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Drudy, D. et al (2006). Emergence and control of Fluoroquinolone Resistant Toxin A Negative Toxin B positive Clostridium difficile. *Infection Control and Hospital Epidemiology*, 28(8), pp. 932-40. doi:10.1086/519181

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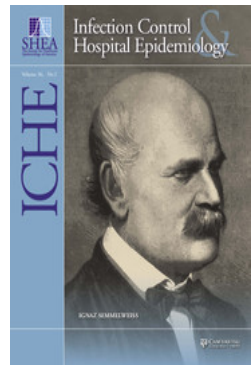
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Infection Control / Volume 28 / Issue 08 / August 2007, pp 932 - 940
DOI: 10.1086/522269, Published online: 02 January 2015

Link to this article: http://journals.cambridge.org/abstract_S0195941700047056

How to cite this article:

Denise Drudy, Norma Harnedy, Séamus Fanning, Margaret Hannan and Lorraine Kyne (2007). Emergence and Control of Fluoroquinolone-Resistant, Toxin A–Negative, Toxin B–Positive *Clostridium difficile*. Infection Control, 28, pp 932-940
doi:10.1086/522269

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ORIGINAL ARTICLE

Emergence and Control of Fluoroquinolone-Resistant, Toxin A–Negative, Toxin B–Positive *Clostridium difficile*

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BACKGROUND. *Clostridium difficile* is a major cause of infectious diarrhea in hospitalized patients. Between August 2003 and January 2004, we experienced an increase in the incidence of *C. difficile*-associated disease. We describe the investigation into and management of the outbreak in this article.

METHODS. A total of 73 consecutive patients with nosocomial *C. difficile*-associated diarrhea were identified. *C. difficile* isolates were characterized using toxin-specific enzyme immunoassays, a tissue-culture fibroblast cytotoxicity assay, polymerase chain reaction (PCR), and antimicrobial susceptibility tests. Rates of recurrence and of *C. difficile* colitis were recorded. Changes in antibiotic use and infection control policies were documented.

RESULTS. The incidence of *C. difficile*-associated diarrhea peaked at 21 cases per 1,000 patient admissions. Of the *C. difficile* isolates recovered, 85 (95%) were identical toxin A–negative and toxin B–positive strains, corresponding to toxinotype VIII and PCR ribotype 017. All clonal isolates were resistant to multiple antibiotics, including ofloxacin, ciprofloxacin, levofloxacin, moxifloxacin, and gatifloxacin (minimum inhibitory concentrations [MICs] of greater than 32 µg/mL) and erythromycin, clarithromycin, and clindamycin (MICs of greater than 256 µg/mL). Recurrent *C. difficile*-associated disease occurred in 26 (36%) of the patients. At least 10 (14%) of the patients developed *C. difficile* colitis. Additional infection control measures introduced included the use of ward memos, a hand-hygiene awareness campaign, increased environmental cleaning, attention to prescribing practices for antibiotics, increased awareness of diarrheal illness, and early isolation of affected patients. Total use of fluoroquinolones did not change throughout the study period. Despite persistence of this toxin-variant strain, the incidence of *C. difficile*-associated disease in our institution decreased to fewer than 5 cases per 1,000 admissions.

CONCLUSIONS. We report on the emergence of a fluoroquinolone- and clindamycin-resistant, toxin A–negative, and toxin B–positive strain of *C. difficile* associated with an outbreak of *C. difficile*-associated disease in our institution during a 6-month period. We found that careful attention to improvement of infection control interventions was the most important means of controlling this nosocomial pathogen.

Infect Control Hosp Epidemiol 2007; 28:932-940

Clostridium difficile is a common nosocomial pathogen and a major cause of infectious diarrhea in hospitalized patients.¹ Patients who receive antibiotic therapy are particularly susceptible to colonization with *C. difficile* and subsequent *C. difficile*-associated disease (CDAD).² The risk of CDAD appears to be greater with certain antimicrobial agents.³⁻⁶ Recent studies also suggest that the risk of CDAD increases if *C. difficile* is resistant to the administered antimicrobial.⁶ Resistance to clindamycin and third-generation cephalosporins has been well documented; however, resistance to fluoroquinolones, in particular the newer generation fluoroquinolones, is a more recent development.⁶⁻⁸ Other worrisome trends in the epidemiology and pathogenesis of CDAD include the emergence of toxin-variant and hypervirulent toxin-produc-

ing strains; the latter have been associated with more-severe disease and increased 30-day mortality.^{3,9-11}

There is ongoing surveillance of all patients with CDAD in our institution (The Mater Misericordiae University Hospital; Dublin, Ireland). From 2001 to 2003, we experienced winter peaks in the incidence of CDAD. From June 2001 to May 2002, levofloxacin was the first-line antimicrobial agent used for treatment of community-acquired pneumonia (CAP). This regimen was switched to cefuroxime plus clarithromycin in May 2002 because of concerns regarding the emergence of fluoroquinolone resistance in *Streptococcus pneumoniae* strains. However, the incidence of CDAD increased to 33 cases per 1,000 patient admissions in January 2003, resulting in the discontinuation of use of cefuroxime as a first-line agent for

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Received September 18, 2006; accepted February 15, 2007; electronically published June 29, 2007.

