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High Frequency of Rifampin Resistance Identified in an Epidemic *Clostridium difficile* Clone from a Large Teaching Hospital

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Abstract

Background—Rifampin is used as adjunctive therapy for *Clostridium difficile*–associated disease, and the drug's derivative, rifaximin, has emerged as an attractive antimicrobial for treatment of *C. difficile*–associated disease. Rifampin resistance in *C. difficile* strains has been reported to be uncommon.

Methods—We examined the prevalence of rifampin resistance among 470 *C. difficile* isolates (51.1% during 2001–2002 and 48.9% during 2005) from a large teaching hospital. Rifampin sensitivity was performed using E-test. The epidemic BI/NAP1 *C. difficile* clone was identified by *tcdC* genotyping and multilocus variable number of tandem repeats analysis. A 200–base pair fragment of the *rpoB* gene was sequenced for 102 isolates. Data on rifamycin exposures were obtained for all patients.

Results—Rifampin resistance was observed in 173 (36.8%) of 470 recovered isolates and 167 (81.5%) of 205 of epidemic clone isolates ($P < .001$). Six *rpoB* genotypes were associated with rifampin resistance. Of 8 patients exposed to rifamycins, 7 had rifampin-resistant *C. difficile,* compared with 166 of 462 unexposed patients (relative risk, 2.4; 95% confidence interval, 1.8–3.3).

Conclusions—Rifampin resistance is common among epidemic clone *C. difficile* isolates at our institution. Exposure to rifamycins before the development of *C. difficile*–associated disease was a risk factor for rifampin-resistant *C. difficile* infection. The use of rifaximin may be limited for treatment of *C. difficile*–associated disease at our institution.

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Clostridium difficile is responsible for hospital-acquired illnesses ranging from mild diarrhea to severe pseudomembranous colitis. An epidemic of severe *C. difficile*–associated disease (CDAD) began at the University of Pittsburgh Medical Center–Presbyterian Hospital (UPMC; Pittsburgh, PA) during 2000–2001 [1,2]. Over the past decade, an increasing number of reports of epidemic CDAD has been noted [3-5]. This change in CDAD epidemiology corresponded with the emergence of a *C. difficile* clone that was rarely encountered previously [4]. This epidemic clone is designated BI/NAP1 on the basis of restriction enzyme analysis and PFGE pattern [4,6]. The increased mortality and morbidity, the poor clinical performance of antimicrobials traditionally used for CDAD, and the high relapse rates associated with the BI/NAP1 epidemic clone highlight the need for new CDAD therapies.

Rifamycins are now being considered for CDAD therapy on the basis of in vitro susceptibility data. Hecht et al. [7] reported rifampin resistance in 3 (2.7%) of 110 clinical isolates from the United States. The rifampin derivative rifaximin has emerged as an attractive potential therapy for CDAD because of its lack of systemic absorption [7,8]. The high levels of rifaximin that can be achieved in the gut are an ideal pharmacologic profile for treatment of CDAD [9]. The rates of spontaneous rifaximin resistance based on agar dilution have been reported to be $<10^{-9}$ at drug concentrations that are 8 times the rifaximin MIC for *C. difficile* [8]. Successful rifaximin treatment of patients experiencing a CDAD relapse has been reported [10].

However, O’Connor et al. [11] have reported that rifaximin resistance in *C. difficile* isolates is associated with point mutations in *rpoB,* the gene encoding the beta subunit of *C. difficile* RNA polymerase; these investigators also showed that rifampin nonsusceptibility accurately predicts rifaximin resistance. In this study, we determined the prevalence and molecular mechanisms of rifampin resistance among *C. difficile* isolates recovered from samples collected from patients with CDAD who were hospitalized at UPMC. In addition, we examined the association of rifampin resistance with rifampin exposure and the BI/NAP1 clone to understand the potential origin of rifampin-resistant *C. difficile* at our institution.

