Fluoroquinolone and Macrolide Exposure Predict *Clostridium difficile* Infection with the Highly Fluoroquinolone- and Macrolide-Resistant Epidemic *C. difficile* Strain BI/NAP1/027

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Antibiotics have been shown to influence the risk of infection with specific *Clostridium difficile* strains as well as the risk of *C. difficile* infection (CDI). We performed a retrospective case-control study of patients infected with the epidemic BI/NAP1/027 strain in a U.S. hospital following recognition of increased CDI severity and culture of stools positive by *C. difficile* toxin immunoassay. Between 2005 and 2007, 72% (103/143) of patients with first-episode CDIs were infected with the BI strain by restriction endonuclease analysis (REA) typing. Most patients received multiple antibiotics within 6 weeks of CDI onset (median of 3 antibiotic classes). By multivariate analysis, fluoroquinolone and macrolide exposure was more frequent among BI cases than among non-BI-infected controls (odds ratio [OR] for fluoroquinolones, 3.2; 95% confidence interval [CI], 1.3 to 7.5; ($P < 0.001$); OR for macrolides, 5.2; 95% CI, 1.1 to 24.0; ($P = 0.04$)). In contrast, clindamycin use was less frequent among the BI cases than among the controls (OR, 0.1; 95% CI, 0.03 to 0.4; ($P = 0.001$)). High-level resistance to moxifloxacin and azithromycin was more frequent among BI strains (moxifloxacin, 49/102 [48%] BI versus 0/40 non-BI, $P = 0.0001$; azithromycin, 100/102 [98%] BI versus 22/40 [55%] non-BI, $P = 0.0001$). High-level resistance to clindamycin was more frequent among non-BI strains (22/40 [55%] non-BI versus 7/102 [7%] BI, $P = 0.0001$). Fluoroquinolone use, macrolide use, and *C. difficile* resistance to these antibiotic classes were associated with infection by the epidemic BI strain of *C. difficile* in a U.S. hospital during a time when CDI rates were increasing nationally due to the highly fluoroquinolone-resistant BI/NAP1/027 strain.

Antibiotic exposure is arguably the most important risk factor for *Clostridium difficile* infection (CDI). Alteration of the normally protective indigenous colonic microbiota by antibiotics is the mechanism most commonly proposed to make the host susceptible to *C. difficile* infection (1). Another proposed role for specific antibiotics is the selection and facilitation of infection by strains of *C. difficile* resistant to those antibiotics. We previously demonstrated that clindamycin use was a specific risk factor for infection with the clindamycin-resistant J strain (PCR ribotype 001), which was responsible for multiple U.S. hospital outbreaks in the early 1990s (2). The epidemic BI strain (PCR ribotype 027, pulsed-field gel electrophoresis [PFGE] type NAP1) has been associated with outbreaks and more severe disease in multiple hospitals throughout North America since 2001 (3, 4). The epidemic BI strain was more likely to be resistant to gatifloxacin and moxifloxacin than other, nonepidemic strains recovered in the outbreak hospitals or “historic” BI strains recovered in the 1980s and 1990s that were not associated with hospital outbreaks (4). Acquisition of a fluoroquinolone resistance-conferring mutation and a related conjugative transposon in this strain group appears to have been a key molecular event preceding these outbreaks (5). In order to study the risk of fluoroquinolones and other antibiotics for infection with the epidemic BI strain, we conducted a retrospective, case-control study of antibiotic exposures among sequential CDI cases in one hospital over a 2-year time period using stool culture, strain typing, and susceptibility testing of the recovered *C. difficile* isolates to define the cases and controls.

MATERIALS AND METHODS

Background. Gatifloxacin was introduced into the hospital formulary of the U.S. Veterans Health Administration (VHA) VISN 12 (Midwest) region in February 2004, replacing levofloxacin as the hospital’s preferred fluoroquinolone. Moxifloxacin was subsequently added to the hospital formulary in March 2006 to replace gatifloxacin, which was voluntarily removed from the U.S. market in May 2006 due to gatifloxacin-related glycemic adverse events (<https://www.federalregister.gov/articles/2008/09/09/E8-20938/determination-that-tequin-gatifloxacin-was-withdrawn-from-sale-for-reasons-of-safety-or-effectiveness>). Following the introduction of gatifloxacin, a marked increase in the incidence of CDI was noted in three hospitals within VISN 12 (6). In January 2005, there were two fatal cases of fulminant CDI in one of these hospitals, prompting the clinical microbiology laboratory to initiate anaerobic culture of stool specimens that were positive by toxin immunoassay for *C. difficile* on selective media. Isolates recovered from specimens were referred to our research laboratory for strain typing analysis.

