A LARGE OUTBREAK OF *CLOSTRIDIUM DIFFICILE*–ASSOCIATED DISEASE WITH AN UNEXPECTED PROPORTION OF DEATHS AND COLECTOMIES AT A TEACHING HOSPITAL FOLLOWING INCREASED FLUOROQUINOLONE USE

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ABSTRACT

BACKGROUND AND OBJECTIVE: Fluoroquinolones have not been frequently implicated as a cause of *Clostridium difficile* outbreaks. Nosocomial *C. difficile* infections increased from 2.7 to 6.8 cases per 1,000 discharges ($P < .001$). During the first 2 years of the outbreak, there were 253 nosocomial *C. difficile* infections; of these, 26 resulted in colectomy and 18 resulted in death. We conducted an investigation of a large *C. difficile* outbreak in our hospital to identify risk factors and characterize the outbreak.

METHODS: A retrospective case–control study of case-patients with *C. difficile* infection from January 2000 through April 2001 and control-patients matched by date of hospital admission, type of medical service, and length of stay; an analysis of inpatient antibiotic use; and antibiotic susceptibility testing and molecular subtyping of isolates were performed.

RESULTS: On logistic regression analysis, clindamycin (odds ratio [OR], 4.8; 95% confidence interval [CI_{95}], 1.9–12.0), ceftriaxone (OR, 5.4; CI_{95}, 1.8–15.8), and levofloxacin (OR, 2.0; CI_{95}, 1.2–3.3) were independently associated with infection. The etiologic fractions for these three agents were 10.0%, 6.7%, and 30.8%, respectively. Fluoroquinolone use increased before the onset of the outbreak ($P < .001$); 59% of case-patients and 41% of control-patients had received this antibiotic class. The outbreak was polyclonal, although 52% of isolates belonged to two highly related molecular subtypes.

CONCLUSIONS: Exposure to levofloxacin was an independent risk factor for *C. difficile*–associated diarrhea and appeared to contribute substantially to the outbreak. Restricted use of levofloxacin and the other implicated antibiotics may be required to control the outbreak (*Infect Control Hosp Epidemiol* 2005;26:273-280).

Clostridium difficile is the most common cause of acute care hospital–acquired diarrhea, accounting for approximately 15% to 30% of all cases of antibiotic-associated diarrhea[1-3] and more than 300,000 cases per year.[4,5] The incidence has been reported to vary from 1 to 30 cases per 1,000 patient discharges.[6-8] Severe disease has been reported to occur in approximately 3% of infected patients and is associated with toxic megacolon, perforation, colectomy, and death.[9,10] The estimated attributable healthcare cost of *C. difficile* infection is $3,669 per case.[11]

The antibiotics considered to be associated with the highest risk of *C. difficile* disease include clindamycin,[6,8,12-15] cephalosporins,[16-20] and ampicillin–amoxicillin.[21,22] Fluoroquinolones were not generally considered to be a major risk factor for antibiotic-associated diarrhea.[23] However, the experience with many of the newer agents in this class is limited, and reports of an association between *C. difficile* colitis and fluoroquinolone use have recently emerged.[7,20,24-32]

From 1999 to 2000–2001, the incidence of nosocomial *C. difficile* disease at our hospital increased from 2.7 to 6.8 cases per 1,000 discharges ($P < .001$) or 0.46 to 1.12 cases per 1,000 patient-days. In 1999, 4 (5.6%) of 72 patients with nosocomial *C. difficile* had severe disease representing 0.15 severe case per 1,000 discharges. During 2000 and 2001, 37 (8.8%) of 419 cases resulted in severe outcomes corresponding to an increased rate of 0.60 severe case per 1,000 discharges ($P = .004$). There were 37 severe nosocomial cases during this 2-year period, 26 patients had *C. difficile*–associated colectomy, and 18 patients had *C. difficile*–associated death.

