ORIGINAL ARTICLE

Host and Pathogen Factors for *Clostridium difficile* Infection and Colonization

Vivian G. Loo, M.D., Anne-Marie Bourgault, M.D., Louise Poirier, M.D., François Lamothe, M.D., Sophie Michaud, M.D., M.P.H., Nathalie Turgeon, M.D., Baldwin Toye, M.D., Axelle Beaudoin, M.Sc., Eric H. Frost, Ph.D., Rodica Gilca, M.D., Ph.D., Paul Brassard, M.D., Nandini Dendukuri, Ph.D., Claire Béliveau, M.D., Matthew Oughton, M.D., Ivan Brukner, Ph.D., and Andre Dascal, M.D.

ABSTRACT

BACKGROUND

Clostridium difficile infection is the leading cause of health care–associated diarrhea, and the bacterium can also be carried asymptomatically. The objective of this study was to identify host and bacterial factors associated with health care–associated acquisition of *C. difficile* infection and colonization.

METHODS

We conducted a 15-month prospective study in six Canadian hospitals in Quebec and Ontario. Demographic information, known risk factors, potential confounding factors, and weekly stool samples or rectal swabs were collected. Pulsed-field gel electrophoresis (PFGE) was performed on *C. difficile* isolates to determine the genotype. Levels of serum antibodies against *C. difficile* toxins A and B were measured.

RESULTS

A total of 4143 patients were included in the study; 117 (2.8%) and 123 (3.0%) had health care–associated *C. difficile* infection and colonization, respectively. Older age and use of antibiotics and proton-pump inhibitors were significantly associated with health care–associated *C. difficile* infection. Hospitalization in the previous 2 months; use of chemotherapy, proton-pump inhibitors, and H_2 blockers; and antibodies against toxin B were associated with health care–associated *C. difficile* colonization. Among patients with health care–associated *C. difficile* infection and those with colonization, 62.7% and 36.1%, respectively, had the North American PFGE type 1 (NAP1) strain.

CONCLUSIONS

In this study, health care–associated *C. difficile* infection and colonization were differentially associated with defined host and pathogen variables. The NAP1 strain was predominant among patients with *C. difficile* infection, whereas asymptomatic patients were more likely to be colonized with other strains. (Funded by the Consortium de Recherche sur le *Clostridium difficile*.)

From McGill University Health Centre (V.G.L., P.B., N.D.) and Jewish General Hospital (M.O., I.B., A.D.), McGill University; Centre Hospitalier de l'Université de Montréal (A.-M.B., F.L.) and Hôpital Maisonneuve-Rosemont (L.P., C.B.), Université de Montréal; and Institut National de Santé Publique du Québec (A.-M.B.) — all in Montreal; Centre Hospitalier Universitaire (CHU) de Sherbrooke, Université de Sherbrooke, Sherbrooke, QC (S.M., A.B., E.H.F.); CHU de Québec–Hôtel-Dieu de Québec (N.T.), Université Laval (R.G.); and Institut National de Santé Publique du Québec (R.G.) — all in Quebec, QC; and Ottawa Hospital, University of Ottawa, Ottawa (B.T.) — all in Canada. Address reprint requests to Dr. Loo at the Department of Microbiology, McGill University Health Centre, 687 Pine Ave. W., Rm. L5.06, Montreal, QC H3A 1A1, Canada.

N Engl J Med 2011;365:1693-703.
Copyright © 2011 Massachusetts Medical Society.

The New England Journal of Medicine
Downloaded from nejm.org on November 5, 2011. For personal use only. No other uses without permission.
Copyright © 2011 Massachusetts Medical Society. All rights reserved.

Clostridium difficile is the leading cause of health care–associated infectious diarrhea.[1] After exposure to *C. difficile*, some patients remain asymptomatic, whereas others have illness ranging from mild diarrhea to fulminant colitis.[2] Outbreaks of *C. difficile* infection in North America and Europe have been attributed to the emergence of an epidemic strain (North American pulsed-field gel electrophoresis [PFGE] type 1 [NAP1]).[3,4]

Risk factors for *C. difficile* infection include antibiotic use, advanced age, increased severity of underlying illness, prior hospitalization, use of feeding tubes, gastrointestinal surgery, and use of proton-pump inhibitors.[5,6] Variability in host factors may explain the wide spectrum of symptoms and course. Colonization with *C. difficile* and high levels of serum antibody against *C. difficile* toxin A appear to provide protection against *C. difficile* infection.[7-9]

The best-described *C. difficile* virulence factors are toxins A and B. The genes encoding toxins A and B (*tcdA* and *tcdB*, respectively) are on the 19.6-kb so-called pathogenicity locus, along with two regulatory genes (*tcdC* and *tcdR*) and a gene (*tcdE*) encoding a protein proposed to function as a porin facilitating the release of toxins A and B.[10-13] It was initially believed that toxin A was the most important toxin in *C. difficile* infection, but studies have shown that toxin B may be the more potent of the two toxins.[14,15] In addition, a binary toxin encoded by two genes (*cdtA* and *cdtB*) has been described in *C. difficile*.[16] The *cdtB* product mediates cell-surface binding and intracellular translocation, and the product of *cdtA* disrupts actin-filament assembly; however, the clinical significance of binary toxin in *C. difficile* infection remains uncertain.[17]

The objective of this study was to examine the relationships among host risk factors, bacterial virulence, and host immunity in health care–associated *C. difficile* infection and health care–associated asymptomatic colonization with *C. difficile*.

Methods

Participating Hospitals

Six Canadian, university-affiliated hospitals participated in the study: five in Quebec and one in Ontario. The research protocol was approved by each institutional review board.

