A Hospital Outbreak of Diarrhea Due to an Emerging Epidemic Strain of Clostridium difficile

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Background: Increased Clostridium difficile–associated disease (CDAD) in a hospital and an affiliated long-term care facility continued despite infection control measures. We investigated this outbreak to determine risk factors and transmission settings.

Methods: The CDAD cases were compared according to where the disease was likely acquired based on health care exposure and characterization of isolates from case patients, asymptomatic carriers, and the environment. Antimicrobial susceptibility testing, strain typing using pulsed-field gel electrophoresis, and toxinotyping were performed, and toxins A and B, binary toxin, and deletions in the tcdC gene were detected using polymerase chain reaction. Risk factors were examined in a case-control study, and overall antimicrobial use was compared at the hospital before and during the outbreak.

Results: Significant increases were observed in hospital-acquired (0.19 vs 0.86; P < .001) and long-term care facility–acquired (0.04 vs 0.31; P = .004) CDAD cases per 100 admissions as a result of transmission of a toxinotype III strain at the hospital and a toxinotype 0 strain at the long-term care facility. The toxinotype III strain was positive for binary toxin, an 18–base pair deletion in tcdC, and increased resistance to fluoroquinolones. Independent risk factors for CDAD included use of fluoroquinolones (odds ratio [OR], 3.22; P = .04), cephalosporins (OR, 5.19; P = .006), and proton pump inhibitors (OR, 5.02; P = .02). A significant increase in fluoroquinolone use at the hospital took place during the outbreak (185.5 defined daily doses per 1000 patient-days vs 200.9 defined daily doses per 1000 patient-days; P < .001).

Conclusions: The hospital outbreak of CDAD was caused by transmission of a more virulent, fluoroquinolone-resistant strain of C difficile. More selective fluoroquinolone and proton pump inhibitor use may be important in controlling and preventing such outbreaks.

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Community acute care hospital in Maine; many of the case patients were admitted from an affiliated LTCF. Measures to control transmission were instituted at both the hospital and the LTCF; however, the increase continued. In November 2003, the Centers for Disease Control and Prevention, Atlanta, Ga, were invited to assist in the outbreak investigation. The objectives of the investigation were to apply epidemiologic and laboratory methods in determining the role of the LTCF and the community in the transmission of *C difficile*, to describe the microbiologic characteristics of outbreak strains of *C difficile*, and to determine the risk factors for CDAD in hospitalized patients.

**METHODS**

**CASE DEFINITIONS AND POTENTIAL PLACE OF ACQUISITION**

We identified CDAD cases that occurred during the preoutbreak (September 2002–April 2003) and outbreak (May 2003–December 2003) periods by reviewing all *C difficile* toxin A–positive records from the hospital clinical microbiology laboratory. Because the hospital laboratory performed *C difficile* toxin testing not only for hospitalized patients but also for LTCF residents and patients of outpatient clinics, our review included persons who resided in the community or the LTCF at the time of testing. Each positive result was linked to a unique person for whom available hospital, LTCF, and outpatient medical records were reviewed.

Three criteria were used to define a CDAD case: (1) a diarrheal illness in a person who tested positive for *C difficile* toxin A by a commercial optical immunoassay (*Cd* TOXA OIA; Thermo Electron Corp, Louisville, Colo); (2) disease onset during the preoutbreak (September 2002–April 2003) or outbreak (May–December 2003) periods; and (3) hospitalization during the preoutbreak or the outbreak period. Although the investigation was limited to hospitalized patients only, hospitalization did not have to coincide with CDAD onset. For those patients meeting all 3 inclusion criteria, medical records were reviewed regarding admission to the hospital, the LTCF, and other inpatient health care settings for the 6 months before CDAD onset. Based on the history of health care exposure before CDAD onset, we assigned each CDAD case patient a possible place of *C difficile* acquisition (Figure 1). If a CDAD case patient had diarrhea onset 48 or more hours after admission to the hospital or the LTCF, the case was classified as hospital or LTCF acquired, respectively. If diarrhea developed before or within 48 hours of admission and there was no history of health care facility exposure over the prior 6 months, the case was classified as community acquired.