These findings prompted a review of the 2,334 patients hospitalized with *C. difficile* colitis from January 1989 to December 2000.[33] The increased incidence of *C.*

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difficile colitis was accompanied by an increased proportion of cases associated with severe adverse outcomes as compared with the historical data, and a 1:3 matched case–control risk factor analysis was done to evaluate 16 patients who required colectomies in 2000.[34] Case-patients were matched to control-patients by age and occurrence of *C. difficile* colitis within the same period. Chronic lung disease and elevated white blood cell count were found to be significant in the regression model. Transplantation and immunosuppression were not significant risk factors. Exposure to levofloxacin was noted to be high (64%) in all patients studied. This study prompted an investigation to identify risk factors for *C. difficile* acquisition and to characterize this outbreak.

METHODS

The University of Pittsburgh Medical Center–Presbyterian Hospital is a 600-bed, tertiary-care teaching facility affiliated with the University of Pittsburgh Schools of the Health Sciences. The University of Pittsburgh Medical Center–Presbyterian Hospital consists of two hospitals connected by a pedestrian bridge. In general, hospital A houses the medical services and hospital B houses the surgical services.

The outbreak began in January 2000 and continued throughout 2002. Before the epidemic period, there was no obvious change in patient population, infection control policies, or laboratory tests used to identify *C. difficile*. The only formulary changes were switches from ceftazidime to cefepime in April 1998 and from ciprofloxacin to levofloxacin in March 1999.

Alcohol sanitizer was introduced into our hospital in July 2000, 7 months after the outbreak began. This is an important point because it has been postulated that disinfecting hands may be less effective in decreasing transmission because alcohol has poor activity against *C. difficile* spores.

Various infection control interventions were implemented in response to the outbreak. These included an education program, efforts to quickly identify and isolate patients with *C. difficile*, verbal and electronic communication of suspected disease along with isolation requirements (private room or cohorting and gown, gloves, and hand hygiene for all patient or environmental contact), development of an electronic isolation code to readily visualize *C. difficile* status on various electronic patient record systems and block placement of non-coded patients in the same room, and the implementation of enhanced hypochlorite disinfection. Additionally, the duration of isolation was extended beyond the recommendations of the Centers for Disease Control and Prevention (duration of illness)[35] to the entire length of stay. Compliance with the infection control strategies was observed (greater than 93% in more than 300 observations for contact isolation signage and supply availability, 60% [76 of 127] for donning of appropriate isolation garb, and 62% for hand hygiene). This did not differ appreciably from the pre-outbreak period.

In general, nosocomial *C. difficile* disease was defined as onset more than 48 hours after admission or within 3 months of last discharge, if no evidence of prior positive results or other healthcare in the interim prior to re-admission. Severe *C. difficile* disease was defined by the presence of *C. difficile* toxin–mediated colitis (toxin, pseudomembranes, or both visualized endoscopically) and *C. difficile*–associated colectomy, death, or both. All potential cases were reviewed by at least two of four trained physicians to determine whether colectomy or death was *C. difficile* associated.

This study consisted of the following three components: (1) a case–control study of risk factors for nosocomial *C. difficile* infection, (2) an analysis of trends of in-patient antibiotic use for the period before and during the outbreak, and (3) a microbiologic component that included antimicrobial susceptibility testing and molecular subtyping.

Case–Control Study Methods

A matched case–control study was performed for cases that occurred during the period of January 1, 2000, to April 30, 2001. A case-patient was defined as a patient who had *C. difficile* toxin identified in his or her stool at least 72 hours after admission to the hospital and signs and symptoms of *C. difficile* disease. For each case-patient, one control-patient who did not have a positive result on *C. difficile* toxin assay was matched on date of admission (± 2 days), type of medical service (medicine vs surgery), and length of stay in the hospital. The length of stay of the control-patient had to be at least as long as the interval between the admission date and the *C. difficile* toxin positivity date of the case-patient. The date of the case-patient's first positive *C. difficile* toxin result was used to define the reference date for the case-patient and control-patient.