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Study design. We conducted a retrospective case-control study at the Edward Hines Jr. Veterans Affairs Hospital to determine if use of specific antibiotic classes was associated with first-episode CDI caused by the epidemic BI strain. All patients admitted to the hospital between February 2005 and February 2007 with first-episode CDI were included in the study. First-episode CDI was defined as an episode of diarrhea with a positive *C. difficile* toxin assay without a history of a positive toxin assay and no previous treatment for CDI. Stool specimens positive for *C. difficile* toxin were subsequently cultured and the recovered isolates typed by restriction endonuclease analysis (REA). Cases and controls were defined as first-episode CDIs in which REA group BI and non-BI strains were recovered, respectively. Clinical and demographic data were collected through review of the computerized patient record system. All medications given to patients admitted to the hospital are scanned prior to administration and consequently listed in the Bar Code Medication Administration (BCMA) log report located in each patient's electronic medical record (EMR). This report was accessed for each patient and used to capture medication usage. For patients not admitted to the hospital, we were able to access outpatient prescription records through each patient's EMR to identify medication usage. We recorded all antibiotics used in the 6-week period prior to the first episode of CDI and included name, indication (if available), dose, and duration. All doses of antibiotics scanned as given were recorded per patient and used to calculate the defined daily doses (DDDs). DDDs were calculated using the methodology endorsed by the World Health Organization (WHO Collaborating Centre for Drug Statistics Methodology ATC/DDD Index, http://www.whocc.no/atc_ddd_index/). The use of gastric acid suppressants, proton pump inhibitors (PPIs), and histamine 2 receptor antagonists (H2RAs) within 1 week of the positive toxin assay was also recorded. Comorbidities, including active malignancy, HIV infection, history of transplantation, and diabetes, were collected, as well as age at the time of positive toxin assay and gender. Routine laboratory data, including white blood cell count (WBC), hemoglobin, hematocrit, and serum creatinine, were collected closest to the positive toxin assay (± 2 days). Albumin level was not routinely available and was collected over a period within 180 days of the positive toxin assay.

Microbiology. During the study period, the clinical laboratory used the Vidas *Clostridium difficile* A and B (bioMérieux) assay on stool specimens of patients suspected to have CDI. Stools with positive toxin assays were then cultured anaerobically on selective taurocholate-cycloserine-cefoxitin fructose agar (TCCFA) medium (7), and recovered isolates with typical *C. difficile* morphology were stored in chopped-meat medium tubes (7). Isolates were then subcultured to blood agar plates and incubated at 37°C for 48 h. Following isolation, REA was performed on the samples. Total cellular DNA was cut with HindIII restriction enzyme and separated by electrophoresis on a 0.7% agarose gel as described previously by Clabots et al. (8). HindIII restriction patterns with a 90% similarity index were included in the same REA group (one- or two-letter designation).

Agar dilution susceptibility testing was performed on all isolates as previously described, using the Clinical Laboratory and Standards Institute (CLSI)-recommended reference agar dilution method for anaerobes (9, 10). MIC values for ciprofloxacin, moxifloxacin, clindamycin, piperacillin-tazobactam, ceftriaxone, and azithromycin were determined and susceptibilities reported as follows. Gatifloxacin powder was not available for agar dilution testing; therefore, we tested moxifloxacin and used moxifloxacin susceptibility as a surrogate for gatifloxacin susceptibility. Fluoroquinolone, piperacillin-tazobactam, ceftriaxone, and clindamycin susceptibilities were based on interpretive categories for anaerobic susceptibilities established by CLSI (10). For fluoroquinolones and clindamycin, an MIC of ≤ 2 $\mu\text{g/ml}$ was considered susceptible, 4 $\mu\text{g/ml}$ intermediate, and ≥ 8 $\mu\text{g/ml}$ resistant. Although no breakpoints are available for macrolides against anaerobes, there is a spectrum of activity suggesting clinical potential (11). Isolates with MICs of ≥ 64 $\mu\text{g/ml}$ for ciprofloxacin, moxifloxacin, clindamycin, and azithromycin were considered to have high-level resistance. For piperacillin-tazobactam, an isolate with an MIC $\leq 32/4$ $\mu\text{g/ml}$ was considered susceptible