Study Population and Recruitment

From March 6, 2006, to June 25, 2007, all consecutive patients 18 years of age or older admitted on selected units were asked to participate in the study. The selected units were those with a historically high or low incidence of *C. difficile* infection. We excluded patients who had hemodynamic instability, who were receiving palliative care, who had neutropenia (an absolute neutrophil count ≤1000 per cubic millimeter), or who were unable to participate in the informed-consent process on their own behalf or represented by a surrogate. All participants gave written informed consent.

Study Definitions

C. difficile infection was defined as follows: the presence of diarrhea and a positive *C. difficile* cytotoxin assay or toxigenic culture, the presence of diarrhea without an alternative explanation and an endoscopic diagnosis of pseudomembranes, or a pathological diagnosis of *C. difficile* infection. Diarrhea was defined as three loose stools within at least one 24-hour period. Asymptomatic *C. difficile* colonization was defined as a positive stool culture for *C. difficile* in the absence of diarrhea.

Colonization or infection was considered to be health care–associated if symptoms began 72 hours or more after admission, if *C. difficile* infection was diagnosed within 4 weeks after discharge from any health care institution, or if the person with colonization or infection was a health care worker in contact with patients.

Recurrence was defined as a second episode of *C. difficile* infection within 60 days after the first episode. An episode of *C. difficile* infection occurring more than 60 days after the first event was considered a new episode.

Clinical Data

Data on demographic information, known risk factors, and potential confounding factors were collected. In particular, information about the use of various medications during the 8 weeks before, as well as during, hospitalization was collected for all patients. For patients in whom health care–associated *C. difficile* infection or colonization developed, the specific start and stop dates of these medications were also recorded to assess whether this exposure occurred before the event of either health care–associated *C. difficile* infection or colo-

The New England Journal of Medicine
Downloaded from nejm.org on November 5, 2011. For personal use only. No other uses without permission.
Copyright © 2011 Massachusetts Medical Society. All rights reserved.

nization. Patients were followed daily until ward discharge, death, or withdrawal from the study. Patients were contacted 60 days after discharge to determine whether diarrhea had developed in the interim.

Outcomes studied were recurrence of *C. difficile* infection, death, need for colectomy, and need for intensive care owing to health care–associated *C. difficile* infection. For any death, two physicians judged independently whether health care–associated *C. difficile* infection was an attributable cause, a contributory cause, or unrelated to the cause of death. In the case of a disagreement, a consensus was reached.

CLINICAL SAMPLES

Rectal swabs or stool samples for toxigenic *C. difficile* culture were obtained on admission, weekly during hospitalization, at the onset of diarrhea (if applicable), and at discharge. A rectal swab was obtained if a stool sample could not be procured on the scheduled day of sampling. Serum samples were obtained on admission for measurement of antibody levels.

LABORATORY ASSAYS

Toxigenic *C. difficile* culture was performed on stool samples or rectal swabs with the use of standard methods.[18] PFGE and assays to detect the binary toxin and the *tcdC* Δ117 deletion were performed on *C. difficile* isolates. Detection of antibodies against toxins A and B was performed with the use of purified recombinant fragments containing the carboxy terminal of toxin A (residues 1753 to 2681) and toxin B (residues 1751 to 2366) of *C. difficile*.[19] An enzyme-linked immunosorbent assay similar to that of Warny and colleagues was used.[20] For additional details, see the Laboratory Assays section in the Supplementary Appendix (available with the full text of this article at NEJM.org).

STATISTICAL ANALYSIS

Epidemiologic and molecular data were collected and interpreted independently. Eligible patients who decided not to participate and those who did participate were compared with respect to mean age and sex.

Participants were categorized into four groups, according to status with respect to *C. difficile* infection or colonization and origin of acquisition: patients with health care–associated *C. difficile* infection, patients with health care–associated *C. difficile* colonization, those with colonization at admission, and those with neither health care–associated *C. difficile* infection nor colonization. The cumulative incidences of health care–associated *C. difficile* infection and colonization were calculated with the use of competing-risks analysis.[21] The SAS software package, version 9.2 (SAS Institute), was used for all statistical analyses.

To study the association between potential risk factors and health care–associated *C. difficile* infection and colonization, we selected control patients admitted to the study units. The control group for health care–associated *C. difficile* infection comprised both patients with health care–associated *C. difficile* colonization only and patients without colonization or infection. Controls for *C. difficile* colonization had neither *C. difficile* infection nor colonization.

To ensure that case patients and control patients had similar risks of exposure to *C. difficile*, a frequency-matching approach was used that linked all affected patients and controls within each stratum defined by a combination of values for hospital and length of stay. The length of stay was defined as the time from admission until diagnosis of *C. difficile* infection or colonization (for infected and colonized patients, respectively) or discharge (for controls).

Univariate and multivariate conditional logistic-regression models were used, with health care–associated *C. difficile* infection and colonization as the outcomes. Analyses included all controls who could be matched to at least one case patient; patients without *C. difficile* infection could serve as controls for more than one patient with health care–associated *C. difficile* infection.

In analyses of health care–associated *C. difficile* infection, 9 patients with infection were excluded because there were no controls with the same length of stay in the same hospital, and another 4 were excluded because they had missing covariate information; the remaining 104 patients with health care–associated *C. difficile* infection were each matched to between 1 and 123 controls with the same length of stay in the same hospital. In analyses of health care–associated *C. difficile* colonization, 7 patients with colonization were excluded because there were no controls with the same length of stay in the same hospital, as well

The New England Journal of Medicine
Downloaded from nejm.org on November 5, 2011. For personal use only. No other uses without permission.
Copyright © 2011 Massachusetts Medical Society. All rights reserved.

as 1 patient with missing covariate information; the remaining 115 case patients were matched to between 1 and 80 controls each.

The prespecified covariates included age, sex, score on the Charlson comorbidity index,[22] status with respect to previous hospitalization, serologic data, and status with respect to medication use in the 8 weeks before hospitalization or before *C. difficile* infection or colonization. Medication use was treated as a dichotomous covariate rather than as time dependent because we had data on dates of medication use only for patients in whom health care–associated *C. difficile* infection or colonization developed. The models included antibiotic use as a single summary variable indicating exposure to any antibiotic.