Most of the data for this study, including medication histories, demographic information, and laboratory data, were obtained from our electronic medical archival retrieval system, composed of hospital databases established for patient care and billing purposes.[36] Information on chronic medical conditions and exposures that occurred outside of our institution was obtained by retrospective chart review.

Antibiotic Trends Analysis Methods

A trend analysis was performed on implicated antibiotics to determine whether there was a change in use associated with the outbreak. Monthly antibiotic data (in units) were abstracted from the medical archival retrieval system. A unit was defined by the specific dose given. All units were calculated and multiplied by the milligram dose of that unit and converted to grams. Defined daily doses (DDDs) for antibiotics were calculated using the standards of the World Health Organization.[37] Monthly DDD rates were calculated per 1,000 patient-days. Monthly DDD rates were averaged for a 12-month period before the fluoroquinolone formulary change (period 1, March 1998 to February 1999) and compared with the rate for the subsequent 12-month

period (period 2, March 1999 to February 2000). Rates were again calculated for the next 12-month period (period 3, March 2000 to February 2001) and compared with those of period 2.

Microbiology Methods

The presence of *C. difficile* toxin in the stool of case-patients was determined using a standard cell culture cytotoxicity assay with MRHF/HFF cells (Diagnostic Hybrids, Inc., Athens, OH) and antitoxin from TechLab (Blacksburg, VA). Toxin testing was done throughout the outbreak period. Culturing for *C. difficile* began in March 2001 and was accomplished using previously published methods.[38]

Antimicrobial susceptibility testing was performed on isolates collected between March and August 2001 by the Etest method (AB Biodisk, Piscataway, NJ) using *C. difficile* strain American Type Culture Collection 9689 as a control. Interpretations were according to National Committee for Clinical Laboratory Standards guidelines[39] for the antibiotics for which guidelines were available. For vancomycin and fluoroquinolones, agents for which National Committee for Clinical Laboratory Standards criteria were not available, the following values were used: resistant, minimum inhibitory concentration (MIC) of 8 μg/mL or greater; intermediate, MIC of 4 μg/mL; and susceptible, MIC of 2 μg/mL or less. We also determined the proportion of isolates that had a MIC of greater than 32 μg/mL to the fluoroquinolones and a MIC of greater than 256 μg/mL to clindamycin.

Restriction enzyme analysis (REA), a common molecular subtyping technique used for *C. difficile* DNA fingerprinting, was performed on *C. difficile* isolates collected between March and December 2001 using a modification of a previously published method.[40] REA types were assigned based on visual inspection. For isolates to be considered to have the same REA type, the banding patterns had to be identical to the prototype isolate for that REA type. In addition, the identical banding pattern had to be confirmed by running the isolates in question on the same gel as the prototype.

Statistical Methods

For the case–control study, matched univariate analysis using matched odds ratios (ORs) and conditional logistic regression was performed on all variables.[41] Exposures were determined for the 28-day period before *C. difficile* positivity for each case-patient and the matched control-patient. A multivariable model was fit using a stepwise model-building approach to identify variables independently associated with being a case. All variables from the univariate analyses were eligible for entry into the model. The stay criterion for the model was a *P* value of less than .05. Etiologic fraction (the proportion of disease presumably caused by the exposure in question) for antibiotics associated with *C. difficile*–associated disease was estimated using the proportion of control-patients exposed to each antibiotic and the matched OR as an approximation for the relative risk, as previously described.[42] For the antibiotic trends analysis, the Cox–Stuart test for trend was performed on antibiotic use data from March 1998 through February 2001.

For analysis of REA typing results, we determined which hospital units case-patients had been on in the 14 days before the case reference date. Analyses were conducted using the SAS system (version 8.2; SAS Institute, Inc., Cary, NC).