TABLE 1 Baseline characteristics of CDI cases (BI) and controls (non-BI)

Characteristic	BI (<i>n</i> = 103)	Non-BI (<i>n</i> = 40)	<i>P</i> value
Median (IQ range) age, yr	74 (65–80)	70 (59–80)	0.1
% male	99	98	
Mean (SD):			
WBC, K/ μl	16.1 (20.9)	11.7 (8.8)	0.02
Hemoglobin, g/dl	11.4 (2.2)	11.4 (1.4)	1.0
Albumin, g/dl	2.9 (0.6)	2.9 (0.9)	0.8
Serum creatinine, mg/dl	1.5 (1.2)	1.5 (1.4)	0.3
No. (%) with:			
Diabetes	39 (37.9)	15 (37.5)	1.0
Transplant	3 (2.9)	1 (2.5)	0.9
Malignancy	25 (24.3)	9 (22.5)	0.8
Proton pump inhibitor use	68 (66)	25 (63)	0.7

and $\geq 64/4$ $\mu\text{g/ml}$ resistant. For ceftriaxone, breakpoints for susceptibility, intermediate, and resistance were ≤ 16 $\mu\text{g/ml}$, 32 $\mu\text{g/ml}$, and >64 $\mu\text{g/ml}$, respectively.

Statistical analysis. Categorical data were compared using Fisher's exact test or the chi-square test. Continuous variables were compared using Student's *t* test for normally distributed data and the Wilcoxon rank sum test for nonnormally distributed data. We used logistic regression to model the relationship between antibiotic exposure and CDI attributed to the BI strain. All variables with *P* values of ≤ 0.1 in univariate analysis were included in the multivariable model. The final model included fluoroquinolones, macrolides, clindamycin, and linezolid.

RESULTS

Case and control characteristics. We identified a total of 143 patients with first-episode CDI, of whom 103 (72%) were infected with the BI strain. The other 40 patients were infected primarily with REA group J (*n* = 21, 15%) strains. Other REA group strains identified included A, G, K, L, X, Y, Z, AH, and DH (*n* ≤ 3 for each). There were no differences in baseline demographic characteristics and underlying comorbidities between the cases (BI associated) and controls (non-BI associated) (Table 1). The median (interquartile [IQ] range) ages for the BI cases versus the non-BI controls were 74 (65 to 80) and 70 (59 to 80) years, respectively. The distributions of comorbid diseases, including diabetes mellitus, HIV infection, history of transplantation, and history of malignancy, were similar in both groups. Laboratory values, including mean serum creatinine and mean serum albumin, were not statistically significantly different between groups, except for the mean white blood cell count, which was significantly higher in the BI cases than in the non-BI controls, (16.1 ± 20.9 versus 11.7 ± 8.8 K/ μl for cases and controls, respectively; *P* = 0.02). Proton pump inhibitor (PPI) use was similar in the two groups, at 66% (*n* = 68) in the BI cases and 62.5% (*n* = 25) in the non-BI controls. Total acid suppressant use was also similar in the two groups, with 70% (*n* = 72) of patients among the BI cases and 70% (*n* = 28) among the non-BI controls using either a PPI or H2RA.

Although CDI treatment and complications were not reviewed, patients infected with BI had poorer outcomes as reflected by mortality. The all-cause mortality at 90 days was higher in the BI cases than in the non-BI controls (31/103 [30%] versus 6/40 [15%]; *P* = 0.05). The mortality at 30 days was 14/103 (13.5%) for the BI cases and 3/40 (7.5%) for the non-BI controls (*P* = 0.24).