An unconditional logistic-regression model with adjustment for length of stay and hospital was used to determine the association between risk factors and health care–associated *C. difficile* infection among patients with positive cultures for *C. difficile*. This strategy was chosen because of the limited numbers of study participants and matched case–control pairs among patients with positive cultures for *C. difficile*, whether they had infection or just colonization. PFGE type, *tcdC* Δ117 deletion status, and presence or absence of binary toxin were used as the genomic covariates. In this analysis, three patients with infection and one patient with colonization were excluded on the basis of missing covariate information.

RESULTS

STUDY UNITS

Each hospital had between 1 and 4 study units, for a total of 14 units: 8 general medicine, 5 general surgery, and 1 hepatobiliary. Each unit had between 23 and 49 beds. The number of admissions ranged from 549 to 1816 per year.

STUDY PATIENTS

A total of 12,304 patients were approached about participation, of whom 2802 were not eligible. Among the 9502 eligible patients, 5422 (57.1%) agreed to participate in the study. Among patients who became infected with *C. difficile* during the study, 75 were excluded because of the development of *C. difficile* infection within 72 hours after admission or within 60 days before admission or because of colonization detected on admission followed by development of infection. Six other patients with a history of *C. difficile* infection in the 60 days before admission were excluded: 3 had asymptomatic colonization and 3 had neither infection nor colonization at the time of admission. A total of 1198 of the 5422 patients (22.1%) could not be evaluated because of incomplete stool or rectal samples. In all, 4143 patients (76.4%) had complete clinical assessments and stool or rectal samples and were included in the analysis (Fig. 1).

Eligible nonparticipants were younger than participants, by 0.77 years (95% confidence interval [CI], −1.45 to −0.09), and were more likely to be women (difference of 2.6 percentage points; 95% CI, 0.6 to 4.7). Participants who could not be evaluated were older than those who could be evaluated, by 1.6 years (95% CI, 0.6 to 2.6), and were more likely to be men (difference of 1.1 percentage points; 95% CI, −2.2 to 4.4).

INCIDENCE AND OUTCOMES

Of the 4143 patients who could be evaluated, 184 (4.4%) had asymptomatic colonization at the time of unit admission, 117 (2.8%) had health care–associated *C. difficile* infection, and 123 (3.0%) had health care–associated *C. difficile* colonization (Fig. 1). The incidences of health care–associated *C. difficile* infection and colonization were 28.1 cases per 10,000 patient-days and 29.5 per 10,000 patient-days, respectively. Table 1 shows the baseline characteristics of the patients. As compared with the other groups, patients with health care–associated *C. difficile* infection tended to be older and were more likely to have been receiving antibiotics or proton-pump inhibitors within 8 weeks before or during hospitalization.

Figure 2 shows the times to health care–associated *C. difficile* infection and colonization. The time to health care–associated *C. difficile* infection was twice that of health care–associated *C. difficile* colonization. For example, colonization had occurred in 2.5% of patients at 7 days, whereas infection had occurred in 2.5% of patients at 14 days.

Among the 117 patients with health care–associated *C. difficile* infection, 14 deaths occurred within 60 days after the diagnosis of *C. difficile* infection, for a crude mortality rate of 12.0%. *C. difficile* infection was the attributable cause of death in 2 of the 117 patients (1.7%), contributed to the cause of death in 6 patients (5.1%), and was unrelated to the cause of death in the remaining 6 patients (5.1%). Because of *C. difficile* infection, 1 of the 117 patients (0.8%) required intensive care. None of the patients required colectomy. Twenty-nine of the 117 infected patients (24.8%) had a

The New England Journal of Medicine
Downloaded from nejm.org on November 5, 2011. For personal use only. No other uses without permission.
Copyright © 2011 Massachusetts Medical Society. All rights reserved.