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CAP. Since April 2003, ofloxacin and benzylpenicillin have been the recommended first-line agents for CAP in our hospital. Despite a decrease in the incidence of CDAD in May and June 2003, we became aware of an increase in the incidence of CDAD in July 2003, when rates increased to more than 10 cases per 1,000 patient admissions. As part of an investigation into this outbreak, we performed cultures, typed strains, and performed antimicrobial-susceptibility testing on all isolates recovered from patients with CDAD during this period. We also documented rates of recurrence and of *C. difficile* colitis.

METHODS

Study Population and Specimens

The Mater Misericordiae University Hospital is a 570-bed, university-affiliated hospital in Dublin, Ireland, that serves a population of approximately 250,000, 11% of whom are aged 65 years or older. There are 37 wards that are arranged into 3-4 bays of 6 beds each. The average ratio of bathrooms to patients is 1 : 6. There are 84 single rooms, 39 of which are isolation rooms with anteroom and gowning facilities. Until 2000, there were 2 infection control nurses; this number increased to 4 in October 2002.

We prospectively studied all newly diagnosed cases of nosocomial CDAD between August 2003 and January 2004. Consecutive cases were identified by daily review of requests for assay of stool samples for *C. difficile* toxin (*C. difficile* Tox A/B II enzyme-linked immunosorbent assay; Techlab) at the clinical microbiology laboratory. Patients with a prior history of CDAD at our institution or of CDAD that was not nosocomially acquired were excluded from the study. A total of 73 patients received a diagnosis of newly acquired nosocomial CDAD in this period. Patients were monitored until hospital discharge or death. For each patient, we recorded age, sex, Charlson comorbidity index,¹² ward of admission, ward transfers, antimicrobial agents received before onset of CDAD, all medications received, type of feeding regimen (oral feeding, tube feeding [by nasogastric, percutaneous gastrostomy, or jejunostomy tube], or total parenteral nutrition), serum albumin levels, and white blood cell (WBC) count, and body temperature at the time of diagnosis of CDAD. Radiological or endoscopic investigations and specific anti-*C. difficile* therapies were recorded. The clinical management after the onset of *C. difficile*-associated diarrhea was also documented. All additional infection control interventions and overall use of quinolone and cephalosporin antibiotics were recorded.

Definitions

Diarrhea was defined as a change in bowel habit with 3 or more unformed bowel movements for at least 2 days. *C. difficile*-associated diarrhea was defined as diarrhea not attributed to any other cause and associated with a stool test positive for *C. difficile* toxin. Nosocomial *C. difficile*-associ-

ated diarrhea was defined as onset of *C. difficile*-associated diarrhea at least 48 hours after admission in a patient with no known previous history of CDAD. Recurrent CDAD was defined as the development of a new episode of CDAD confirmed by a positive results of *C. difficile* toxin test of stool during a 60-day follow-up period, after resolution of the episode that began at enrollment for at least 48 hours and after discontinuation of therapy with metronidazole or vancomycin. Patients were classified as having *C. difficile* colitis on the basis of radiological or endoscopic findings.

Culture of Isolates

C. difficile isolates were cultured on cyloserine-cefoxitin-fructose agar. Ninety *C. difficile* isolates were recovered from 64 of 73 patients in this study and included a single isolate from each patient with 1 episode of diarrhea and 1 isolate representing each recurrent episode of diarrhea. We were unable to culture *C. difficile* from fecal specimens from 9 patients who tested positive for *C. difficile* toxin, because these patients had received metronidazole. However, these patients were still regarded as having tested positive for *C. difficile* in the epidemiological evaluation.

Testing for *C. difficile* Toxins

Toxin-specific immunoassays and cytotoxicity assays were used to detect toxin production after culture of *C. difficile* in brain-heart infusion broth for 72 hours. Toxins A and B were detected using the *C. difficile* Tox A/B II test (Techlab) and a tissue-culture cytotoxin assay (IMR-90; European Collection of Cell Cultures), respectively.