Outbreak-Associated Costs

The number and rates of *C. difficile* infections were calculated at baseline (1999) and during the outbreak (2000 and 2001). The excess numbers of annual and total infections during the outbreak were determined, and the estimated attributable healthcare cost of these additional infections was calculated by multiplying the number of excess infections by the estimate of $3,669 per episode.[11]

RESULTS

Case–Control Study

Of a total of 447 *C. difficile* toxin–positive patients who were identified during the study period, 227 (50.8%) were positive 72 hours or more after hospital admission. Matched control-patients were identified for 203 (89.4%) of these case-patients. The remaining 24 case-patients (10.6%) were excluded because no patients who were admitted within 2 days of them had stays at least as long as the time to the case reference date.

The demographics and clinical characteristics of the enrolled case-patients and control-patients are provided in Table 1. On univariate analysis, case-patients were more likely than control-patients to have had a transplant (OR, 4.3; 95% confidence interval [CI_{95}], 2.0 to 9.2), diabetes mellitus (OR, 1.7; CI_{95}, 1.1 to 2.7), chronic lung disease (OR, 1.6; CI_{95}, 1.1 to 2.5), or diverticulosis (OR, 3.0; CI_{95}, 1.2 to 7.6) or be immunosuppressed (OR, 1.9; CI_{95}, 1.2 to 3.1). Multiple medications were associated with being a case-patient on the univariate analysis (Table 2).

On the multivariable conditional logistic regression analysis, age (OR, 1.02 for each year of age; CI_{95}, 1.006 to 1.037), diabetes mellitus (OR, 2.1; CI_{95}, 1.2 to 3.6), organ transplantation (OR, 5.8; CI_{95}, 2.3 to 14.6), H_2 blocker use (OR, 2.0; CI_{95}, 1.1 to 3.5), and use of proton pump inhibitors (OR, 2.4; CI_{95}, 1.3 to 4.4) were associated with being a case-patient. Clindamycin (OR, 4.8; CI_{95}, 1.9 to 12.0), ceftriaxone (OR, 5.4; CI_{95}, 1.8 to 15.8), and levofloxacin (OR, 2.0; CI_{95}, 1.2 to 3.3) were the antibiotics associated with *C. difficile* infection. The etiologic fractions for these three agents were 10.0%, 6.7%, and 30.8%, respectively. Therapy with single antibiotics was relatively uncommon among both case-patients and control-patients, which did not allow for an assessment of the risk of monotherapy with these agents.

Antibiotic Trends

In the analysis of trends of antibiotic use, fluoroquinolone use increased significantly from an average of

TABLE 1
MATCHED UNIVARIATE ANALYSIS OF SELECTED DEMOGRAPHICS AND CLINICAL CHARACTERISTICS OF CASE-PATIENTS AND CONTROL-PATIENTS

Characteristic	Case-Patients (n = 203)	Control-Patients (n = 203)	Matched OR	CI_{95}	*P*
Male	104 (51.2%)	106 (52.2%)	0.96	0.6–1.5	.75
White	166 (82.2%)	164 (81.2%)	1.1	0.6–1.8	.80
Surgery service	122 (60.1%)	122 (60.1%)	Matching criterion		
Chronic medical condition					
Organ transplantation	44 (21.7%)	18 (8.9%)	4.3	2.0–9.2	.0002
Diabetes	83 (40.9%)	59 (29.1%)	1.7	1.1–2.7	.01
Malignancy	54 (26.6%)	47 (23.2%)	1.2	0.8–1.9	.42
Chronic lung disease	68 (33.5%)	47 (23.2%)	1.6	1.1–2.5	.03
HIV infection	3 (1.5%)	4 (2.0%)	0.8	0.2–3.4	.71
Inflammatory bowel disease	4 (2.0%)	12 (5.9%)	0.3	0.1–1.03	.06
Diverticulosis	18 (8.9%)	6 (3.0%)	3.0	1.2–7.6	.02
Colostomy	3 (1.5%)	11 (5.4%)	0.2	0.04–0.9	.04
Short gut syndrome	1 (0.5%)	6 (3.0%)	0.2	0.02–1.4	.10
Chronic renal failure	37 (18.2%)	42 (20.7%)	0.8	0.5–1.4	.52
Immunosuppressed	70 (34.5%)	48 (23.6%)	1.9	1.2–3.1	.01
Previous history of *Clostridium difficile* infection	3 (1.5%)	2 (1.0%)	1.5	0.3–9.0	.66
Median LOS before case infection, d (range)	14 (3–100)	14 (1–102)	Matching criterion		
Median total LOS, d (range)	28 (5–234)	25 (3–174)			.53
Median age, y (range)	64 (17–95)	59 (16–93)			.10