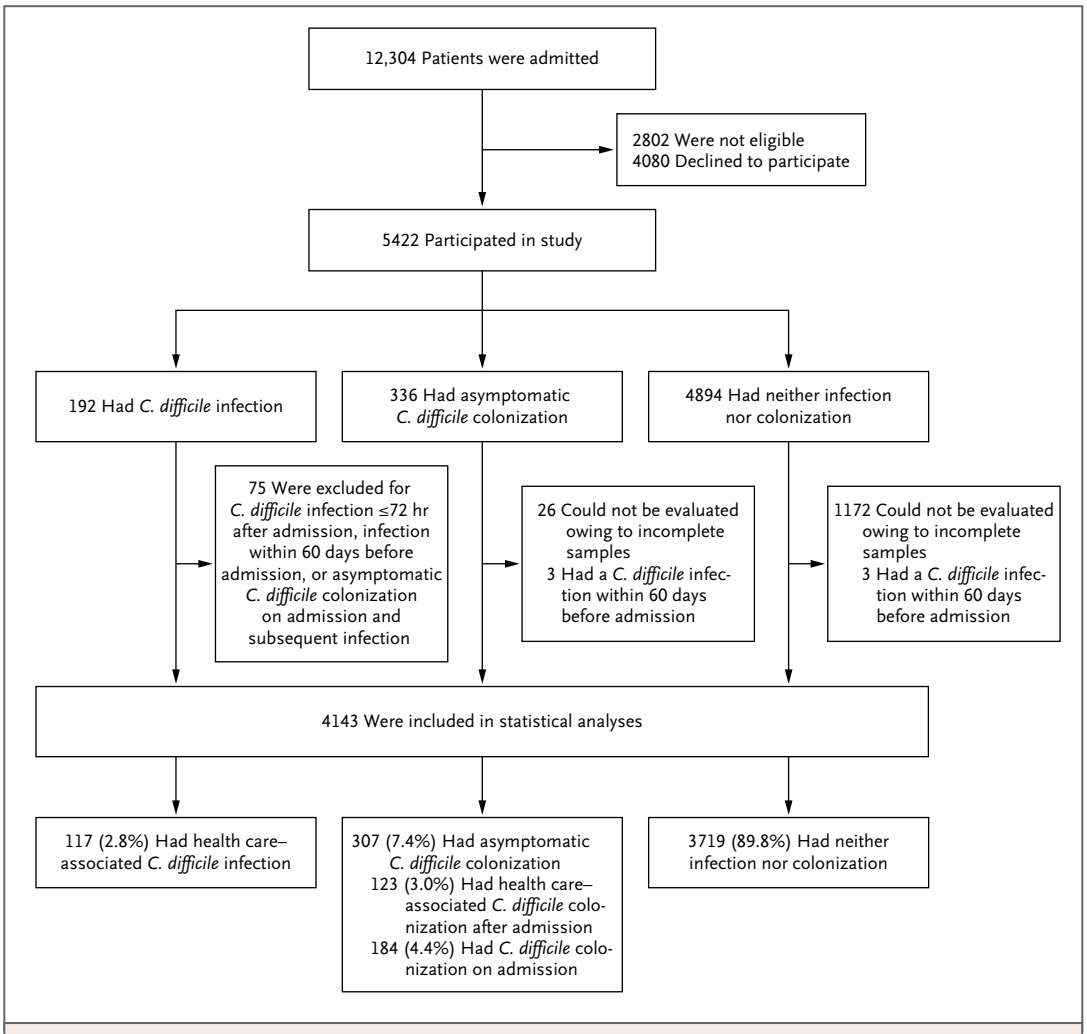

Figure 1. Enrollment and Follow-up of the Study Patients.

recurrence, with 21 (17.9%) having one recurrence, 6 (5.1%) having two recurrences, and 2 (1.7%) having more than two recurrences.

Among the 60 excluded patients who became infected with *C. difficile* within 72 hours after admission, 42 (70.0%) had been hospitalized during the previous 3-month period, 2 (3.3%) had been admitted from a rehabilitation center and long-term care, and 16 (26.7%) had either never been hospitalized or had been hospitalized more than 12 months previously.

C. DIFFICILE ISOLATES

Laboratory analyses were performed on 383 available isolates. Patients with health care–associated *C. difficile* infection were more likely to be infected with NAP1 strains, strains that contained the binary toxin, or strains of the *tcdC* Δ117 genotype than were patients with *C. difficile* colonization (Table 1). Stool samples obtained from 3 patients with *C. difficile* infection had nontoxigenic strains but had positive results on a direct stool cytotoxin assay sent in parallel for routine testing. Isolates were available for 119 (96.7%) of 123 patients with health care–associated *C. difficile* colonization, 30 (25.2%) of whom had nontoxigenic strains (neither NAP1 nor NAP2).

RISK FACTORS FOR HEALTH CARE–ASSOCIATED *C. DIFFICILE* INFECTION OR COLONIZATION

Older age, use of antibiotics, and use of proton-pump inhibitors were all significant risk factors for health care–associated *C. difficile* infection (Table 2). Hospitalization in the previous 2 months;

The New England Journal of Medicine
Downloaded from nejm.org on November 5, 2011. For personal use only. No other uses without permission.
Copyright © 2011 Massachusetts Medical Society. All rights reserved.

Table 1. Baseline Characteristics of the Study Patients and Characteristics of Samples and Pathogens, According to Clinical Status.*

Variable	Health Care–Associated *C. difficile* Infection (N=117)	Health Care–Associated *C. difficile* Colonization (N=123)	*C. difficile* Colonization on Admission (N=184)	Neither *C. difficile* Infection nor Colonization (N=3719)
Age — yr	67.4±14.1†	63.3±14.7	63.4±14.8	62.1±15.6
Male sex — no. (%)	57 (48.7)	62 (50.4)	94 (51.1)	1871 (50.3)
Score on Charlson comorbidity index‡	2.4±3.9	2.6±2.7	2.3±2.2	1.9±2.2
Hospitalization before current admission — no. (%)§				
Never or >12 mo before	53 (45.3)	59 (48.0)	66 (35.9)	2263 (60.9)
2–12 mo before	34 (29.1)	35 (28.5)	68 (37.0)	925 (24.9)
<2 mo before	30 (25.6)†	29 (23.6)¶	50 (27.2)	530 (14.3)
Medication use — no. (%)‖				
Antibiotic	111 (94.9)†	102 (82.9)¶	122 (66.3)	2612 (70.2)
Chemotherapy	6 (5.1)	9 (7.3)	7 (3.8)	159 (4.3)
Proton-pump inhibitor	74 (63.2)†	62 (50.4)¶	90 (48.9)	1209 (32.5)
H_2 blocker	24 (20.5)	32 (26.0)¶	34 (18.5)	620 (16.7)
Glucocorticoid	13 (11.1)	16 (13.0)	20 (10.9)	262 (7.0)
NSAID	72 (61.5)	82 (66.7)	100 (54.4)	2176 (58.5)
Nasogastric tube — no. (%)‖	15 (12.8)	20 (16.3)	10 (5.4)	437 (11.8)
Samples available for serologic analysis — no./total no. (%)	113/117 (96.6)	122/123 (99.2)	176/184 (95.7)	3559/3719 (95.7)
Positive for antibody against toxin A	17/113 (15.0)	25/122 (20.5)	32/176 (18.2)	607/3559 (17.1)
Positive for antibody against toxin B	34/113 (30.1)	45/122 (36.9)¶	59/176 (33.5)	902/3559 (25.3)
Samples available for isolate analysis — no./total no. (%)	83/117 (70.9)	119/123 (96.7)	181/184 (98.4)	Not applicable
PFGE type				Not applicable
NAP1	52/83 (62.7)	43/119 (36.1)	24/181 (13.3)	
NAP2	1/83 (1.2)	7/119 (5.9)	8/181 (4.4)	
Neither	30/83 (36.1)	69/119 (58.0)	149/181 (82.3)	
Binary toxin				Not applicable
Positive	55/83 (66.3)	50/119 (42.0)	30/181 (16.6)	
Negative	27/83 (32.5)	67/119 (56.3)	149/181 (82.3)	
Discordant**	1/83 (1.2)	2/119 (1.7)	2/181 (1.1)	
tcdC Δ117 Genotype	50/83 (60.2)	45/119 (37.8)	25/181 (13.8)	Not applicable

* Plus–minus values are means ±SD. NAP1 denotes North American PFGE (pulsed-field gel electrophoresis) type 1, NAP2 North American PFGE type 2, and NSAID nonsteroidal antiinflammatory drug.