TABLE 2 Antibiotic exposures for CDI cases (BI) and controls (non-BI)

Antibiotic	<i>n</i> (%)		Univariate analysis		Multivariate analysis	
	BI (<i>n</i> = 103)	Non-BI (<i>n</i> = 40)	OR (95% CI)	<i>P</i> value ^a	OR (95% CI)	<i>P</i> value
Fluoroquinolones						
Total	63 (61)	17 (43)	2.1 (1.0–4.5)	0.05	3.2 (1.3–7.5)	<0.001
Ciprofloxacin	29 (28)	6 (15)	2.2 (0.8–5.8)	0.1		
Gatifloxacin and moxifloxacin	38 (37)	12 (30)	1.7 (0.5–2.7)	0.7		
Macrolides						
Total	24 (23)	2 (5)	5.8 (1.3–25.7)	0.02	5.2 (1.1–24.0)	0.04
Azithromycin	21 (20)	1 (2.5)		0.001		
Clindamycin	4 (3.9)	10 (25)	0.12 (0.04–0.41)	0.0005	0.1 (0.03–0.4)	0.001
First-generation cephalosporins	24 (23.3)	10 (25)	0.9 (0.4–2.4)	1.0		
Second- and third-generation cephalosporins	29 (28.2)	5 (12.5)	2.7 (0.9–7.7)	0.06		
Piperacillin-tazobactam	22 (20.3)	10 (25)	0.8 (0.3–1.8)	0.5		
Aminopenicillins	18 (17.5)	7 (17.5)	1.0 (0.4–2.6)	1.0		
Vancomycin	26 (25.2)	8 (20)	1.4 (0.6–3.3)	0.5		
Linezolid	2 (1.9)	3 (7.5)	0.2 (0.04–1.5)	0.1	0.2 (0.02–1.3)	0.09
Aminoglycosides	3 (2.9)	4 (10)	0.3 (0.06–1.3)	0.1	0.4 (0.08–2.0)	0.3
Median (95% CI) no. of antibiotics per patient	3 (2–3)	3 (2–3)		0.8		

^a By the Mann-Whitney test.

Antibiotic exposures. Antibiotic exposure was frequent in both cohorts, with all but one patient in each group receiving one or more antimicrobial agents. The majority of patients received >1 antibiotic prior to onset of CDI, with similar frequencies observed in the BI and non-BI cohorts (74% versus 70%). The median number of antibiotic classes prescribed prior to onset of CDI was three in each group. Overall, fluoroquinolones were the second most commonly used class of antibiotics in both groups, and exposure was more frequent in the BI cases than in the controls (odds ratio [OR], 2.1; 95% confidence interval [CI], 1.0 to 4.5; *P* = 0.05) (Table 2). Specific fluoroquinolone exposures tended to be more frequent in the BI cases as well, but these differences were not significant. Gatifloxacin or moxifloxacin exposure was recorded in 37% of the BI cases compared to 30% of the controls, and ciprofloxacin exposure was 28% and 15% in the cases and controls, respectively. Additionally, the majority of patients who received fluoroquinolones also received antibiotics from another class. Only 19% of patients infected with the BI strain received fluoroquinolone monotherapy, compared to only one patient in the non-BI group (*P* = 0.28). Macrolide exposure was also more frequent in the BI cases than in the controls (OR, 5.8; 95% CI, 1.3 to 25.7; *P* = 0.02). Azithromycin was the most common macrolide and was used in 20% of the BI cases. Only one non-BI control patient was treated with azithromycin. Of the 21 BI cases who received azithromycin, 19 also received other antibiotics, including 13 who received a fluoroquinolone, 14 who received a cephalosporin (ceftriaxone in 12), and 10 who received a fluoroquinolone and a cephalosporin as well as azithromycin. In contrast, clindamycin exposure was less frequent in the BI cases than in the non-BI controls (OR, 0.12; 95% CI, 0.04 to 0.41; *P* = 0.0005).

The multivariate and final model, which included fluoroquinolones, clindamycin, macrolides, and linezolid, continued to demonstrate a strong correlation between fluoroquinolone exposure (OR, 3.2; 95% CI, 1.3 to 7.5; *P* < 0.001) as well as macrolide exposure (OR, 5.2; 95% CI, 1.1 to 24.0; *P* = 0.04) and the in-

creased risk of acquiring the BI strain (Table 2). Clindamycin exposure was a risk for infection with non-BI strains, as opposed to infection with the BI strains, by multivariate analysis and final models (OR, 0.1; 95% CI, 0.03 to 0.4; *P* = 0.001) (Table 2). No other antibiotic class demonstrated a correlation with infection by either the BI or non-BI strains.