OR = odds ratio; CI_{95} = 95% confidence interval; HIV = human immunodeficiency virus; LOS = length of stay.

217 DDDs per 1,000 patient-days in period 1 to 275 DDDs per 1,000 patient-days for period 2 ($P < .001$). This increase in fluoroquinolone use preceded the beginning of the outbreak by 9 months. Cephalosporin use did not change significantly, with 51 DDDs per 1,000 patient-days in period 1 versus 66 DDDs per 1,000 patient-days in period 2 ($P = .19$), and clindamycin use averaged 28 DDDs per 1,000 patient-days for period 1 and 30 DDDs per 1,000 patient-days for the second 12-month period ($P = .01$). For period 3, quinolone use was 310 DDDs per 1,000 patient-days ($P = .003$ for increase from period 2 to 3), cephalosporin use was 66 DDDs per 1,000 patient-days ($P = .6$), and clindamycin use was 32 DDDs per 1,000 patient-days ($P = .19$).

Antimicrobial Susceptibility

Antimicrobial susceptibility testing was performed on 91 isolates: 46 nosocomial isolates and 45 isolates acquired elsewhere. None of the isolates had resistance to metronidazole or vancomycin. However, 78 (85.7%) of the isolates were resistant to clindamycin and 61 (67.0%) had a clindamycin MIC of greater than 256 µg/mL. Eighty-six (94.5%) also displayed resistance to levofloxacin; 84 (92.3%) of 91 had a MIC greater than 32 µg/mL. A subset of 10 isolates was similarly tested for susceptibility to other quinolone antibiotics. All were resistant to ofloxacin and ciprofloxacin and 70% were resistant to moxifloxacin and gatifloxacin. Of the 45 isolates acquired elsewhere, 38 (84.4%) were resistant to clindamycin and 26 (57.8%) had a clindamycin MIC of greater than 256 µg/mL. For the 46 nosocomial isolates, 40 (87.0%) were resistant to clindamycin and 35 (76.1%) had a clindamycin MIC of greater than 256 µg/mL. There was no significant difference in antimicrobial susceptibility between the nosocomial isolates and those acquired elsewhere.

Of the 91 isolates available for antimicrobial susceptibility testing, 11 were type 2 and 14 were type 4. All 11 type 2 isolates were resistant to both levofloxacin (MIC > 32 µg/mL) and clindamycin (MIC > 256 µg/mL). Of the 14 type 4 isolates, all were resistant to levofloxacin (MIC > 32 µg/mL), and 13 (93%) were resistant to clindamycin (MIC > 256 µg/mL). The remaining isolate had a MIC of 6 µg/mL for clindamycin, indicating intermediate resistance.