PATIENTS AND METHODS

Study design and setting

We performed a retrospective cohort analysis of patients who had positive results of *C. difficile* stool toxin tests at UPMC, an 834-bed tertiary care teaching facility. UPMC serves as a referral center for patients from institutions throughout western Pennsylvania, eastern Ohio, and northern West Virginia. Isolates recovered from samples obtained from inpatients at UPMC, patients seen at affiliated outpatient clinics, and patients seen at the UPMC emergency department were included.

The study cohort comprised all patients with a positive *C. difficile* stool toxin test result during 2 periods. The first and second periods comprised all toxin tests performed from 21 March 2001 through 6 March 2002 and from 11 February 2005 through 9 September 2005, respectively, because these periods allowed the staff at UPMC to assess its CDAD control efforts [12]. During each period, only the result of the first toxin test for each patient was considered, to exclude most cases of CDAD relapse. Nosocomial CDAD (acquired at or attributable to UPMC) was defined using an expanded epidemiologic definition that is described elsewhere [12] and was classified by infection control practitioners. UPMC electronic pharmacy records were reviewed for all patients in the cohort to determine the incidence of rifamycin exposure (rifampin, rifapentine, rifabutin, and rifaximin) during the 6 months before the date of CDAD onset. The University of Pittsburgh Institutional Review Board approved this study.

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CDAD diagnostic testing and isolation of C. difficile

For all laboratory assays used in this study, research laboratory personnel were blinded to patient identity. *C. difficile* stool toxin tests were performed by cytotoxicity assay (Diagnostic Hybrids). All stool samples sent for toxin testing were also cultured for *C. difficile* on cefoxitin, cycloserine, and fructose agar (Remel), as described elsewhere [2]. Presumptive *C. difficile* isolates were identified by Gram stain and odor and colony morphology, were subcultured on prereduced CDC anaerobe 5% sheep blood agar (Becton Dickinson), and were biochemically identified with a RapID ANA II panel (Remel). Chopped meat broth culture supernatants recovered from all *C. difficile* isolates were confirmed to be toxigenic by cytotoxicity assay [13].

Rifampin susceptibility testing of C. difficile *isolates*

Isolates were cultured from frozen stocks to tryptone (3%), yeast extract (2%), and arginine (0.87%) agar containing 0.1% sodium taurocholate and were incubated anaerobically at 37°C for 48 h. Suspensions of *C. difficile* isolates with a minimum 50% transmittance at 540 nm were prepared in prereduced thioglycolate medium without dextrose or Eh indicator (Becton Dickinson). This suspension was applied to prereduced 150-mm Brucella agar plates (Anaerobe Systems) by streaking a cotton swab with the suspension in 3 directions. Rifampin E-test strips (AB Biodisk) were placed on each plate according to the manufacturer's instructions. The plates were sealed and incubated anaerobically in AnaBags (Hardy Diagnostics) at 37°C for 48 h. E-tests were read according to the manufacturer's instructions. Rifampin susceptibility was categorized according to MICs ($\leq$0.002 μg/mL, 0.003–32 μg/mL, and >32 μg/mL). Although Clinical Laboratory Standards Institute breakpoints for *C. difficile* are not defined for rifampin, these categories are used to refer to isolates as rifampin susceptible, rifampin intermediate, and rifampin resistant, respectively. A rifampin-susceptible *C. difficile* isolate from our collection (CD40) and the CD630 genome reference strains were used as controls for *C. difficile* rifampin susceptibility analysis [14].

C. difficile *genotyping*

C. difficile isolates belonging to the BI/NAP1 clone were identified by multilocus variable number tandem repeat analysis (MLVA) and by *tcdC* genotyping, as described elsewhere [13,15]. Isolates bearing the *tcdC-sc1* genotype were classified as BI/NAP1 [13,15]. Isolates bearing other *tcdC* genotypes were classified as non-BI/NAP1 *C. difficile.*

Sequence analysis of rpoB

A subset of 102 isolates was selected for *rpoB* sequence analysis, including 18 rifampin-susceptible, 4 rifampin-intermediate, and 80 rifampin-resistant isolates. Of the 18 rifampin-susceptible isolates selected, 6 were closely related to rifampin-resistant isolates by MLVA.

