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Use of Rifamycin Drugs and Development of Infection by Rifamycin-Resistant Strains of *Clostridium difficile*

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The relationship between rifamycin drug use and the development of resistant strains of *Clostridium difficile* was studied at a large university hospital in Houston, TX, between May 2007 and September 2011. In 49 of 283 (17.3%) patients with *C. difficile* infection (CDI), a rifamycin-resistant strain of *C. difficile* was identified that compares to a rate of 8% using the same definitions in 2006-2007 ($P = 0.59$). The 49 patients infected by a resistant organism were matched by date of admission to 98 control patients with CDI from whom a rifamycin-susceptible *C. difficile* strain was isolated. Cases and controls did not differ according to demographic and clinical characteristics and showed similar but low rates of prior rifamycin use. Similar rates of rifamycin resistance were seen in cases of hospital-acquired CDI (38/112 [34%]) versus community-acquired CDI (7/20 [35%]). At a university hospital in which rifaximin was commonly used, infection by rifamycin-resistant strains of *C. difficile* was not shown to relate to prior use of a rifamycin drug or to acquiring the infection in the hospital, although the rate of overall resistance appeared to be rising.

Clostridium difficile is the leading definable cause of antibiotic-associated diarrhea (AAD) acquired in health care settings. Antibiotic exposure has been identified as one of the three most important risk factors in the pathogenesis of *C. difficile* infection (CDI), with nearly all classes of antibiotics being associated with increased risk of development of CDI (1).

Rifampin and rifaximin are derivative of rifamycins, which bind to the β subunit of the bacterial DNA-dependent RNA polymerase leading to inhibition of protein synthesis. The mechanism of developing rifamycin resistance differs from plasmid-mediated resistance that affects other antibiotics (2). The presence of mutational alterations in the chromosomal *rpoB* gene in rifamycin-resistant *C. difficile* has been described for rifaximin and rifampin (3–6). Rifampin is a systemically absorbed rifamycin used for the treatment of tuberculosis and *Neisseria meningitidis* prophylaxis and has been used in combination therapy with other drugs to treat infection caused by methicillin-resistant *Staphylococcus aureus* (7, 8). Rifaximin is a nonabsorbed (<0.4%) rifamycin with *in vitro* activity against Gram-positive, Gram-negative, and anaerobic bacteria. Rifaximin has been used to treat traveler's diarrhea (9), CDI (10), diarrhea predominant irritable bowel syndrome (11), and hepatic encephalopathy (HE) (12). Hospital use of rifaximin for liver disease began in 1998 when the drug was given orphan status by the U.S. Food and Drug Administration (FDA) for use in HE, with usage further increasing in early 2010 when the FDA licensed rifaximin for the condition.

Previous studies demonstrated that approximately 3 to 8% of *C. difficile* isolates are resistant to rifamycin drugs (13, 14). It is assumed that the major risk factor in the development of rifamycin-resistant *C. difficile* is exposure to a rifamycin drug. With widespread and chronic use of rifaximin in gastroenterology, concern has been expressed about promoting dissemination of rifaximin-resistant strains of CDI limiting the value of this class of drugs in management of CDI.

The aim of the present study was to examine the frequency of rifamycin resistance and associated risk factors for developing resistance, including prior use of a rifamycin drug or acquisition of CDI in a hospital where the important use of rifaximin was taking place.

MATERIALS AND METHODS

Study setting and subjects. The study was carried out at a 700-bed university-affiliated hospital in Houston, TX. Patients at the hospital with AAD and a positive fecal toxin test for *C. difficile* in the hospital diagnostic laboratory were approached by our staff to enroll in a study of CDI pathogenesis and recurrence. Approximately half of the patients at the hospital with CDI volunteered for this research program. Once enrolled in the study, stools were transported three blocks to the Enteric Microbiology Laboratory at the University of Texas School of Public Health for culture and susceptibility testing.

Study design. From May 2007 to September 2011, all subjects with AAD and positive fecal assay(s) for *C. difficile* toxin B enrolled in our study provided stool samples that were cultured *in vitro* for *C. difficile* and tested for susceptibility to rifamycins (rifaximin and rifampin). Those found to be infected by a rifamycin-resistant strain of *C. difficile* were then included in a case-control study with patients with CDI from whom a rifamycin-susceptible strain was identified to determine whether rifaximin or rifampin exposure in the past 6 months affected rifamycin susceptibility of the infecting strain. A case was identified as a patient with AAD from whom a *C. difficile* isolate obtained from culture of stool that was resistant *in vitro* to either rifaximin or rifampin. Resistant isolates of *C. difficile* were matched to rifamycin-susceptible strains by date of admission in a ratio of cases to controls of 1:2.