REA Type

REA was performed on 135 *C. difficile* isolates: 92 from patients with nosocomial infection and 43 from patients who acquired their *C. difficile* infection elsewhere. Among the nosocomial isolates, there were 36 distinct REA patterns. REA types 2 and 4, which differed from each other by a single band, were the most frequent,

TABLE 2
Matched Univariate Analysis of Selected Drugs Administered to Case-Patients and Control-Patients During the 4 Weeks Before the Detection of *Clostridium difficile* Toxin in the Stool of the Case-Patient

Drug	Case-Patients (n = 203)	Control-Patients (n = 203)	OR	CI_{95}	*P*
Antimicrobial agent					
Ceftriaxone	21 (10.3%)	8 (3.9%)	3.2	1.3–7.9	.014
Clindamycin	32 (15.8%)	13 (6.4%)	2.7	1.4–5.4	.004
Cefuroxime	5 (2.5%)	2 (1.0%)	2.5	0.5–12.9	.27
Amoxicillin–clavulanate	5 (2.5%)	2 (1.0%)	2.5	0.5–12.9	.27
Antiviral class	43 (21.2%)	24 (11.8%)	2.5	1.3–4.7	.006
Levofloxacin	120 (59.1%)	83 (40.9%)	2.2	1.4–3.4	.0003
Sulfa class	33 (16.3%)	18 (8.9%)	2.2	1.1–4.2	.02
Antifungal class	71 (35.0%)	50 (24.6%)	2.1	1.2–3.6	.008
Cefepime	49 (24.1%)	28 (13.8%)	2.1	1.2–3.6	.008
Macrolide class	30 (14.8%)	16 (7.9%)	2.0	1.1–3.8	.03
Metronidazole	52 (25.6%)	39 (19.2%)	1.5	0.9–2.5	.10
Cephalosporin class	124 (61.1%)	109 (53.7%)	1.4	0.9–2.1	.11
Ampicillin	9 (4.4%)	7 (3.4%)	1.3	0.5–3.5	.62
Vancomycin (intravenous)	92 (45.3%)	81 (39.9%)	1.3	0.8–1.9	.27
Imipenem	8 (3.9%)	7 (3.4%)	1.1	0.4–3.2	.80
Ceftazidime	2 (1.0%)	2 (1.0%)	1.0	0.1–16.0	1.00
Ciprofloxacin	15 (7.4%)	15 (7.4%)	1.0	0.5–2.2	1.00
Tetracycline class	2 (1.0%)	2 (1.0%)	1.0	0.1–7.1	1.00
Piperacillin–tazobactam	62 (30.5%)	64 (31.5%)	0.95	0.6–1.5	.82
Cefazolin	81 (39.9%)	87 (42.9%)	0.9	0.6–1.3	.50
Ampicillin–sulbactam	15 (7.4%)	17 (8.4%)	0.9	0.4–1.8	.71
Cefotetan	13 (6.4%)	14 (6.9%)	0.9	0.4–2.0	.84
Aztreonam	8 (3.9%)	10 (4.9%)	0.8	0.3–2.0	.64
Rifampin	4 (2.0%)	5 (2.5%)	0.8	0.2–3.0	.74
Aminoglycoside class	33 (16.3%)	42 (20.7%)	0.7	0.4–1.2	.25
Cefotaxime	2 (1.0%)	3 (1.5%)	0.7	0.1–4.0	.66
Vancomycin (oral)	1 (0.5%)	2 (1.0%)	0.5	0.05–5.5	.57
Quinupristin–dalfopristin, linezolid, or both	1 (0.5%)	3 (1.5%)	0.3	0.04–3.2	.34
Other medications					
Immunosuppressive drugs	52 (25.6%)	26 (12.8%)	3.2	1.7–6.1	.0005
Proton pump inhibitor	78 (38.4%)	54 (26.6%)	1.8	1.2–2.9	.009
H_2 blocker	159 (78.3%)	141 (69.5%)	1.6	1.0–2.5	.04
Corticosteroids	92 (45.3%)	79 (38.9%)	1.4	0.9–2.1	.17
Anti-diarrheal medication	18 (8.9%)	12 (5.9%)	1.5	0.7–3.3	.26
Antacids	31 (15.3%)	36 (17.7%)	0.8	0.5–1.4	.49
Sucralfate	14 (6.9%)	11 (5.4%)	1.3	0.6–3.0	.53
Narcotic	182 (89.7%)	178 (87.7%)	1.2	0.7–2.3	.53

OR = odds ratio; CI_{95} = 95% confidence interval.

accounting for 18 (19.5%) and 29 (31.5%) of the isolates, respectively. The other 34 REA types among the remaining 45 isolates occurred at frequencies ranging from 1 (1.1%) to 5 (5.4%) of the isolates and only 5 (5.4%) of our isolates were REA type J9. In a comparison of nosocomial isolates and isolates acquired elsewhere, 47 (51.2%) versus 12 (27.9%), respectively, were REA types 2 or 4 (P = .01).