Molecular Analysis of Isolates

Genomic DNA was extracted from broth cultures of *C. difficile* and was quantified using the PicoGreen dsDNA Quantitation Kit (Molecular Probes). Polymerase chain reaction (PCR) restriction-fragment-length polymorphism (RFLP) "toxintyping" was used to investigate all 90 *C. difficile* isolates.^{13,14} PCR ribotyping was performed in accordance with Stubbs et al.,¹⁵ with some minor modifications. DNA fingerprints were stored as tagged-image-file-format (TIFF) files and were imported into BioNumerics software, version 4.0 (Applied Maths), with which dendrograms were created using the Dice coefficient and the unweighted pair-group method with arithmetic mean for cluster correlation.

Antibiotic Susceptibility Testing

Susceptibility to clindamycin, erythromycin, clarithromycin, ofloxacin, ciprofloxacin, levofloxacin, gatifloxacin, and moxifloxacin was determined using the Etest (AB Biodisk). The minimum inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic that prevented visible growth after 48 hours under anaerobic conditions and was interpreted according to Clinical and Laboratory Stan-

TABLE 1. Infection Control Interventions Used Before and During an Outbreak of *Clostridium difficile* Infection, by Year

2002
Daily surveillance of patients with gastrointestinal symptoms
Purchase of new bedpans and commodes
Additional ward cleaning
Increase in the number of infection control personnel
Introduction of Spirigel alcohol-based hand rub
2003
Distribution of ward memos
Topic: hand hygiene
Topic: early recognition of symptoms
Topic: early isolation and cohorting on the basis of clinical symptoms
Increased awareness and improved reporting of infection
Lectures given at grand rounds and postgraduate lectures on infection control
Hand-hygiene awareness campaign
Enhanced environmental cleaning

dards Institute (CLSI) breakpoints for clindamycin and trovofloxacin.

Statistical Analysis

Patients with severe CDAD and patients with nonsevere CDAD were compared. We used the χ^2 test or 2-tailed Fisher exact test, for categorical variables, and the Student *t* test or Wilcoxon rank-sum test, for continuous variables. We used multivariable logistic regression analyses to determine the independent predictors of severe disease. Age and sex were entered into each model, along with variables associated with the outcomes in univariate analyses ($P < .1$). Only variables statistically significantly associated with the outcomes were included in the final models. All analyses were performed using the SAS software system, version 6.12 (SAS Institute). The α level was set at 0.05. All *P* values were 2 sided.

RESULTS

Incidence of CDAD

From July 2003 through January 2004, there were 73 consecutive patients with CDAD and the corresponding *C. difficile* isolates analyzed. In September 2003, the incidence of CDAD peaked at 21 cases per 1,000 patient admissions. Heightened infection control measures were introduced and are outlined in Table 1 and below. These measures included recommendations that all antibiotic prescriptions should be reviewed with regard to specific start and stop dates and the necessity for antibiotic treatment. However, fluoroquinolone use was not discouraged, and ofloxacin and benzylpenicillin remained the first-line agents for treatment of CAP. By January 2004, incidence rates had returned to baseline levels and have remained stable up to the time that the analysis was

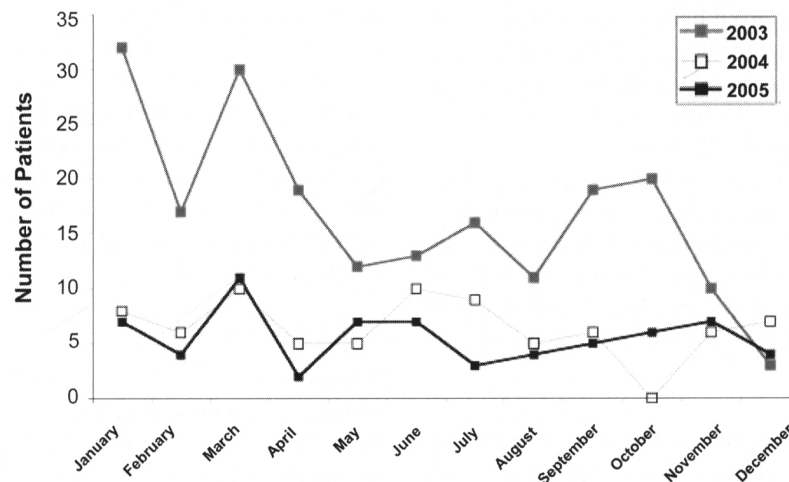


FIGURE 1. Annual time line showing the number of new patients with nosocomial *Clostridium difficile*-associated disease at Mater Misericordiae University Hospital in 2003 (gray boxes), 2004 (open boxes), and 2005 (black boxes), by month.

