.
- Morris AM, Jobe BA, Stoney M, Sheppard BC, Deveney CW, Deveney KE. *Clostridium difficile* colitis: an increasingly aggressive iatrogenic disease? *Arch Surg* 2002;137: 1096-100.
- Dallal RM, Harbrecht BG, Boujoukas AJ, et al. Fulminant *Clostridium difficile*: an underappreciated and increasing cause of death and complications. *Ann Surg* 2002;235: 363-72.
- Muto CA, Pokrywka M, Shutt K, et al. A large outbreak of *Clostridium difficile*-associated disease with an unexpected proportion of deaths and colectomies at a teaching hospital following increased fluoroquinolone use. *Infect Control Hosp Epidemiol* 2005;26:273-80.
- Kyne L, Merry C, O'Connell B, Kelly A, Keane C, O'Neill D. Factors associated with prolonged symptoms and severe disease due to *Clostridium difficile*. *Age Ageing* 1999;28: 107-13.
- Lai KK, Melvin ZS, Menard MJ, Kotilainen HR, Baker S. *Clostridium difficile*-associated diarrhea: epidemiology, risk factors, and infection control. *Infect Control Hosp Epidemiol* 1997;18:628-32.
- McCusker ME, Harris AD, Perencevich E, Roghmann MC. Fluoroquinolone use and *Clostridium difficile*-associated diarrhea. *Emerg Infect Dis* 2003;9:730-3.
- Gaynes R, Rimland D, Killum E, et al. Outbreak of *Clostridium difficile* infection in a long-term care facility: association with gatifloxacin use. *Clin Infect Dis* 2004;38:640-5.
- Johnson S, Samore MH, Farrow KA, et al. Epidemics of diarrhea caused by a clindamycin-resistant strain of *Clostridium difficile* in four hospitals. *N Engl J Med* 1999;341: 1645-51.
- Samore MH, Venkataraman L, DeGiro-lami PC, Arbeit RD, Karchmer AW. Clinical and molecular epidemiology of sporadic and clustered cases of nosocomial *Clostridium difficile* diarrhea. *Am J Med* 1996;100:32-40.
- McFarland LV, Surawicz CM, Stamm WE. Risk factors for *Clostridium difficile* carriage and *C. difficile*-associated diarrhea in a cohort of hospitalized patients. *J Infect Dis* 1990;162:678-84.
- Green J, Wintfeld N, Sharkey P, Passman LJ. The importance of severity of illness in assessing hospital mortality. *JAMA* 1990; 263:241-6.

Copyright © 2005 Massachusetts Medical Society.

CORRECTION

A Predominantly Clonal Multi-Institutional Outbreak of *Clostridium difficile*-Associated Diarrhea with High Morbidity and Mortality

A Predominantly Clonal Multi-Institutional Outbreak of *Clostridium difficile*-Associated Diarrhea with High Morbidity and Mortality . In Table 3 on page 2446, for the category "Any exposure to antibiotics," the number (and percentage) of case patients should have read 223 (94.1) and the number (and percentage) of controls should have read 159 (67.1), rather than 188 (79.3) for case patients and 141 (59.5) for controls, as printed. On the same page, line 8 of the left-hand column should have read "94.1 percent vs. 67.1 percent," rather than "79.3 percent vs. 59.3 percent," as printed. The P value for this comparison ($P < 0.001$) remains unchanged.