Data collection. For the initial study, CDI was defined as diarrhea (passage of ≥3 unformed stools) with *C. difficile*-toxin B-positive stools. For the case-control study, information was obtained by review of patients' records for demographic characteristics (age, sex, and race), length of hospital stay associated with CDI, hospital-acquired infection or com-

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munity-acquired infection, hospital admission within the previous 60 days, and preinfection medical comorbidities. Receipt of rifamycin therapy for the 6 months preceding CDI diagnosis was obtained through an examination of hospital records and a review of home medications. Diagnoses of conditions or infections during 6 months before study known to be associated with rifamycin use were sought including presence of infection caused by methicillin-resistant *S. aureus*, tuberculosis, heart valve infection or endocarditis, traveler's diarrhea, irritable bowel syndrome, inflammatory bowel disease, Crohn's disease, liver disease, liver failure or hepatic encephalopathy, and chronic diarrhea.

Hospital-acquired CDI was defined as diarrhea plus *C. difficile* toxin B-positive stools by an approved assay, either tissue culture cytotoxicity assay or real-time PCR assay, >48 h after admission to or <4 weeks after discharge from the study hospital (1). Community-acquired CDI was defined as CDI occurring without previous hospitalization or >12 weeks after the last hospital discharge (1). Patients admitted with CDI diagnosed within 48 h were evaluated to determine whether they met the criteria for hospital- or community-acquired CDI. Indeterminate CDI was defined when CDI diagnosis was made between 4 and 12 weeks after discharge from the hospital (1).

Laboratory investigations. The presence of CDI at the hospital was determined by documenting the presence of fecal *C. difficile* toxin B in the hospital diagnostic laboratory by tissue culture cytotoxicity assay from May 2007 to April 2011 and by a commercial real-time PCR method (Becton Dickinson, Franklin Lakes, NJ) after April 2011. Samples positive for toxin B by cytotoxicity assay or real-time PCR were submitted to the Enteric Infectious Diseases Laboratory at the University of Texas School of Public Health within 3 days of collection. The samples were inoculated onto prereduced *C. difficile*-selective cycloserine-cefoxitin fructose agar plates (Remel, Lenexa, KS). The plates were cultured under anaerobic conditions at 37°C for 48 to 72 h. Colonies were identified by morphology and odor. The isolates were subcultured on blood agar plates to assure purity of the culture. *C. difficile* isolates were stored at −80°C in Trypticase soy broth (Becton Dickinson, Sparks, MD) with 7% horse blood and 10% glycerol as previously published.

Susceptibility testing. Rifampin Etest strips (Etest; AB Biodisk, Piscataway, NJ) were used to test the susceptibility of *C. difficile* isolates to rifampin. Agar dilution methodology based on standards developed by the Clinical and Laboratory Standards Institute (CLSI) (15) was applied to determine the susceptibility of *C. difficile* strains to rifaximin (Xifaxan; Salix Pharmaceuticals, Morrisville, NC). Rifaximin was dissolved in acetone at a concentration of 10,240 μg/ml before making rifaximin 2-fold dilutions ranging from 1,024 to 0.016 μg/ml. Rifaximin dilution was added to Mueller-Hinton agar, followed by the instructions of the manufacturer (Becton Dickinson, Sparks, MD). After the preparation, *C. difficile* isolates were inoculated onto the Mueller-Hinton agar plates and incubated at 37°C for 48 h under anaerobic conditions. *Escherichia coli* (ATCC 25922), *S. aureus* (ATCC 29213), and *C. difficile* (ATCC 700057) were used as the quality control strains for the MICs (14). The MIC breakpoint for *in vitro* resistance of *C. difficile* strains to rifaximin or rifampin was ≥32 μg/ml. Strains resistant to either rifamycin were considered resistant for the study.