**CASE-CONTROL STUDY**

To determine risk factors for infection, we conducted a matched case-control study in which cases and controls were patients admitted to the hospital during the preoutbreak or outbreak period. Two controls per case were selected to match case patients with regard to sex, age (±5 years), and admission date (±3 days). The medical records for case and control patients were reviewed for demographic information, exposures to health care facilities, and comorbid conditions. The comorbidities were analyzed using the method suggested by Johnston et al, which involves analysis of principal and secondary diagnostic codes of the *International Classification of Diseases, Clinical Modification*, associated with the index hospitalization. Assessment of risk factors for CDAD included exposures to antimicrobial agents, chemotherapy, antacids, or tube feeding in the 30 days before the onset of CDAD; radiation therapy, gastric surgery, colon surgery, or colon disease at any time in the patient’s life; and use of a rectal thermometer in the hospital emergency department.

**MICROBIOLOGIC ASSESSMENT OF PATIENTS AND THE ENVIRONMENT**

To determine contamination of the environment with *C difficile*, we collected surface swabs from commodes and frequently touched areas in case patients’ rooms in the hospital and the LTCF. To determine strain types of *C difficile* circulating in the hospital and the LTCF, we collected a convenience sample of stool swabs from asymptomatic hospital patients and LTCF residents as well as a convenience sample of case-patient, toxin-positive stool specimens. All stool swabs from asymptomatic patients and stool samples from case patients were shipped to the laboratories of the Maine Bureau of Health, Augusta, where the specimens were cultured on cycloserine-cefoxitin-fructose agar for *C difficile* after alcohol shock treatment to kill vegetative cells. All *C difficile* isolates were sent to the Centers for Disease Control and Prevention for confirmation of species identification followed by molecular characterization and antimicrobial susceptibility testing.

**LABORATORY INVESTIGATION**

Isolates collected from case patients, asymptomatic patients, and the environment were analyzed for strain relatedness, toxin gene variations, and the presence of the binary toxin gene. Strain relatedness was determined by both pulse-field gel electrophoresis (PFGE) and toxinotyping. Toxinotyping consists of analysis of restriction fragment length polymorphisms of segments of the toxin A and toxin B genes. Polymerase chain re-

**Figure 1.** Algorithm for assigning case patients with *Clostridium difficile*-associated disease (CDAD) to place of acquisition of infection. LTCF indicates long-term care facility.
action was used to determine the presence of one of the genes for binary toxin CDT\(^1\)\(^7\) (\(cdtB\)) and deletions in \(tcdC\). Finally, we determined antimicrobial susceptibility to clindamycin and fluoroquinolones (ie, levofloxacin, gatifloxacin, and moxifloxacin) using the E-test method (AB Biodisk, Solna, Sweden).\(^18\) Because no resistance break points for these fluoroquinolones have been set by the Clinical Laboratory Standards Institute for \(C\) difficile, we used the Clinical Laboratory Standards Institute’s break points for trovafloxacin as surrogate break points. Quality control of antimicrobial susceptibility testing was performed with each test run using the following standard strains: \(Enterococcus faecalis\) ATCC 29212, \(Pseudomonas aeruginosa\) ATCC 27583, \(Bacteroides fragilis\) ATCC 25285, and \(Bacteroides thetaiotaomicron\) ATCC 29741.

HOSPITAL-WIDE ANTIMICROBIAL USE EVALUATION

Hospital-wide antimicrobial use information was collected for the preoutbreak and outbreak periods from computerized administrative billing claims data. The amount of drug represented by each claim was divided by an adult standard dose as previously described.\(^19\) The resulting defined daily doses were then summed and divided by the number of patient days for each antimicrobial class and compared between preoutbreak and outbreak periods.