† P<0.05 for the comparison with patients who had health care–associated *C. difficile* colonization plus patients who had neither *C. difficile* infection nor colonization, calculated with the use of univariate conditional logistic-regression modeling.

‡ The score on the Charlson comorbidity index reflects the number of coexisting conditions, weighted according to their relative effects on mortality, with scores ranging from 0 to 33 and higher scores indicating a greater burden of illness.

§ Information about prior hospitalization was unknown for 1 of the 3719 patients without health care–associated *C. difficile* infection or colonization.

¶ P<0.05 for the comparison with neither *C. difficile* infection nor colonization, calculated with the use of univariate conditional logistic-regression modeling.

‖ Medication use and nasogastric-tube use were defined as use within 8 weeks before hospitalization or during hospitalization but before health care–associated *C. difficile* infection or colonization.

** Discordant refers to discordant results regarding the presence of *cdtA* or *cdtB* genes or both across repeated tests.

use of chemotherapy, proton-pump inhibitors, or H_2 blockers; and the presence of antibodies against toxin B were significant risk factors for health care–associated *C. difficile* colonization.

Among patients with positive cultures for *C. difficile*, patients with health care–associated *C. difficile* infection were more likely than those with health care–associated *C. difficile* colonization to be older,

The New England Journal of Medicine
Downloaded from nejm.org on November 5, 2011. For personal use only. No other uses without permission.
Copyright © 2011 Massachusetts Medical Society. All rights reserved.

to have used antibiotics or proton-pump inhibitors, and to have the NAP1 strain (Table 3). Two other multivariate logistic-regression models were studied that included the same variables except that *tcdC* Δ117 genotype or binary toxin was included instead of NAP1 strain, but the model with NAP1 as the genomic variable provided a better fit with the data and had a more favorable discriminatory value (i.e., a higher concordance [C] statistic) (data not shown).

DISCUSSION

Health care–associated *C. difficile* infection and health care–associated *C. difficile* colonization were differentially associated with defined host and pathogen variables. Older age, use of antibiotics, and use of proton-pump inhibitors were significantly associated with health care–associated *C. difficile* infection, whereas previous hospitalization, chemotherapy, use of proton-pump inhibitors or H_2 blockers, and antibodies against toxin B were associated with health care–associated *C. difficile* colonization. Patients with health care–associated *C. difficile* infection were more likely to be infected with the NAP1 strain than were patients with health care–associated *C. difficile* colonization.

Our study not only confirms the finding in other studies that older age is a risk factor for health care–associated *C. difficile* infection but also provides a quantitative estimate of the association.[4,23] For every additional year of age after age 18, the risk of health care–associated *C. difficile* infection increases by approximately 2%. Use of antibiotics or proton-pump inhibitors was also found to be a risk factor for health care–associated *C. difficile* infection.[4-6,24] The incidence of *C. difficile* infection might be decreased if use of these medications were reduced. We measured levels of antibodies against toxins A and B at the time of admission and did not find a significant association between these levels and subsequent health care–associated *C. difficile* infection; neither did Kyne and colleagues,[9] although they also measured antibody levels serially during hospitalization and found that patients with higher IgG antibody levels against toxin A after the acquisition of *C. difficile* are more likely to become asymptomatic carriers and less likely to become infected with *C. difficile* than are patients with lower IgG antibody levels. Other studies have examined levels of antibodies against toxin A within a certain period before or after infection and colonization but not at the time of admission, and therefore they are not comparable to our study.[20,25]

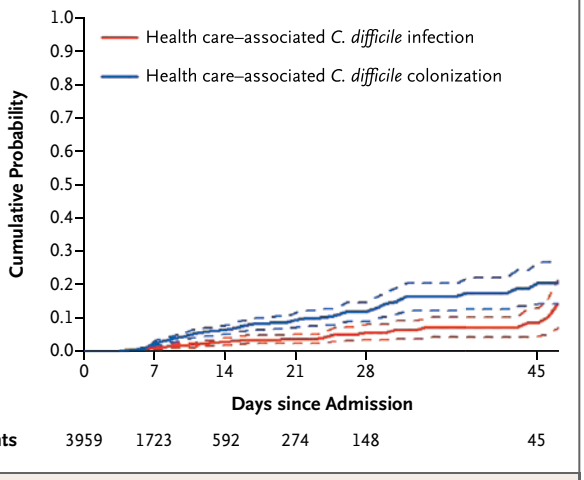

Figure 2. Times to Health Care–Associated *Clostridium difficile* Infection and Colonization during Hospitalization.

Analyses of the cumulative probability of *C. difficile* infection or colonization excluded the 184 patients with *C. difficile* colonization on admission. The dashed lines indicate 95% confidence intervals.