Data analysis. Analyses were performed using Stata version 12 (StataCorp LP, College Station, TX). The data are presented as the median (interquartile range [IQR]) or the percent frequency. Previous usage of rifamycins and other categorical variables were compared using χ^2 analysis or the Fisher exact test, where appropriate. Continuous variables were compared using Wilcoxon rank-sum (Mann-Whitney) test. The threshold for statistical significance was considered to be $P < 0.05$ for all analyses.

RESULTS

Of the 283 *C. difficile* strains identified from 292 stool specimens and tested for MICs against rifaximin and rifampin from May 2007 to September 2011, rifamycin resistance was seen in 49 (17.3%) strains. Twelve strains were resistant only to rifaximin, 12 strains were resistant only to rifampin, and 25 strains were resistant to both rifamycin drugs.

TABLE 1 Baseline demographic and clinical characteristics of patients with CDI from which *Clostridium difficile* strains were obtained for culture

Patient characteristics	No. (%) of patients		
	Rifamycin resistant ($n = 49$)	Rifamycin susceptible ($n = 98$)	P^b
Median age (IQR) in yrs[a]	66 (55–76)	64 (53–77)	0.47
Female	22 (45)	50 (51)	0.48
Race			
Caucasian	31 (63)	52 (53)	0.45*
African-American	10 (20)	27 (28)	
Hispanic	8 (16)	10 (10)	
Asian	0 (0)	2 (2)	
Median length of hospital stay (IQR) in days	13 (8–28)	14 (8–25)	0.76
Type of CDI			
Hospital acquired	38 (78)	74 (76)	0.91*
Community acquired	7 (14)	13 (13)	
Indeterminate	4 (8)	11 (11)	
Hospital admission within previous 60 days	30 (61)	50 (52)	0.27
Comorbidity			
Congestive heart failure	10 (20)	15 (15)	0.44
Obesity	2 (4)	1 (1)	0.26*
Hypertension	29 (59)	53 (54)	0.56
Diabetes	15 (31)	31 (30)	1.00
Renal insufficiency	14 (29)	34 (35)	0.47
Respiratory insufficiency	13 (27)	14 (14)	0.07
Malignancy	10 (20)	13 (13)	0.26
Rifamycin use in previous 90 days	2 (4)	3 (3)	1.00*

[a] Interquartile range (IQR) is reported.
[b] *, Determined using the Fisher exact test. All other *P* values were determined using χ^2 analysis.

For the case-control study, the 49 rifamycin-resistant cases were matched to 98 rifamycin-susceptible cases with the same date of admission. The characteristics of case and control patients are summarized in Table 1. Cases and controls did not differ according to basic demographic characteristics, length of hospital stay, known risk factors of CDI, origin of CDI, and pre-CDI medical comorbidities. No significant association between prior rifamycin use and development of rifamycin-resistant CDI was identified. Looking for a history of a condition that could be associated with rifamycin use in the 6 months before CDI diagnosis, we identified 3 of 49 case patients as having been treated for endocarditis and 1 of 49 gave a history chronic diarrhea. In 2 of the 49 (4%) patients with a rifamycin-resistant *C. difficile*, a rifamycin drug had been administered in the previous 6 months preceding the diagnosis of CDI. Similarly, among the 98 patients infected by a rifamycin-susceptible strain of *C. difficile*, 6 patients had liver disease or liver failure, 2 had been treated for tuberculosis, and 1 had a history of chronic diarrhea. The medical records indicated that 3 of the 98 (3%) patients infected by rifamycin-susceptible *C. difficile* actually had received a rifamycin drug within the same time period before CDI diagnosis. Of the 112 patients included in the case-control

study with hospital-acquired CDI, 38 (34%) were infected by a rifamycin-resistant strain of *C. difficile* compared to 7 of 20 (35%) for community-acquired cases of CDI ($P = 0.93$).

At the study hospital in 2010 there were 643 inpatients who received a course of rifaximin, and in 2011 the number of inpatients receiving a course of rifaximin was 1,030. Based on the dosing, it appeared that most of these patients were receiving the drug for various degrees of hepatic encephalopathy, with a smaller percentage being treated for CDI.