STATISTICAL ANALYSIS

We compared rates of CDAD by place of acquisition during preoutbreak and outbreak periods using the \(\chi^2\) test (Epi Info 6.0; Centers for Disease Control and Prevention). Matched univariate and multivariable analyses of the case-control study were performed using SAS logistic regression (SAS Institute Inc, Cary, NC). We compared the incidence density of antimicrobial use between preoutbreak and outbreak periods using the Pearson \(\chi^2\) test.

RESULTS

From September 2002 through December 2003, 77 hospital inpatients met the CDAD case definition (Figure 2). The CDAD attack rate, calculated as the number of CDAD cases per 100 hospital admissions, increased from 0.33 in the preoutbreak period to 1.55 (\(P<.001\)) in the outbreak period. Comparison of the CDAD attack rates by place of acquisition showed a significant increase in the number of hospital-acquired and LTCF-acquired cases (Table 1).

CASE-CONTROL STUDY

In the case-control study, 68 cases were compared with 127 controls; full information on risk factors was available for both groups. Cases and controls were similar in regard to age and sex (Table 2). Comparison of comorbid conditions showed a higher prevalence for chronic obstructive pulmonary disease, presence of fluid or electrolyte disorders, and psychoses or depression among cases. Analysis of health care exposures showed that case patients who developed disease after admission to the hospital spent on average 7 days (range, 1-45 days) in the hospital before the onset of CDAD. Comparison of exposures to the hospital for cases (expressed as days from admission until CDAD onset) and controls (expressed as length of stay) showed that cases spent significantly more days in the hospital before the development of...
CDAD than the total number of days that control patients spent in the hospital (OR, 1.2; \( P < .001 \)).

Comparison of antimicrobial exposure in the 30 days before CDAD onset revealed no significant difference in clindamycin use. However, use of any cephalosporins or fluoroquinolones was significantly associated with CDAD, with matched ORs of 3.53 and 4.71, respectively. The use of histamine2 (H2) blockers (OR, 2.69; \( P = .02 \)) or proton pump inhibitors (OR, 3.14; \( P = .003 \)) in the 30 days before CDAD onset was also significantly associated with the risk of CDAD. Finally, case patients were significantly more likely to die than were controls (OR, 14.12; \( P = .01 \)). Comparison of surgical procedures, chemotherapy, and use of rectal thermometers did not show statistical association with disease. To determine the independent effect of the CDAD risk factors, we performed a multivariable analysis by including the significant risk factors in a regression model simultaneously. Four risk factors remained significant: use of proton pump inhibitors, cephalosporins, or fluoroquinolones and length of stay (Table 3).

### LABORATORY INVESTIGATION

A total of 19 isolates were collected from 11 patients with CDAD, from the hospital and LTCF environments, and from 3 asymptomatic carriers each in the hospital and the LTCF. The 6 asymptomatic carriers represented 14%
of 43 stool swabs collected at the hospital and the LTCF. Comparison of isolates for which PFGE results were available (n = 16) revealed 2 groups of indistinguishable isolates (Figure 3). One group consisted of isolates collected from the hospital: 1 environmental isolate, 1 isolate from an asymptomatic carrier, and 6 isolates from case patients whose CDAD was classified as hospital acquired. The second group of indistinguishable isolates consisted of isolates collected from the LTCF: 1 environmental isolate, 2 isolates from asymptomatic carriers, and 1 isolate from a case patient whose CDAD was classified as LTCF acquired.

Using another strain-typing method, toxinotyping, we identified 3 toxinotypes: toxinotype 0 (n = 9), toxinotype III (n = 9), and toxinotype XIV (n = 1). The PFGE-indistinguishable isolates collected from the hospital were identified as toxinotype III, and the PFGE-indistinguishable isolates collected from the LTCF were toxinotype 0. The toxinotype XIV isolate was collected from a case patient with community-acquired CDAD.