The factors we found to be associated with health care–associated *C. difficile* colonization were previous hospitalization; use of chemotherapy, proton-pump inhibitors, or H_2 blockers; and the presence of antibodies against toxin B at the time of admission. Previous hospitalization suggests previous exposure to *C. difficile* and possibly the subsequent development of immunity. Chemotherapy, proton-pump inhibitors, and H_2 blockers may disrupt the bowel flora and allow for *C. difficile* colonization. Antibodies against toxin B may permit colonization by *C. difficile* but prevent infection. Antibodies against toxin A were not significantly associated with health care–associated *C. difficile* colonization. This result is supported by a study of *C. difficile* infection showing that toxin B is essential, and toxin A is less important, for virulence[15]; other studies have shown that *C. difficile* strains that are negative for toxin A but positive for toxin B can cause disease.[26,27] In addition, low levels of serum antibodies against the receptor-binding epitope domain of toxin B have been significantly associated with recurrent disease.[28] Hence, antibodies against toxin B may have protective effects and may be a potential target for vaccine development.

Bacterial factors also affected outcomes in our study. Patients with health care–associated *C. difficile* infection were more likely to have the NAP1 strain than were patients with health care–associ-

The New England Journal of Medicine
Downloaded from nejm.org on November 5, 2011. For personal use only. No other uses without permission.
Copyright © 2011 Massachusetts Medical Society. All rights reserved.

Table 2. Odds Ratios for Health Care–Associated *Clostridium difficile* Infection and Colonization According to Various Patient and Pathogen Characteristics.*

Variable	Odds Ratio (95% CI)	
	Health Care–Associated *C. difficile* Infection	Health Care–Associated *C. difficile* Colonization
Age — per increase of 1 yr	1.02 (1.00–1.04)	1.00 (0.99–1.02)
Score on Charlson comorbidity index — per unit	1.01 (0.93–1.10)	1.02 (0.95–1.10)
Male sex vs. female sex	0.98 (0.64–1.49)	1.11 (0.75–1.64)
Hospitalization before current admission		
Never or >12 mo before	Reference	Reference
2–12 mo before	1.25 (0.76–2.07)	1.19 (0.74–1.90)
<2 mo before	1.61 (0.94–2.75)	2.18 (1.31–3.61)
Colonization with *C. difficile* ≤3 days before health care–associated infection	1.32 (0.57–3.02)	NA
Use of nasogastric tube†	1.28 (0.56–2.92)	0.81 (0.37–1.73)
Medication use†		
Antibiotic	5.25 (2.15–12.82)	1.04 (0.61–1.78)
Chemotherapy	1.33 (0.49–3.65)	2.37 (1.09–5.14)
Proton-pump inhibitor	2.64 (1.71–4.09)	1.71 (1.15–2.53)
H_2 blocker	0.98 (0.55–1.73)	2.14 (1.24–3.70)
Glucocorticoid	0.97 (0.48–1.97)	1.33 (0.72–2.45)
NSAID	0.85 (0.55–1.30)	1.21 (0.79–1.84)
Serologic analysis		
Positive for antibody against toxin A vs. negative	0.72 (0.41–1.29)	1.02 (0.62–1.67)
Positive for antibody against toxin B vs. negative	1.27 (0.80–2.02)	1.75 (1.15–2.66)

* Odds ratios were calculated with the use of conditional logistic-regression analysis of patients with health care–associated *C. difficile* infection or colonization as compared with matched controls. There were 104 patients with infection and 1989 controls (for a total of 2093 patients) and 115 patients with colonization and 1425 controls (for a total of 1540 patients). For analysis of infection, controls were selected from patients admitted to the study units who had colonization only or neither colonization nor infection; for analysis of colonization, controls were selected from patients who had neither infection nor colonization. NA denotes not applicable.

† Medication use and nasogastric-tube use were defined as use within 8 weeks before hospitalization or during hospitalization but before health care–associated *C. difficile* infection or colonization.

ated *C. difficile* colonization only. We found that the NAP1 strain was an independent risk factor for health care–associated *C. difficile* infection after taking potential confounders into account. The NAP1 strain is postulated to be more virulent than others because of a deletion in the *tcdC* gene leading to increased toxin A and B production.[29] Colonization with a non-NAP1 strain may result in the development of antibodies against toxin B that then confer protection against acquisition of the NAP1 strain. Two studies have shown that colonization with nontoxigenic or toxigenic *C. difficile* strains is associated with a decreased risk of *C. difficile* infection, but the effect of antibodies was not studied.[8,30]

The incidence of health care–associated *C. difficile* colonization was approximately 29 cases per 10,000 patient-days in our study, which was similar to the incidence of health care–associated *C. difficile* infection. As compared with previous studies, our study showed a higher incidence of health care–associated *C. difficile* infection and a lower incidence of health care–associated *C. difficile* colonization; therefore, the ratio of infection to colonization was higher than that in previous studies.[7,31] McFarland and colleagues studied 399 patients, of whom 52 (13%) had health care–associated *C. difficile* colonization and 31 (8%) had health care–associated *C. difficile* infection.[31] One strain accounted for 28% of the isolates. Johnson and

The New England Journal of Medicine
Downloaded from nejm.org on November 5, 2011. For personal use only. No other uses without permission.
Copyright © 2011 Massachusetts Medical Society. All rights reserved.

Table 3. Odds Ratios for Health Care–Associated *Clostridium difficile* Infection among Study Patients Who Had Positive Cultures for *C. difficile*, According to Various Patient and Pathogen Characteristics and Type of Analysis.*