TABLE 2. Demographic and Clinical Characteristics of Patients With *Clostridium difficile*-Associated Disease (CDAD) and Factors Associated With Severe Disease

Characteristic	Patients with severe disease (n = 10)	Patients with nonsevere disease (n = 63)	All patients (n = 73)	P
Male sex	7 (70)	26 (41)	33 (45)	.09
Age, years				
Mean \pm SD	70.6 \pm 14.6	72.7 \pm 12.9	72.4 \pm 13.0	
Median (range)	72.5 (38-88)	73 (29-95)	73 (29-95)	NS
Albumin level at admission, g/L				
Mean \pm SD	29.9 \pm 7.5	26.7 \pm 6.5	27.1 \pm 6.7	
Median (range)	29.5 (15-39)	28 (10-40)	28 (10-40)	NS
No. of antibiotics received before diagnosis of CDAD				
Mean \pm SD	1.6 \pm 0.7	1.6 \pm 0.8	1.6 \pm 0.8	
Median (range)	1.5 (1-3)	2 (0-3)	2 (0-3)	NS
Use of H ₂ -receptor antagonists	1 (10)	8 (13)	9 (12)	NS
Use of proton-pump inhibitors	5 (50)	39 (62)	44 (66)	NS
Intensive care unit	5 (50)	14 (22)	19 (26)	.06
Enteral feeding ^a				
Any	8 (80)	28 (44)	36 (49)	.04
Nasogastric feeding	8 (80)	26 (41)	34 (47)	.02
Charlson comorbidity index				
Mean \pm SD	1.6 \pm 1.7	4.5 \pm 2.2	4.1 \pm 2.3	
Median (range)	1.5 (0-5)	4 (0-12)	4 (0-12)	.0003
No. of comorbidities				
Mean \pm SD	3.8 \pm 1.9	6.1 \pm 2.6	5.8 \pm 2.6	
Median (range)	6 (1-12)	6 (1-12)	6 (0-12)	.01

NOTE. Data are no. (%) of patients, unless otherwise indicated. NS, not significant.

^a Feeding by nasogastric, percutaneous gastrostomy, or jejunostomy tube.

completed (fewer than 5 cases per 1,000 patient admissions). Figure 1 shows all new cases of CDAD from 2003 through 2005.

Patient Characteristics and Outcomes

The 73 patients affected were predominantly older white patients (median age, 73 years; range, 29-95 years). Most had clinically significant comorbid disease (median Charlson comorbidity score, 4; range, 0-12). Nineteen (26%) of the patients had severe underlying disease and spent some time in the intensive care unit (ICU) or high-dependency unit. Other baseline characteristics are shown in Table 2.

Recurrent CDAD

Of the 73 patients studied, 47 (64%) had a single episode of CDAD, 26 (36%) had a single recurrent episode of CDAD, and 7 (27%) had a second recurrence of CDAD during the outbreak period. All patients with recurrent CDAD were given treatment with antibiotics for 10 days. The frequency of recurrence based on the type of treatment received was not examined.

C. difficile Colitis

At least 10 (14%) of the patients developed *C. difficile* colitis. All these patients showed evidence of colitis on abdominal computed tomography (CT) and had median WBC counts of 18.8×10^9 cells/L (range, 10.2×10^9 - 67.2×10^9 cells/L). One of these patients had a total colectomy for extensive pseudomembranous colitis. He survived the initial illness but subsequently died of unrelated causes. Of the other 9 patients with colitis, 4 died within 30 days after the onset of symptoms. Patients with severe disease were more likely to be male ($P = .09$) and to have severe underlying disease that necessitated treatment in the ICU ($P = .06$) (Table 2). Although patients with more-severe disease had significantly lower scores on the Charlson comorbidity index ($P = .0003$), they were significantly more likely to have received enteral feeding ($P = .04$), in particular nasogastric feeding ($P = .02$), compared with patients with less severe disease. The unadjusted odds ratio (OR) for severe disease associated with nasogastric feeding was 5.69 (95% confidence interval [CI], 1.12-29.00); however, in a multivariable logistic regression analysis, after adjustment for age and ICU stay, the OR was 4.69 (95% CI, 0.81-28.62). No other independent predictors of severe dis-

TABLE 3. Minimum Inhibitory Concentration (MIC) Values for Fluoroquinolone and Macrolide Antibiotics for 85 Toxin-Variant *Clostridium difficile* Isolates (TV) and 5 Nonvariant Isolates (NV)