As previously reported, analysis of isolates for the presence of potential virulence factors showed that all toxinotype III isolates had the binary toxin CDT, gene cdtB, and an 18–base pair deletion in tcdC. In contrast, all toxinotype 0 isolates were negative for both cdtB and a tcdC deletion. The toxinotype XIV isolate from a community case patient had cdtB but did not have a deletion in tcdC. Antimicrobial susceptibility testing showed differences in susceptibility between toxinotype III and 0 strains. Although both toxinotypes were largely resistant to clindamycin (in 73% of isolates) and levofloxacin, they were different in terms of their susceptibility to the other 2 fluoroquinolones: toxinotype III isolates were resistant to both gatifloxacin and moxifloxacin (minimum inhibitory concentration >32 µg/mL), while toxinotype 0 isolates were susceptible (minimum inhibitory concentration <2 µg/mL) to both drugs.

### COMPARISON OF OVERALL ANTIMICROBIAL USE

Comparing preoutbreak and outbreak periods, we found an insignificant decrease in overall antimicrobial use (from 649.7 to 634.0 defined daily doses [DDDs] per 1000 patient-days; P = .78) and a significant decrease in cephalosporin use (from 245.3 to 226.2 DDDs per 1000 patient-days; P < .001). In contrast, there was a significant increase in overall fluoroquinolone use (from 185.5 to 200.9 DDDs per 1000 patient-days; P < .001), which consisted of a significant increase in levofloxacin use (from 118.8 to 134.5 DDDs per 1000 patient-days; P < .001).

### Table 3. Multivariable Model of Risk Factors for Clostridium difficile–Associated Disease (CDAD) in 68 Case Patients and 127 Control Subjects

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Matched OR (95% CI)</th>
<th>P Value</th>
<th>Attributable Risk, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalosporins</td>
<td>5.19 (1.61-16.77)</td>
<td>.006</td>
<td>80.7</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>3.22 (1.03-10.09)</td>
<td>.04</td>
<td>68.9</td>
</tr>
<tr>
<td>Histamine, blockers</td>
<td>3.33 (0.70-15.72)</td>
<td>.13</td>
<td>69.9</td>
</tr>
<tr>
<td>Length of stay</td>
<td>1.25 (1.11-1.39)</td>
<td>&lt;.001</td>
<td>20.0</td>
</tr>
<tr>
<td>Proton pump inhibitors</td>
<td>5.02 (1.30-19.36)</td>
<td>.02</td>
<td>80.1</td>
</tr>
<tr>
<td>COPD</td>
<td>1.62 (0.59-4.46)</td>
<td>.26</td>
<td>38.3</td>
</tr>
<tr>
<td>Psychoses and depression</td>
<td>2.93 (0.99-9.53)</td>
<td>.07</td>
<td>65.9</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; COPD, chronic obstructive pulmonary disease; OR, odds ratio.

### Figure 3. Pulsed-field gel electrophoresis patterns for Clostridium difficile isolates from case patients, from asymptomatic carriers in the hospital and the long-term care facility (LTCF), and from the hospital and LTCF environments (12 of these 16 patterns have previously been published, with the location of the isolates designated Maine, Facility A or Facility B).13
We found that the outbreak of CDAD in a Maine community hospital was the result of nosocomial transmission of a more virulent, fluoroquinolone-resistant epidemic strain of *C difficile*. This strain has been recently reported to cause outbreaks in several acute care hospitals in the United States and to be associated with particularly severe disease. \(^1\^\) In addition to nosocomial transmission of the epidemic strain, we hypothesized that other sources of infection had contributed. The close affiliation of the hospital with the LTCH raised the question of whether transmission was also occurring in the LTCH and contributing to the increased rate of CDAD in the hospital owing to the frequent transfer of LTCH residents to the hospital. Comparison of cases by place of acquisition in the preoutbreak and outbreak periods showed a significant increase in hospital-acquired and LTCH-acquired cases, while the other health care–acquired and community-acquired cases stayed at similar levels.