Variable	Odds Ratio (95% CI)	
	Univariate Analysis	Multivariate Analysis
Age — per increase of 1 yr	1.03 (1.00–1.05)	1.04 (1.01–1.07)
Charlson comorbidity index score — per unit	1.01 (0.92–1.10)	0.99 (0.90–1.09)
Male sex vs. female sex	0.91 (0.49–1.67)	1.07 (0.50–2.29)
Hospitalization before current admission		
Never or >12 mo before	Reference	Reference
2–12 mo before	1.23 (0.60–5.52)	1.18 (0.52–2.67)
<2 mo before	1.15 (0.53–2.50)	1.40 (0.53–3.69)
Use of nasogastric tube†	0.62 (0.18–2.06)	1.59 (0.35–7.19)
Medication use†		
Antibiotic	6.24 (1.82–21.44)	6.67 (1.76–25.31)
Chemotherapy	0.88 (0.25–3.09)	0.99 (0.23–4.24)
Proton-pump inhibitor	1.65 (0.88–3.09)	2.16 (1.03–4.56)
H_2 blocker	0.48 (0.21–1.09)	0.55 (0.21–1.49)
Glucocorticoid	1.05 (0.43–2.58)	0.95 (0.33–2.72)
NSAID	0.82 (0.43–1.58)	0.90 (0.41–1.98)
Serologic analysis		
Positive for antibody against toxin A vs. negative	0.57 (0.24–1.35)	0.47 (0.17–1.30)
Positive for antibody against toxin B vs. negative	0.75 (0.39–1.43)	0.93 (0.43–2.03)
PFGE type		
NAP1 vs. non-NAP1	3.84 (1.87–7.92)	3.90 (1.69–8.99)

* Odds ratios were calculated with the use of univariate or multivariate conditional logistic-regression analysis of cases of health care–associated *C. difficile* infection as compared with cases of health care–associated *C. difficile* colonization. The multivariate model included all the variables shown and used data from 80 patients with infection and 118 controls (for a total of 198 patients with positive cultures). The univariate model included data from 83 patients with infection and 119 controls. All analyses were adjusted for length of stay and hospital center.

† Medication use and nasogastric-tube use were defined as use within 8 weeks before hospitalization or during hospitalization but before health care–associated *C. difficile* infection or colonization.

colleagues studied 282 patients, of whom 51 (18%) had health care–associated *C. difficile* colonization and 9 (3%) had health care–associated *C. difficile* infection, with either restriction-endonuclease (REA) type B or REA type B2 accounting for all cases.[7]

The differences between these two studies and ours can be explained by several factors. The NAP1 strain was predominant in our study, accounting for approximately 63% of isolates among patients with health care–associated *C. difficile* infection. The NAP1 strain may be more likely than other strains to cause symptomatic disease. Also, the incidence of health care–associated *C. difficile* colonization in our study is likely to be underestimated because the proportion of incomplete stool or rectal samples may have been higher among patients with asymptomatic health care–associated *C. difficile* colonization than among those with symptomatic infection. Since we did not perform stool-specimen culture for asymptomatic patients at 60 days after discharge, we may have underestimated the incidence of health care–associated *C. difficile* colonization relative to infection. Finally, admission criteria and case severity may have differed substantially between our study and the prior studies.

The time to health care–associated *C. difficile* infection was twice the time to health care–associated *C. difficile* colonization. A possible explanation is that both toxigenic and nontoxigenic strains colonize patients. Many of the toxigenic strains do

The New England Journal of Medicine
Downloaded from nejm.org on November 5, 2011. For personal use only. No other uses without permission.
Copyright © 2011 Massachusetts Medical Society. All rights reserved.

not cause *C. difficile* infection because the patient has an appropriate anamnestic antibody response. Therefore, for a given *C. difficile* exposure, it is likely that colonization will result rather than infection.

Our study has several limitations. First, the participation rate of 57% was lower than anticipated, and there was a large number of patients who could not be evaluated because of incomplete laboratory samples. The patients with and those without data included in the analysis differed significantly in age; however, the difference (1.6 years) was small and of minimal clinical importance. The absence of clinically significant differences in baseline characteristics between participants and nonparticipants suggests that other confounding factors were also distributed evenly between the two groups, and no major bias was introduced. Second, we did not perform cultures of environmental samples or skin samples from the hands of personnel — two potential sources of health care–associated *C. difficile* infection and colonization in patients. Finally, our findings are limited to hospitalized patients and may not be applicable to patients with community-associated *C. difficile* infection.

In conclusion, our study shows differential effects of age, medication use, and host immunity and pathogen variables on health care–associated *C. difficile* infection and health care–associated *C. difficile* colonization. The findings add to the understanding of *C. difficile* infection and colonization and have implications for prevention and therapy.

Supported by the Consortium de Recherche sur le *Clostridium difficile*; the consortium consists of the following partners: Fonds de la Recherche en Santé du Québec, Canadian Institutes of Health Research, Ministère de la Santé et des Services Sociaux du Québec, Institut National de Santé Publique du Québec, Health Canada, Centre Hospitalier de l'Université de Montréal, McGill University Health Centre, CHU de Québec, and CHU de Sherbrooke.

Dr. Loo reports receiving consulting fees from Merck; Dr. Turgeon, receiving lecture fees from Merck; Dr. Toye, receiving lecture fees from Pfizer Canada and owning stock in Spartan Biosciences; Dr. Oughton, receiving consulting fees from Cubist Pharmaceuticals; and Dr. Dascal, receiving grant support from and owning stock in Cepheid and receiving grant support from Genome Canada. No other potential conflict of interest relevant to this article was reported.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank all the patients who agreed to participate in the study; all the research assistants and technologists at the participating institutions for performing patient enrollment, data collection, and specimen collection and processing; Dr. Marcel Behr for reviewing a draft of the manuscript; Dr. Ken Dewar and the McGill University Genome Quebec Innovation Centre for performing DNA sequencing; Mr. Ian Schiller and Ms. Sarah Vahey for assisting with data analysis; Ms. Luciana Porfilio for assisting with the preparation of the manuscript; and bioMérieux France for providing purified recombinant toxins.