DISCUSSION

Rifamycin-resistant *C. difficile* strains have been shown to have mutations in the *rpoB* gene (16, 17). Both *in vitro* and *in vivo* studies have shown that exposure to rifampin can select for resistant microorganisms (3–6). Rifaximin is an orally nonabsorbed (<0.4%) antibiotic used widely for gastrointestinal disorders. Concern has been expressed about the development of antimicrobial resistance among enteric bacteria, including *C. difficile*, by the widespread use of rifaximin. In 2006 and 2007, we studied the susceptibility of *C. difficile* strains to rifaximin/rifampin in patients with CDI at our institution and found a resistance rate of 8%. In the present study for strains isolated 2007-2011 the resistance rate was found to be 17%. Here, 5 of 147 (3.4%) enrolled patients gave a history of prior use of a rifamycin drug during the 6 months before CDI diagnosis. However, no relationship was seen between rifamycin exposure and the development of CDI due to a rifamycin-resistant strain of *C. difficile*. Of five patients developing CDI after receiving a rifamycin drug, two (40%) were infected by a resistant strain. Curry et al. (16) studied rifampin resistance in *C. difficile* isolates at their hospital by using a rifampin Etest and found that in 7 (88%) of 8 patients with CDI who previously received a rifamycin drug in the preceding 6 months, the infecting strain was rifampin resistant compared to 166/462 (36%) for patients not having received a rifamycin (relative risk = 2.4; 95% confidence interval, 1.8 to 3.3).

It appears that rifamycin has a therapeutic role in the prevention and treatment of CDI. Monitoring for emergence of resistance is advisable. The present study showed that 34 to 35% of hospital and community associated cases of CDI were infected by a rifamycin resistant strain of *C. difficile*. Resistance rates were similar from a single center study (16). In the present study, 173 of 470 (36.8%) *C. difficile* strains displayed reduced susceptibility to a rifamycin. From the present study it appears that there are factors other than exposure to rifamycin drugs that can be associated with the development of rifamycin resistance in strains of *C. difficile*. One factor may be the strains of *C. difficile* causing CDI. In one study, the rate of rifamycin resistance was more than twice as high for BI/NAP1 strains than for other strains encountered at one institution (16). A second study in Austria also found that rifaximin resistance was higher in the more virulent ribotype 027 strain (26%) compared to all other *C. difficile* isolates (7.5%) (18). A limitation of the present study is that we did not look for the presence of NAP1 in the strains of *C. difficile* identified.

At our study hospital, there is an active liver transplantation center, and rifaximin is used in many patients with various degrees of liver disease. We failed to see a difference in rifamycin susceptibility between patients acquiring CDI at our institution and those who were admitted after acquiring their CDI in the community. Our study failed to identify CDI among the many liver patients being managed on rifaximin at our hospital. We are now planning molecular strain typing studies of *C. difficile* strains to better understand the reason for the rise in rifamycin resistance among our isolates of *C. difficile*.

Most published studies have used an MIC of ≥32 μg/ml as a breakpoint for rifamycin susceptibility (14, 17, 18). A majority of the resistant strains in our institution show high-level resistance (MIC ≥ 1,024 μg/ml). Huhulescu et al. (18) detected very high-level rifaximin resistance in some strains of *C. difficile*, with MICs ranging from 4,096 to 32,678 μg/ml. The breakpoints of an MIC of ≥32 μg/ml may be too low to determine rifamycin resistance for an enteric infection where a drug concentrates to very high levels in the gut. Further studies are needed on the proper breakpoint for rifaximin, including a correlation between MIC values and clinical response to treatment of enteric infection.

This study has limitations. Prior rifamycin exposure history was very low in both cases and controls. Although the sample size for rifamycin-resistant *C. difficile* isolates appeared to be adequate, we found a surprisingly small number of patients in the group who had previous rifamycin exposure. We did not study all patients with CDI at our institution, and bias may have been seen in the enrollment criteria, although we did enroll approximately half of all patients with CDI at our institution. There is no apparent reason why the half of the subjects who did enroll would differ from the ones not enrolling.

In conclusion, the frequency of occurrence of rifamycin-resistant strains of *C. difficile* in patients with CDI at our large university hospital, with an active liver transplantation program, was moderately high (17%) and had increased from our prior studies. We failed to show a relationship between prior exposure to a rifamycin drug and the acquisition of infection by a rifamycin-resistant strain of *C. difficile*. Based on findings of the present study, we recommend carefully monitoring *C. difficile* for increases in resistance to rifamycins and other antibiotics with potential value in the therapy of CDI.

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