Antibiotic, by class	MIC, µg/mL ^a							
	MIC ₅₀		MIC ₉₀		Range		Median	
	TV	NV	TV	NV	TV	NV	TV	NV
Fluoroquinolone								
Ofloxacin	>32	>32	>32	>32	>32	>32	>32	>32
Ciprofloxacin	>32	>32	>32	>32	>32	>32	>32	>32
Levofloxacin	>32	2	>32	2	>32	1.5-2	>32	2
Moxifloxacin	>32	0.25	>32	0.5	>32	0.125-0.5	>32	0.315
Gatifloxacin	>32	0.25	>32	0.38	>32	0.25-0.38	>32	0.25
Macrolide								
Clindamycin	>256	4	>256	4	>256	4	>256	4
Erythromycin	>256	1.5	>256	1.5	>256	1.5	>256	1.5
Clarithromycin	>256	0.5	>256	0.5	>256	0.5	>256	0.5

NOTE. The 85 toxin-variant *C. difficile* isolates were negative for toxin A and positive for toxin B. The 5 nonvariant isolates were positive for toxins A and B. MIC₅₀, minimum inhibitory concentration required to inhibit growth of 50% of organisms; MIC₉₀, minimum inhibitory concentration required to inhibit growth of 90% of organisms.

^a MICs that were beyond the detection limits of the test used are so indicated: >32 µg/mL for fluoroquinolones and >256 µg/mL for macrolides.

ease were identified (Table 2). All patients with severe disease were infected with a fluoroquinolone-resistant toxin-variant *C. difficile* strain.

Clinical Management of CDAD

Whenever possible, use of antibiotics that predispose to CDAD was discontinued; however, many patients had concurrent bacterial infections that necessitated continued use of antibiotics. One patient with a single episode of CDAD received supportive care only. The remaining 72 patients were treated with metronidazole and/or vancomycin. Use of proton-pump inhibitors and H₂-receptor antagonists was continued. Patients did not receive probiotics. Contact isolation was implemented when possible. With the rare exception of patients who could not be isolated, the ward was quarantined and no patients were admitted or discharged from that ward until they were symptom free for longer than 48 hours and until laboratory results confirmed no evidence of CDAD. If a case of CDAD was identified on a 6-bed unit, additional cases were sought.

Trends in Antibiotic Use

Sixty-nine (95%) of the patients received 1 or more antimicrobial before the onset of CDAD (Table 3). Thirty (41%) of the patients received treatment with a single antibiotic, and 30 patients (41%) received treatment with 2 antibiotics. Four patients did not receive any antibiotics while in the hospital. Of the patients with CDAD, 33 (27%) had received augmentin and ofloxacin, alone or in combination. Trends in antibiotic prescribing since 2001 for individual fluoroquinolones and cephalosporins and for each class as a whole are

shown in Figure 2. Total cephalosporin use in 2002 (16,992 g) was almost twice that in 2001 (31,132 g). In contrast, total use of cephalosporins from 2003 through 2005 showed a downward trend, with 15,140 g used in 2003, 13,719 g used in 2004, and 11,696 g used in 2005. Total use of fluoroquinolones in 2002 through 2005 was almost half that in 2001 (Figure 2).

Infection Control Interventions

A number of changes were introduced to the hospital's infection control policy in 2002, after the first case of norovirus gastroenteritis was diagnosed in the hospital. These included the following measures: (1) an audit of all sinks, soaps, and moisturizers used throughout the hospital; (2) a switch from a liquid hand gel to alcohol-based hand rub (Spirigel; Spiral), to increase sanitizer coverage; (3) an increase in infection control personnel; and the resulting (4) increased patient surveillance, in which every ward was visited on a daily basis and every patient with undiagnosed diarrhea was surveyed. For each patient with diarrhea, a surveillance form was filled out by the infection control staff, with a detailed medical history and information about prior diarrheal episodes, antibiotics and/or stool looseners received, and prior CDAD. All patients with diarrhea had stool samples routinely screened for *Salmonella*, *Shigella*, and *Campylobacter* species, as well as *Escherichia coli* serotype 0157, *C. difficile*, and norovirus. New equipment was purchased; this included new commodes that could be easily cleaned and bedpans that fit both the commodes and the standard bedpan washers. In addition, bedpan washers were monitored to ensure that the correct washing temperature was consistently reached.