REFERENCES

1. Barbut F, Corthier G, Charpak Y, et al. Prevalence and pathogenicity of *Clostridium difficile* in hospitalized patients. Arch Intern Med 1996;156:1449-54.
2. Johnson S, Gerding DN. *Clostridium difficile*-associated diarrhea. Clin Infect Dis 1998;26:1027-36.
3. McDonald LC. *Clostridium difficile*: responding to a new threat from an old enemy. Infect Control Hosp Epidemiol 2005; 26:672-5.
4. Loo VG, Poirier L, Miller M, et al. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*–associated diarrhea with high morbidity and mortality. N Engl J Med 2005;353:2442-9. [Erratum, N Engl J Med 2006;354:2200.]
5. Bignardi GE. Risk factors for *Clostridium difficile* infection. J Hosp Infect 1998; 40:1-15.
6. Howell MD, Novack V, Grgurich P, et al. Iatrogenic gastric acid suppression and the risk of nosocomial *Clostridium difficile* infection. Arch Intern Med 2010;170: 784-90.
7. Johnson S, Clabots CR, Olson MM, Linn FV, Peterson LR, Gerding DN. Nosocomial *Clostridium difficile* colonisation and disease. Lancet 1990;336:97-100.
8. Shim JK, Johnson S, Samore MH, Bliss DZ, Gerding DN. Primary symptomless colonisation by *Clostridium difficile* and decreased risk of subsequent diarrhoea. Lancet 1998;351:633-6.
9. Kyne L, Warny M, Qamar A, Kelly CP. Asymptomatic carriage of *Clostridium difficile* and serum levels of IgG antibody against toxin A. N Engl J Med 2000;342: 390-7.
10. Cohen SH, Tang YJ, Silva J Jr. Analysis of the pathogenicity locus in *Clostridium difficile* strains. J Infect Dis 2000;181:659-63.
11. Hammond GA, Johnson JL. The toxigenic element of *Clostridium difficile* strain VPI 10463. Microb Pathog 1995;19:203-13.
12. 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.
13. Tan KS, Wee BY, Song KP. Evidence for holin function of *tcdE* gene in the pathogenicity of *Clostridium difficile*. J Med Microbiol 2001;50:613-9.
14. Drudy D, Fanning S, Kyne L. Toxin A-negative, toxin B-positive *Clostridium difficile*. Int J Infect Dis 2007;11:5-10.
15. Lyras D, O'Connor JR, Howarth PM, et al. Toxin B is essential for virulence of *Clostridium difficile*. Nature 2009;458:1176-9.
16. Popoff MR, Rubin EJ, Gill MD, Boquet P. Actin-specific ADP-ribosyltransferase produced by a *Clostridium difficile* strain. Infect Immun 1988;56:2299-306.
17. 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.
18. Clabots CR, Gerding SJ, Olson MM, Peterson LR, Gerding DN. Detection of asymptomatic *Clostridium difficile* carriage by an alcohol shock procedure. J Clin Microbiol 1989;27:2386-7.
19. Letourneur O, Ottone S, Delauzun V, Bastide M-C, Foussadier A. Molecular cloning, overexpression in *Escherichia coli*, and purification of 6x his-tagged C-terminal domain of *Clostridium difficile* toxins A and B. Protein Expr Purif 2003;31:276-85.
20. Warny M, Vaerman J-P, Avesani V, Delmee M. Human antibody response to *Clostridium difficile* toxin A in relation to clinical course of infection. Infect Immun 1994;62:384-9.
21. Kim HT. Cumulative incidence in

The New England Journal of Medicine
Downloaded from nejm.org on November 5, 2011. For personal use only. No other uses without permission.
Copyright © 2011 Massachusetts Medical Society. All rights reserved.

competing risks data and competing risks regression analysis. Clin Cancer Res 2007; 13:559-65.
22. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987;40:373-83.
23. Miller M, Gravel D, Mulvey M, et al. Health care-associated *Clostridium difficile* infection in Canada: patient age and infecting strain type are highly predictive of severe outcome and mortality. Clin Infect Dis 2010;50:194-201.
24. Dial S, Alrasadi K, Manoukian C, Huang A, Menzies D. Risk of *Clostridium difficile* diarrhea in hospital inpatients prescribed proton pump inhibitors: cohort and case-control studies. CMAJ 2004; 171:33-8.
25. Johnson S, Gerding DN, Janoff EN. Systemic and mucosal antibody responses to toxin A in patients infected with *Clostridium difficile.* J Infect Dis 1992;166: 1287-94.
26. Alfa MJ, Kabani A, Lyerly D, et al. Characterization of a toxin A-negative, toxin B-positive strain of *Clostridium difficile* responsible for a nosocomial outbreak of *Clostridium difficile*-associated diarrhea. J Clin Microbiol 2000;38:2706-14.
27. Martin H, Willey B, Low DE, et al. Characterization of *Clostridium difficile* strains isolated from patients in Ontario, Canada, from 2004 to 2006. J Clin Microbiol 2008;46:2999-3004.
28. Leav BA, Blair B, Leney M, et al. Serum anti-toxin B antibody correlates with protection from recurrent *Clostridium difficile* infection (CDI). Vaccine 2010;28:965-9.
29. Warny M, Pepin 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.
30. Merrigan MM, Sambol SP, Johnson S, Gerding DN. New approach to the management of *Clostridium difficile* infection: colonisation with non-toxigenic *C. difficile* during daily ampicillin or ceftriaxone administration. Int J Antimicrob Agents 2009;33:Suppl:S46-S50.
31. McFarland LV, Mulligan ME, Kwok R, Stamm WE. Nosocomial acquisition of *Clostridium difficile* infection. N Engl J Med 1989;320:204-10.
Copyright © 2011 Massachusetts Medical Society.

JOURNAL ARCHIVE AT NEJM.ORG

Every article published by the *Journal* is now available at **NEJM.org**, beginning with the first article published in January 1812. The entire archive is fully searchable, and browsing of titles and tables of contents is easy and available to all. Individual subscribers are entitled to free 24-hour access to 50 archive articles per year. Access to content in the archive is available on a per-article basis and is also being provided through many institutional subscriptions.

The New England Journal of Medicine
Downloaded from nejm.org on November 5, 2011. For personal use only. No other uses without permission.
Copyright © 2011 Massachusetts Medical Society. All rights reserved.