The intensity and duration of antibiotic exposure were also measured by calculating the defined daily dose (DDD) and days of therapy (DOT) (Table 3). Both cases and controls had similar pooled mean DDDs and DOTs per 1,000 patient days among patients who received fluoroquinolones and ciprofloxacin. Mean macrolide DDDs/1,000 patient days and DOTs/1,000 patient days were higher among BI cases than among non-BI controls (DDD, 155.07 ± 98.76 versus 83.33 ± 16.84 [*P* = 0.01]; DOT, 142.86 ± 84.54 versus 59.52 ± 16.84 [*P* = 0.004], respectively).

Antibiotic susceptibilities. Susceptibilities were tested for 102 BI and 40 non-BI isolates (Table 4). A significantly greater proportion of BI strains were resistant to moxifloxacin (100/102 [98%] versus 22/40 [55%] for BI and non-BI, respectively; *P* = 0.0001), with 48% (49/102) of BI strains possessing high-level resistance compared to 0% (0/40) of non-BI strains (*P* = 0.0001). Similarly, high-level resistance to azithromycin was more common in BI strains (100/102, 98%) than in non-BI strains (22/40, 55%) (*P* = 0.0001). In contrast, resistance to clindamycin tended to be more frequent in non-BI strains (28/40 [70%] versus 59/102 [58%] for non-BI and BI, respectively; *P* = 0.25), with a greater proportion of non-BI isolates possessing high-level resistance to clindamycin (22/40 [55%] versus 7/102 [7%] for non-BI and BI, respectively; *P* = 0.001). All isolates were resistant to ceftriaxone, and all but one in each group were susceptible to piperacillin-tazobactam.

A secondary analysis comparing antibiotic exposure risk factors to isolate resistance patterns regardless of REA strain type was also performed. In general, there was a trend for antibiotic exposures and corresponding resistance patterns of the isolates (data not shown). There was a significant association with exposure to

TABLE 3 Antibiotic exposures by defined daily dose and days of therapy per 1,000 patient days for CDI cases (BI) and controls (non-BI)

Antibiotic	Mean DDDs per 1,000 patient days $\pm$ SD		<i>P</i> value	Mean DOTs per 1,000 patient days $\pm$ SD		<i>P</i> value
	BI (<i>n</i> = 103)	Non-BI (<i>n</i> = 40)		BI (<i>n</i> = 103)	Non-BI (<i>n</i> = 40)	
Fluoroquinolones						
Total	162.97 $\pm$ 178.73	139.36 $\pm$ 59.49	0.37	167.03 $\pm$ 169.38	156.86 $\pm$ 86.69	0.77
Ciprofloxacin	191.63 $\pm$ 243.13	160.71 $\pm$ 63.78	0.56	193.76 $\pm$ 234.13	182.54 $\pm$ 113.36	0.86
Gatifloxacin and moxifloxacin	132.52 $\pm$ 91.64	117.06 $\pm$ 62.34	0.51	137.84 $\pm$ 79.48	142.86 $\pm$ 70.63	0.85
Macrolides						
Total	155.07 $\pm$ 98.76	83.33 $\pm$ 16.84	0.01	142.86 $\pm$ 84.54	59.52 $\pm$ 16.84	0.004
Azithromycin	153.41 $\pm$ 85.99	71.4		130.39 $\pm$ 60.99	71.43	
Clindamycin	168.15 $\pm$ 186.74	177.38 $\pm$ 112.30	0.93	154.76 $\pm$ 136.78	204.08 $\pm$ 121.29	0.64
First-generation cephalosporins	82.69 $\pm$ 74.94	165.32 $\pm$ 189.57	0.24	84.25 $\pm$ 71.98	139.19 $\pm$ 109.47	0.22
Second- and third-generation cephalosporins	133.21 $\pm$ 192.84	203.14 $\pm$ 305.68	0.64	159.61 $\pm$ 165.72	523.81 $\pm$ 269.37	0.45
Piperacillin-tazobactam	156.80 $\pm$ 120.38	181.80 $\pm$ 145.41	0.64	173.47 $\pm$ 107.58	207.14 $\pm$ 139.76	0.43
Aminopenicillins	371.36 $\pm$ 348.79	1,119.48 $\pm$ 2,136.97	0.35	198.88 $\pm$ 132.28	370.75 $\pm$ 316.42	0.27
Vancomycin	165.75 $\pm$ 318.78	88.54 $\pm$ 87.16	0.28	219.78 $\pm$ 291.40	101.19 $\pm$ 93.30	0.08
Linezolid	47.62 $\pm$ 33.67	422.62 $\pm$ 345.14	0.37	47.62 $\pm$ 33.67	428.57 $\pm$ 336.72	0.35
Gentamicin	27.74 $\pm$ 28.12	214.29		35.71 $\pm$ 16.84	214.29	