During the outbreak in 2003, several additional infection control measures were included, along with heightened enforcement and awareness of existing measures. Efforts were increased for the early identification and isolation of patients with CDAD. During the outbreak period, a container of alcohol-based hand rub was placed at the end of the bed of every patient with diarrhea. Healthcare workers with minor contact with patients and clinically clean hands (according to World Healthcare Organization guidelines) used alcohol-based hand rub to clean their hands between caring for different patients. When extensive patient contact had occurred, HCWs were encouraged to wash their hands with soap and water. A multifaceted education program was introduced, and a major emphasis was placed on education and awareness of this new hand-hygiene policy throughout the hospital. This included a hand-hygiene campaign that targeted existing staff, new staff, and patients. Lectures on hygiene were given by infection control staff and consultants at grand rounds, staff-induction meetings, and meetings of the infection control committee. Regular random checks with the ultraviolet test for adequate hand hygiene were performed on staff throughout the hospital. Enhanced measures to control the environmental transmission of *C. difficile* were also introduced. These included increasing the frequency of environmental cleaning from once to twice daily with use of the standard chlorine-releasing disinfectant (1,000 ppm); enhanced cleaning of patient furniture, in which armchairs were cleaned as part of daily cleaning procedure; and enhanced cleaning of shared equipment, such as sphygmomanometer cuffs for measuring blood pressure. Use of shared equipment was restricted to similarly affected patients if used by a patient with diarrhea.

Molecular Analysis of *C. difficile* Isolates

Ninety *C. difficile* isolates were cultured from 64 of the 73 patients in this study. Sixty patients were infected with toxin A-negative, toxin B-positive *C. difficile* isolates, whereas 4 patients were infected with toxin A-positive, toxin B-positive *C. difficile* isolates. All sample isolates were positive for *C. difficile* toxin B by use of the tissue-culture cytotoxicity assay (data not shown). Eighty-five (95%) of the isolates induced a differential cytopathic effect, with complete rounding of the fibroblast body in comparison with wild-type cytopathic effect, in which protrusions remain attached to the cell body.¹⁶ None of these isolates produced detectable toxin A (data not shown). Toxinotyping identified a 1.7-kb deletion located in the repeating domain of the *tcdA* gene in these isolates. PCR identified RFLPs in the A3, B1, and B3 domains, and isolates were designated toxinotype VIII, in accordance with the scheme of Rupnik et al.¹⁴ The 5 remaining isolates carried the entire *tcdA* and *tcdB* genes, corresponding to toxinotype 0. All 90 isolates were analyzed by 16S-23S PCR ribotyping, producing DNA banding patterns consisting of 10-14 bands, with a size range of approximately 100-1,000 bp. One major cluster of 85 toxin A-negative, toxin B-positive *C. difficile* isolates was identified on the basis of a quantitative analysis of the banding patterns obtained, and all isolates demonstrated banding patterns indistinguishable from strain type 1470 and PCR ribotype 017 (data not shown).

Antibiotic Susceptibility Testing

The MICs for the 90 *C. difficile* isolates from this study are shown in Table 3. All isolates negative for toxin A and positive for toxin B demonstrated a high level of resistance to the 5

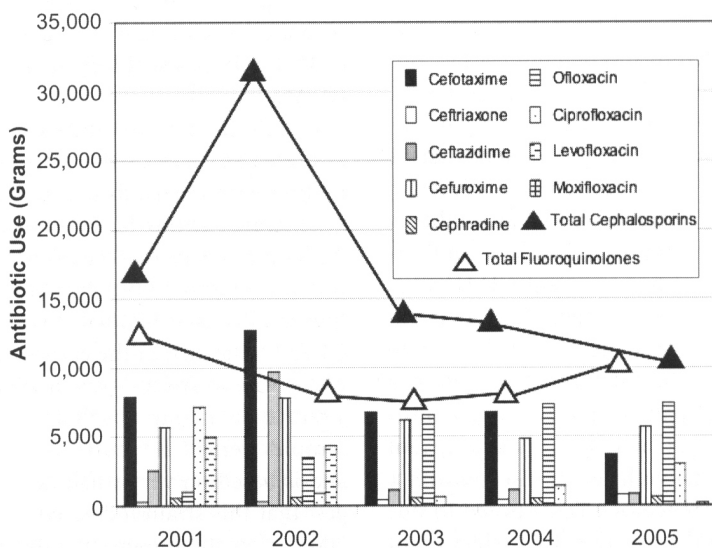


FIGURE 2. Fluoroquinolone and cephalosporin usage at Mater Misericordiae University Hospital, 2001-2005. Total use of fluoroquinolones (open triangles) and cephalosporins (black triangles) is overlaid as a line graph.

fluoroquinolones tested, with MICs of greater than 32 µg/mL. Resistance to clindamycin, erythromycin, and clarithromycin was also identified, with MICs of greater than 256 µg/mL. In contrast, the MICs of the unrelated isolates positive for toxins A and B were markedly reduced for levofloxacin, moxifloxacin, and gatifloxacin, with median MICs of 3, 0.3, and 0.2 µg/mL, respectively. Likewise, these isolates positive for toxins A and B were up to 9-fold more susceptible to clindamycin, erythromycin, and clarithromycin, with median MICs of 4, 1.5, and 0.5 µg/mL, respectively.