Among the 92 nosocomial isolates for which REA data were available, hospital location data were available for 87. REA type 2 was associated with case-patients housed on the tenth floor of hospital A (P = .01), as well as hospital A in general (P = .01). Of the 17 REA type 2 isolates, 13 (76.5%) of the patients had been admitted to hospital A, with 7 on the tenth floor, 3 on the ninth floor, and an additional 2 patients spending time on both the ninth and tenth floors. Type 4 was associated with hospital B: 21 (80.8%) of 26 patients with REA type 4 spent time in hos-

pital B versus 29 (47.5%) of 61 patients with other REA types ($P = .01$).

Outbreak-Associated Costs

In 1999, prior to the onset of the outbreak, 72 *C. difficile* infections occurred at our institution. In 2000 and 2001, the average annual incidence of *C. difficile* disease increased 2.5-fold in our institution from 2.7 cases per 1,000 discharges in 1999 to 6.8 cases per 1,000 discharges, resulting in a total excess of 253 infections or an average of 127 infections annually. The estimated attributable healthcare cost of these additional *C. difficile* infections during 2000–2001 was $928,257. Additional costs were likely to have been incurred as the proportion of severe cases also increased significantly from 4 (5.6%) of 72 in 1999 to 18 (8.4%) of 214 and 19 (9.3%) of 205 in 2000 and 2001, respectively, with 26 *C. difficile*–associated colectomies and 18 *C. difficile*–associated deaths noted.

DISCUSSION

The epidemiology of *C. difficile* at our institution has changed. Although several recent case reports[24-30] and a few case–control studies[7,20,31,32] proposed an association between quinolone use and *C. difficile*–associated disease, no single large study has identified increased quinolone use as a primary precipitory agent of an ongoing *C. difficile* outbreak. The major finding of our investigation was an independent association between *C. difficile*–associated disease and exposure to levofloxacin.

Although causation is difficult to infer from observational studies, we believe that the relationship between *C. difficile* and levofloxacin was causal for several reasons. First, fluoroquinolones, like most other antibiotics, have recently been found to be associated with *C. difficile* infection at various institutions; suggesting that quinolone exposure may be a promoter of this illness is biologically plausible. Second, our particular outbreak was preceded by the introduction of levofloxacin to our formulary and a large increase in quinolone use; temporality is perhaps the most fundamental criterion for inferring a causal relationship from observational data. The reason for the occurrence of a lag of approximately 9 months between the increase and the onset of the outbreak is not clear.

Ceftriaxone and clindamycin were also identified as risk factors for disease in our study. Although exposure to third-generation cephalosporins,[16-20] specifically ceftriaxone,[17] and clindamycin is regarded as a well-established risk factor for *C. difficile* infection,[1,6,8,12-15,43] the magnitude of the increased use of these antibiotics was small and the proportion of cases that received these antibiotics was less than 11% and 16%, respectively. The OR for the association between levofloxacin and *C. difficile*–associated diarrhea was relatively modest (2.0) and lower than those for clindamycin and ceftriaxone (4.8 and 5.4, respectively). However, the etiologic fraction estimates clearly suggested that levofloxacin was responsible for a greater proportion of cases than were the other two agents. The large number of patients included in the case–control study was a major strength of this outbreak investigation and may have permitted us to detect an association between levofloxacin and *C. difficile*–associated diarrhea that might otherwise have gone unnoticed in a smaller study.