A 200–base pair region of the *rpoB* gene known to contain point mutations associated with high-level rifampin and rifaximin resistance in *C. difficile* was targeted for PCR amplification and sequence analysis [11]. In brief, genomic DNA was PCR amplified with primers CDrpoB2-F (5′-ATGGAAGCTATAACGCCTCAA-3′) and CDrpoB2-R (5′-ACAGCACCATTTACAGTTCTA-3′) in a reaction mixture containing AmpliTaq 10 × PCR buffer, 125 nmol of magnesium chloride, 10 pmol each of CDrpoB2-F and CDrpoB2-R primers, 10 nmol of each deoxynucleotide triphosphate, and 1.5 U of AmpliTaq Gold (Applied Biosystems). The cycling conditions were initial incubation at 95°C for 5 min, followed by 35 cycles consisting of 1 min at 95°C, 1 min at 49.9°C, and 1 min at 72°C. The resulting PCR products were purified by polyethylene glycol precipitation and were sequenced with the CDrpoB2-F and CDrpoB2-R primers with use of the BigDye terminator kit, version 3.1 (Applied Biosystems). Capillary sequence analysis was performed on a 3730 DNA Analyzer

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(Applied Biosystems). The *rpoB* fragment of each isolate was sequenced off both DNA strands from 2 independent PCR reactions, and sequences that differed from the CD630 reference gene were translated to identify amino acid substitutions within RpoB with use of the Sequencher software package, version 4.8 (Gene Codes) [14]. The CD630 reference strain also underwent *rpoB* sequencing as an internal control, and sequences conforming to the CD630 *rpoB* sequence were considered to be wild type.

Statistical methods

Fisher's exact test was used to test the association between categorical variables. SAS, version 9.1 (SAS Institute), was used for all analyses.

RESULTS

Molecular subtyping

Of the 706 patients who met the study inclusion criteria, 470 (66.6%) had toxigenic *C. difficile* isolates recovered from their samples; 240 isolates (51.1%) were recovered during 2001–2002, and 230 isolates (48.9%) were recovered during 2005. Two hundred five (43.6%) of 470 isolates were identified as BI/NAP1 by MLVA and *tcdC* genotyping.

Rifampin susceptibility of C. difficile *isolates from UPMC, 2001–2002 and 2005*

Of the 470 isolates tested for rifampin susceptibility, 291 (61.9%) were susceptible (MIC, ≤0.002 μg/mL), 173 (36.8%) were resistant (MIC, >32 μg/mL), and 6 (1.3 %) showed intermediate (MIC, 0.003–32 μg/mL) susceptiblity to rifampin; the 6 intermediate isolates had rifampin MICs of 0.38–0.75 μg/mL. The proportion of *C. difficile* isolates resistant to rifampin decreased during the study period from 100 (41.7%) of 240 during 2001–2002 to 73 (31.7%) of 230 during 2005 ($P = .028$).

Rifampin resistance in the BI/NAP1 epidemic clone

Of 205 BI/NAP1 isolates identified by MLVA and *tcdC* genotyping, 167 (81.5%) were rifampin resistant, whereas only 6 (2.3%) of 265 non-BI/NAP1 isolates were resistant to rifampin ($P < .001$) (table 1). Rifampin-intermediate isolates were infrequent ($n = 6$) and showed no association with the BI/NAP1 clone. The proportion of BI/NAP1 isolates resistant to rifampin decreased during the study period from 95 (96.0 %) of 99 during 2001–2002 to 72 (67.9 %) of 106 during 2005 ($P < .001$).