DISCUSSION

We experienced a significant increase in the incidence of CDAD at our institution over a 6-month period in 2003; there were 73 patients diagnosed with CDAD, at least 14% of whom had evidence of colitis. During this period, we saw the emergence of a toxin-variant clone (negative for toxin A and positive for toxin B) that was resistant to fluoroquinolones and macrolide, lincosamide, and streptogramin antibiotics. The trigger for the clonal expansion of this strain type is not known, but we speculate that it may have been the widespread use of fluoroquinolones and cephalosporins in our institution in the preceding years, 2001 and 2002. Control of this toxin-variant strain was achieved through infection control measures other than curtailing the use of fluoroquinolones and cephalosporins. The hand-hygiene policy was changed in 2002, when alcohol-based hand rub was introduced, and was not associated with an increase in the incidence of *C. difficile* infection, which is similar to findings reported by Boyce et al.¹⁷ and Gordin et al.¹⁸ Although the hand-hygiene policy was not altered with regard to the use of alcohol hand rub, heightened awareness and enforcement of this policy is likely to have contributed to control of this outbreak.

Toxin-variant (toxin A–negative and toxin B–positive) *C. difficile* strains have been isolated in several countries worldwide. Prevalence rates vary from 0.2% to 37% of isolates.^{19–22} Although several sporadic cases of CDAD due to toxin-variant strains have been reported, only 2 hospitals have previously documented outbreaks of infection with this strain type. Al-Barrak et al.²³ were the first to report an outbreak of infection with a toxin-variant strain of *C. difficile*, in Canada in 1998, in which 14 cases of toxin-variant CDAD were diagnosed. In that study, 5 (36%) of the patients had recurrent diarrhea, whereas 3 (20%) had severe colitis, associated with a 66% mortality rate. Kuijper et al.²⁴ described an outbreak in The Netherlands in which 24 patients received a diagnosis of toxin-variant CDAD. Three patients (13%) had recurrent disease, and there were 7 patients with severe disease, with 1 death (14%). The rate of recurrence of CDAD noted in our study (36%) and in other outbreaks due to toxin-variant strains are similar to recurrence rates reported by us and others for disease caused by isolates positive for toxins A and B.²

The rate of severe disease documented in our study is

higher than that reported elsewhere for nonvariant *C. difficile*. In an earlier prospective study of patients who acquired toxin A–positive, toxin B–positive *C. difficile* in Boston, we found that less than 5% developed severe pseudomembranous colitis.² Because 95% of the isolates we found were toxin variant, we were unable to compare disease severity rates associated with variant and nonvariant strains in our hospital. The prospective nature of our investigation into this outbreak did allow us to assess each patient closely for symptoms of severe disease. We used criteria for disease severity similar to those used by McEllistrem et al.¹¹ in a recent investigation of a hospital outbreak of CDAD. In that study, the rate of severe disease was 16.3%, which is similar to the rate in our study (14%).¹¹ It is possible that we underestimated the rate of severe disease because the decision to investigate patients by use of CT was determined by the physician responsible for the healthcare provision to those patients and was generally reserved for sicker patients. We feel that it is unlikely that we overestimated the rate of severe disease, because in addition to having CT evidence of colitis, all patients classified as having severe disease had markedly elevated WBC counts, elevated temperatures, and abdominal pain or distension.

We found that patients who developed more-severe disease had fewer comorbidities than did patients who developed less-severe disease. However, the former were sicker patients; 50% of them were cared for in the ICU, and 80% of them received nasogastric feeding. Although nasogastric feeding was significantly associated with more-severe disease on univariate analysis, this was a marker for severe underlying disease, since the association lost significance once we controlled for age and ICU stay. A limitation of our study was that patients were studied after their diagnosis of CDAD was confirmed by laboratory testing. Consequently we were unable to assess the severity of underlying diagnosis before the onset of symptoms. We showed, in a previous study, that severity of underlying disease is a strong independent risk factor for CDAD.¹² It is also likely to be a risk factor for severity of CDAD.