Identification of 2 distinct sets of isolates by PFGE and toxinotyping methods and the matching of isolates from asymptomatic carriers and the environment to those from case patients provide support for our classification of patients by place of acquisition based on health care exposure history. Analysis of virulence factors of the 3 strains identified in our investigation showed that the hospital-associated toxinotype III strain had genetic determinants associated with increased virulence, ie, *cdlB* and an 18-bp deletion in *tcdC*. Also, compared with toxinotype 0 isolates, the toxinotype III isolates were more resistant to fluoroquinolones. Increased resistance to fluoroquinolones, combined with increased virulence, may have provided this strain with a greater potential to transmit within the hospital. Recent studies have shown this strain to have been the cause of multiple outbreaks in US health care facilities. \(^9,11,13,20,21\)

Similar to our findings, the dominant epidemic strain in those outbreaks was more resistant to the fluoroquinolones than nondominant strains. Although the manifestation of this increased resistance consists of higher rates of resistance to the C-8-methoxyfluoroquinolones (ie, gatifloxacin and moxifloxacin), there is also evidence to suggest that the toxinotype III epidemic strain has higher minimum inhibitory concentrations within the resistant range to levofloxacin. \(^13\) The small number of isolates tested as part of our study precluded a comparison of categorical resistance to the C-8-methoxyfluoroquinolones or a minimum inhibitory concentration comparison for levofloxacin. However, our finding of fluoroquinolones as an independent risk factor for disease, along with a temporal association between the outbreak and an increase in levofloxacin hospital-wide use, is consistent with the study of Muto et al, \(^21\) who found that increased levofloxacin use was associated with an outbreak. Our results, together with those of Muto and colleagues, suggest an etiologic role for the fluoroquinolones that is not limited to C-8-methoxyfluoroquinolones, which were not in use at the hospital during the outbreak period.

Our finding of proton pump inhibitors as an independent risk factor for CDAD is consistent with some recent reports of hospital outbreaks involving the toxinotype III epidemic strain \(^21,22\) but with not others. \(^23\) Because *C difficile* spores are relatively acid resistant, the alteration of gastric pH is thought to have little impact on spore survival during passage through the alimentary tract. Therefore, the role of medications that suppress gastric acidity on transmission remains controversial. However, proton pump inhibitors also have antibacterial properties that could affect the lower intestinal flora and increase the risk of CDAD in that way. \(^24\) A recent report of risk factors for community CDAD offers additional evidence that these medications increase a patient’s risk. \(^25\) In the hospital study by Muto et al \(^21\) and the recent community CDAD study by Dial et al \(^22\) both H\(_2\) blockers and proton pump inhibitors were independent risk factors for CDAD. Our sample size may have been simply too small to detect the smaller increased risk associated with H\(_2\) blockers relative to more potent acid-suppressing proton pump inhibitors.

Our study has several limitations. First, we had to rely on a small number of *C difficile* isolates from outbreak case patients, and we had no isolates from preoutbreak patients to determine whether the outbreak was due to the introduction of a new strain. Second, the sampling at the hospital environment and the LTCH environment was performed after intensive infection control interventions had been implemented, including bleach cleaning of case patients’ rooms and equipment. This cleaning may have reduced any environmental contamination that may have been present during the outbreak and resulted in very few environmental isolates.

In light of our findings, as well as reports of increased disease severity \(^24\) and the widespread emergence of this epidemic strain, \(^13,20\) certain measures should be taken. First, the infection control response to outbreaks must be multifaceted, coordinated with local departments of health and other hospitals, and include contact isolation precautions and enhanced environmental cleaning. It is important that hospitals institute surveillance to detect outbreaks. Evaluation of prior health care exposure in patients with CDAD may be an easy and practical way of determining the likely location of *C difficile* acquisition and may help direct early implementation of targeted infection control interventions. Second, transmission prevention measures alone may not be enough to control an outbreak of *C difficile*. Judicious antimicrobial use in hospitalized patients, especially in those at risk for disease (eg, because of old age, comorbid conditions, surgery, or chemotherapy), is critical. Finally, clinicians should become more aware of potential risks associated with gastric acid–suppressive medications and administer them judiciously.

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