any fluoroquinolone and moxifloxacin resistance: 73/122 (60%) of moxifloxacin-resistant strain cases (MIC $\geq$ 8) had fluoroquinolone exposure, compared to 6/20 (30%) of moxifloxacin-non-resistant cases (MIC $<$ 8) ($P = 0.012$). In addition, exposure to clindamycin was more frequent among cases infected with high-level-resistant (HLR) clindamycin isolates (MIC $\geq$ 64) than among those with infected with non-HLR isolates (MIC $<$ 64) (10/29 [34.5%] versus 4/113 [3.5%]; $P = 0.0003$).

DISCUSSION

In our hospital, where fluoroquinolones were the second most common antibiotic class prescribed prior to development of CDI (544.4 total DDDs, compared to 630.8 total DDDs for aminopenicillins), total fluoroquinolone exposure was a risk factor for infection with the epidemic BI strain among patients with CDI. Macrolide exposure, and azithromycin use specifically, was also a risk for infection with BI among patients with CDI. In contrast, clindamycin use among patients with CDI was a risk for non-BI strains, of which the previously epidemic J strain (PCR ribotype 001) predominated. The *in vitro* susceptibilities of BI and non-BI strains also correlated with the prior antibiotic exposures of the infected patients; high-level resistance to ciprofloxacin, moxifloxacin, and azithromycin was more common in the BI strains, whereas high-level resistance to clindamycin was more common in the non-BI strains. These data support the hypothesis that use

of specific antibiotics in hospitalized patients predisposes to infection with specific strains that are resistant to those antibiotics and thereby facilitates epidemic spread.

Although our increase in CDI rates followed a hospital antibiotic formulary shift from levofloxacin to gatifloxacin, it was total fluoroquinolone use that was associated with infection by the BI strain over other strains. Two studies in other VA hospital systems also noted an increase in CDI rates during the same shift in formularies to gatifloxacin (12, 13) but could not attribute the increased rates to gatifloxacin specifically. The molecular strain epidemiology of *C. difficile* in these institutions was not known, but it is possible that the total burden of fluoroquinolone use in addition to the introduction of quinolones with increased antianaerobic activity (e.g., gatifloxacin and moxifloxacin) contributed to these increased CDI rates. Adams et al. were able to show that gatifloxacin and moxifloxacin exert selective pressure favoring fluoroquinolone-resistant *C. difficile* strains in a mouse model (14). In addition, ciprofloxacin and levofloxacin also exerted selective pressure in the mouse when given in combination with other antianaerobic antibiotics. We showed in a hamster model of CDI that an epidemic BI strain (BI17) with high-level fluoroquinolone resistance had similar colonization rates following treatment with ciprofloxacin, levofloxacin, and moxifloxacin (15). In contrast, an historic BI strain (BI1) that was not resistant to moxi-

TABLE 4 Antibiotic susceptibilities of epidemic BI strain isolates and non-BI isolates

Antibiotic	BI (<i>n</i> = 102 ^a)				Non-BI (<i>n</i> = 40)				<i>P</i> value (HLR)
	MIC (μg/ml)			No. (%) with HLR ^b	MIC (μg/ml)			No. (%) with HLR	
	MIC ₅₀	MIC ₉₀	Range		MIC ₅₀	MIC ₉₀	Range		
Ciprofloxacin	>64	>64	8–>64	99 (97)	32	64	4–>64	16 (40)	0.0001
Moxifloxacin	32	>64	2–>64	49 (48)	16	32	1–32	0	0.0001
Clindamycin	8	8	1–>64	7 (7)	>64	>64	1–>64	22 (55)	0.0001
Azithromycin	>64	>64	4–>64	100 (98)	>64	>64	1–>64	22 (55)	0.0001
Ceftriaxone	32	64	16–64		64	128	16–128		
Piperacillin-tazobactam	8	8	4–16		4	8	4–16		

^a One BI isolate was not available for susceptibility testing.

^b High-level resistance (HLR) was defined as >64 μ g/ml.

floxacin colonized hamsters efficiently only after moxifloxacin treatment, supporting acquisition of high-level fluoroquinolone resistance as a risk for CDI for all fluoroquinolones.