C. difficile is not cultured routinely in microbiology laboratories in Ireland; therefore, we have little historic information with regard to the molecular epidemiology of *C. difficile* strains at our facility and at other Irish hospitals. This limitation means that we cannot predict when this strain type was introduced to our hospital or when it increased in frequency. Limited historical strain typing from outbreaks of CDAD indicate that the earliest documentation of a PCR type 017 isolate in Ireland was in 1996, and the first record of this strain type at our hospital was in July 2001 (when 3 of 8 samples were PCR type 017). Although our evidence is limited and is based on very small random-sample sizes, it does suggest that this strain increased in frequency between 2001 and 2003. After the present investigation, we identified this strain type in a number of other Irish hospitals.^{25,26} Unfortunately, *C. difficile*–positive status is shown only in a patient's medical record at a given healthcare facility, and this information is

not transmitted routinely when patients are transferred between facilities. This lack of epidemiological data makes it impossible to trace the emergence of this *C. difficile* clone throughout the greater Dublin area.

Prior antibiotic use is an important individual risk factor for CDAD. Fluoroquinolone use has recently emerged as a predominant risk factor in epidemics in Pittsburgh, Pennsylvania, and Quebec, Canada.^{5,9} In April 2003, before the observed increase in CDAD, the antibiotic formulary had changed such that ofloxacin and benzyl penicillin were recommended as first-line agents for the treatment of CAP. Before this, in 2001 and the first 5 months of 2002, levofloxacin was used extensively in our hospital to treat CAP. This was switched to cefuroxime and clarithromycin in May 2002. The overall use of fluoroquinolones was halved in 2002 and did not alter significantly between 2002 and 2005. However, we speculate that use of levofloxacin, which accounted for almost half of the total use of fluoroquinolones in 2001 and 2002, may have promoted the acquisition of fluoroquinolone resistance by *C. difficile*, leading to the emergence of this variant strain. Fluoroquinolone use continued in our hospital throughout the outbreak, and despite this, the incidence of CDAD declined. However, the fluoroquinolones used were predominantly older classes (eg, ofloxacin and ciprofloxacin) with lower antianaerobic activity than that of the newer classes of fluoroquinolones recently associated with increased risk of CDAD (levofloxacin and gatifloxacin).^{5,8,9} Although fluoroquinolones may have contributed to the emergence of this variant strain, we achieved control of this variant strain through much greater attention to infection control interventions, rather than through a change in the antibiotic use policy.

Ongoing surveillance at our hospital indicates the persistence of this toxin A-negative, toxin B-positive *C. difficile* strain, even though the incidence rate remains low. Recently, investigators have determined that greater sporulation capacity, along with increased antimicrobial resistance, may contribute to the persistence of certain nosocomial *C. difficile* isolates; however, the mechanisms by which the toxin A-negative, toxin B-positive strain persists have not yet been examined. The increased resistance to macrolide, lincosamide, and streptogramin antibiotics as well as fluoroquinolones seen among the isolates in our outbreak is likely to contribute to their persistence in both the hospital environment and the human gut. Isolates from the 2 previously documented outbreaks of CDAD due to toxin A-negative, toxin B-positive strains do not appear to have been tested for fluoroquinolone resistance. However, as in the outbreak at our hospital, isolates from the outbreak in The Netherlands had an *erm B* gene that coded for clindamycin resistance.²⁴ Surveillance after the outbreak in The Netherlands demonstrated that the toxin A-negative, toxin B-positive isolates did not persist, and those toxin A-negative, toxin B-positive strains are no longer found at that institution (E. Kuijper, oral communication).

In summary, we found that a *C. difficile* strain negative for

toxin A and positive for toxin B and resistant to fluoroquinolones and macrolide, lincosamide, and streptogramin antibiotics is prevalent at our institution. Studies that address the molecular epidemiology and antibiotic susceptibility of *C. difficile* strains will help increase our understanding of the factors that lead to institutional outbreaks. The virulence factors associated with epidemic strain types must be precisely defined if the incidence of CDAD is to decline substantially.

ACKNOWLEDGMENTS

We thank the Scientific Staff and the Infection Control Team at the Department of Microbiology, Mater Misericordiae University Hospital, for their assistance with sample collection and for useful comments.

Financial support. Supported in part by a K23 Patient-Oriented Career Development award from the US National Institute on Aging for a project entitled "Aging and the Human Antibody Response to *C. difficile*," a Health Research Board Ireland grant (RP/2005/72 [to L.K.]), and the Newman Scholarship Programme "Diageo Newman Scholarship in Food Safety" (to D.D.).

Potential conflicts of interest. All authors report no conflicts of interest relevant to this article.

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