A recent small study by Gaynes et al.[31] also found an association between fluoroquinolones and *C. difficile* disease. Their findings differed substantially from ours. They demonstrated a difference in *C. difficile* colitis risk during periods when two different fluoroquinolones (levofloxacin and gatifloxacin) were used. Of note, the *C. difficile* disease attack rate associated with each fluoroquinolone was high. The authors speculated that the enhanced anaerobic spectrum of gatifloxacin may have had a more disruptive effect on fecal flora and accounted for their increased rate. Historically, the newer fluoroquinolone agents were found to be more active against *C. difficile* than were the older fluoroquinolones.[44] However, this study, like ours, found most isolates to be resistant to all quinolones tested. As levofloxacin does not have the enhanced anaerobic spectrum of gatifloxacin, we believe that perhaps the inability of levofloxacin to exhibit an inhibitory effect on *C. difficile* isolates coupled with its increased use, along with concomitant use of other antimicrobials, accounted for our increased *C. difficile* rate. One additional case–control study of *C. difficile*–associated disease done in 2001[32] also found fluoroquinolone use (OR, 12.7; CI_{95}, 2.6 to 61.6) to be the only significant risk factor for *C. difficile*–associated disease. The primary fluoroquinolone used was levofloxacin (60% of subjects), and only 15% of subjects were exposed to gatifloxacin. Gaynes et al. did not report quinolone use during their study. Perhaps their changing rates were associated with changing fluoroquinolone use and were less specific to which quinolone was used.

In an editorial accompanying the study by Gaynes et al.,[45] Gerding concurred that the anaerobic activity of newer fluoroquinolones is likely to be more disruptive of gut flora, but also believed that the additional factors of acquired fluoroquinolone resistance in *C. difficile* and proliferation of these resistant clones are the critical events leading to hospital outbreaks. In addition, he suggested that with this selected resistance and continued exposure to any fluoroquinolone, infection rates may not decrease.

Several studies have documented clonal spread of *C. difficile* in healthcare settings.[8,46-49] REA type J9 is often found as the predominant subtype in these outbreaks.[46] Unlike other facilities, our most prevalent REA types (2 and 4) had not been previously described, and REA type J9 was infrequently isolated during our outbreak. Although 28 distinct nosocomial REA types were identified, more than half of the isolates belonged to 2 REA types that differed from each other by only 1 band; preliminary plasmid analysis did not suggest that this difference was due to the presence of a plasmid (data not shown). The analysis of these REA types by geographic location indicates that these two strains behaved differently epidemiologically despite being highly related

genetically. Taken together, the molecular and case–control study data suggest that both nosocomial transmission and antibiotic use contributed to this outbreak.

There are several limitations of this study. The large number of variables we analyzed increased the likelihood that some of the associations we observed were due to chance. However, the temporal relationship between levofloxacin use and the onset of the outbreak, as well as the well-established relationship between clindamycin and the third-generation cephalosporins and *C. difficile*–associated disease, suggests that our main findings were not spurious. The case–control study and molecular epidemiologic analysis were from essentially two different, albeit contiguous, time periods. However, we doubt that our findings would have differed substantially if both study components had been from overlapping periods. Finally, as most of our patients who received levofloxacin also received other antibiotics, we were not able to examine the risk of levofloxacin monotherapy in this study.[23]

Data from other settings suggest that aggressive antibiotic restriction may be required to stem an outbreak.[6,8] Many other hospitals are now reporting epidemic rates of *C. difficile*–associated colitis. Conceivably, the answer may not relate to changing from one quinolone to another. Rate reduction might be accomplished only when the use of fluoroquinolones, in general, is significantly reduced.[45] We have recently established an antibiotic management program that will focus on promoting the judicious use of antibiotics, as well as restricting the specific antibiotics implicated in this investigation and other broad-spectrum antibiotics. Ongoing surveillance of *C. difficile* disease will continue to assess whether this intervention will control the outbreak.

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