Other case-control studies performed in Europe and Canada have looked for an association with prior fluoroquinolone exposure and infection with epidemic strains in comparison to other circulating strains (16–20). These studies had somewhat different strain prevalences and antibiotic associations, but a meta-analysis supported fluoroquinolone use as a risk factor for infection with PCR ribotype 027 (REA group BI) (21). The Canadian study (19) most closely resembled the strain epidemiology and antibiotic associations that we found in our study. These investigators surveyed clinical isolates and reviewed patient charts from two time periods in one Montreal hospital. From 2000 to 2001, 84% of *C. difficile* clinical isolates were ribotype 001 (REA group J) and were highly resistant to clindamycin. From 2003 to 2004, the epidemiology was markedly different, as 80% of the isolates were now ribotype 027 (REA group BI) and highly resistant to fluoroquinolones but susceptible to clindamycin. Their conclusion was that clindamycin use selected for infection with ribotype 001 strains in the setting of preexisting high endemicity and that fluoroquinolones selected for ribotype 027 during the epidemic period noted for increased CDI rates and increased morbidity and mortality. Also, fluoroquinolone but not clindamycin exposure was a risk for infection with the epidemic 027 strain at 12 Quebec hospitals in 2004 (3). The results of our study conducted in a U.S. hospital 1 to 3 years later were markedly similar to these findings. Although we did not have clinical isolates saved prior to 2005 from our hospital, the case-control study identified the same antibiotic risk factors for CDI due to BI (ribotype 027) and non-BI (predominantly REA J, ribotype 001) infections. It is interesting that clindamycin use still predisposed to non-BI infections at a time in our hospital when the predominant infecting strain was BI and for which fluoroquinolone use was a risk. Relative antibiotic usage patterns might explain part of the epidemiology in our hospital. Among patients with CDI, 9.8% of patients received clindamycin (102.5 total DDDs) compared to 56% who received fluoroquinolones (544.4 total DDDs).

Most of the patients in our study who received fluoroquinolones also received other antibiotics. It is possible that other antibiotics, some with more profound effects on normal intestinal flora, in addition to heavy fluoroquinolone use combined to drive the BI epidemic in our hospital. Sundram et al. found that ciprofloxacin use for >7 days (but not <7 days) was associated with CDI in a case-control study where cases were matched with control patients without diarrhea by age, sex, ward location, underlying disease severity, and length of stay (22). Forty-five percent of the *C. difficile* isolates from the cases in that study were ribotype 027 (REA group BI).

BI isolates were more likely than non-BI isolates to be highly resistant to azithromycin *in vitro*, as defined by an MIC of ≥ 64 $\mu\text{g/ml}$. To our knowledge, this is the first study to identify macrolides as a risk factor for infection with the BI strain, although their use was frequently accompanied by fluoroquinolone and ceftriaxone use. One case-control study found an association of macrolides with CDI in univariate analysis during an 027 (BI) strain outbreak but not in the multivariate analysis which controlled for other potential factors, including comedication and use of multiple antibiotics (23).

A strength of this study was the ability to comprehensively

review the antibiotic exposures prior to developing CDI, given that our veteran patients preferentially use the VA system for their inpatient and outpatient care and these data are captured in the electronic medical record. Analysis of DDDs and DOTs suggested that both BI and non-BI cases received intensive and prolonged antibiotic exposure prior to CDI, but categorical exposures to specific antibiotics predicted infection with specific *C. difficile* strains. Specific resistance patterns of the infecting isolates also demonstrated some association with antibiotic exposures, particularly moxifloxacin resistance and high-level clindamycin resistance. However, other potential virulence factors in the fluoroquinolone-resistant BI strain and the highly clindamycin-resistant J strain may contribute to their epidemic potential in addition to the resistance determinants which facilitate dissemination of these strains.

In summary, fluoroquinolone and macrolide use were risk factors for infection with the fluoroquinolone-resistant epidemic BI strain in patients with CDI, whereas clindamycin use was a risk factor for non-BI strains that, while not epidemic, were highly resistant to clindamycin. These data suggest that similar antibiotic usage patterns may have contributed to the major outbreaks of the epidemic BI/027/NAP1 strain documented in Canada, Europe, and the United States since 2000. These findings have obvious potential implications for antibiotic stewardship interventions, but prospective studies in outbreak settings where this strain predominates are needed.

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