RpoB amino acid polymorphisms associated with rifampin resistance

There were 6 different RpoB polymorphisms identified in the investigated subset of rifampin-intermediate and -resistant *C. difficile* isolates (table 2). All of the identified amino acid substitutions resided between amino acid 488 and 548 of RpoB. The majority of these amino acid substitutions have been previously reported [11]. However, the H502N substitution alone and the combination of S498T/R505K amino acid substitutions are both novel. All of the rifampin-resistant isolates contained the R505K substitution, which was the most frequent single substitution (38 [47.5%] of 80 rifampin-resistant isolates tested) among rifampin-resistant isolates tested. The 4 rifampin-intermediate isolates contained only the H502N substitution (table 2). Of the 79 rifampin-resistant BI/NAP1 isolates that underwent *rpoB* sequence analysis, 37 (46.8%) contained the R505K substitution alone and the remaining rifampin-resistant BI/NAP1 isolates contained additional amino acid substitutions (table 2). The 1 non-BI/NAP1 rifampin-resistant isolate that was examined for RpoB mutations had only the R505K amino acid substitution. Five rifampin-susceptible BI/NAP1 isolates and 13 rifampin-susceptible non-BI/NAP1 isolates that were examined for RpoB mutations contained wild-type RpoB.

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Rifamycin exposure and subsequent development of rifampin-resistant C. difficile

Of the 706 patients who met the study criteria, 11 (1.6%) had medical records indicating rifamycin exposures during the 6 months before their first positive *C. difficile* stool toxin test result in the study periods (table 3). Isolates were available for 8 (72.7%) of these patients, and an isolate was obtained during a CDAD relapse from 1 of the remaining patients (patient 4) (table 3). Only 1 (9.1%) of the rifamycin-exposed patients received this class of antimicrobials for a CDAD-related indication (patient 4), and this was for a severe primary CDAD episode for which no isolate was recovered. Rifampin was prescribed for 10 patients (90.9%) and was administered for 0.5–54.5 defined daily doses before recovery of *C. difficile.* One patient received 384 defined daily doses of rifabutin over 128 days (patient 1) (table 3) before recovery of *C. difficile.* Patient 11 (table 3) received both rifabutin and rifampin sequentially for 52 and 45 defined daily doses, respectively, before development of CDAD, but no isolate was recovered. For 3 patients, rifampin use was concomitant with development of rifampin-resistant *C. difficile.* However, in 7 cases, the last recorded rifampin exposure was 3–93 days before recovery of *C. difficile* from stool samples (table 3). An isolate found in a sample obtained from patient 2 was rifampin susceptible.

Of the 470 patients with CDAD for whom there were available *C. difficile* isolates, 462 had no recorded history of rifamycin exposure. Of these patients, 166 (35.9%) had infection due to rifampin-resistant *C. difficile,* whereas 7 (87.5%) of 8 rifamycin-exposed patients had infection due to rifampin-resistant *C. difficile.* Patients with CDAD who were exposed to rifamycins during the 6 months before their first positive *C. difficile* stool toxin test result were more than twice as likely as nonexposed patients to develop infection due to rifampin-resistant *C. difficile* (relative risk, 2.4; 95% CI, 1.8–3.3).

DISCUSSION

This study demonstrates that more than one-third of *C. difficile* isolates recovered at our institution during the study period were rifampin resistant. This finding is significant because, to our knowledge, such a high frequency of resistance to rifampin in *C. difficile* during a CDAD outbreak has not been previously reported. Although rifampin exposure was associated with an increased risk of subsequent development of rifampin-resistant *C. difficile,* only 11 patients with CDAD in our study were known to have been exposed to rifampin, and in 10 cases, rifampin exposure was incidental in that it was intended for infections other than CDAD. Therefore, the proportion of primary rifampin-resistant and rifampin-intermediate *C. difficile* isolates that can be directly attributed to prior rifampin exposure in this study was 7 (4.0%) of 179. These data suggest that, although rifamycin use may drive the selection of resistant isolates, transmission of rifampin-resistant *C. difficile* to patients without a history of rifamycin use is much more common at UPMC. This observation is supported by the finding that most rifampin-resistant *C. difficile* isolates belonged to the BI/NAP1 clone. Our observations build on similar findings recently reported by O'Connor et al. [11].

Sequence analysis of the *rpoB* gene from a subset of *C. difficile* cohort isolates did not reveal a single RpoB polymorphism associated with rifampin resistance, although all of the rifampin-resistant isolates tested in this study shared the R505K substitution, which is sufficient to confer high-level rifampin resistance (MIC, 128 μg/mL) by agar dilution [11]. Rifaximin levels in the gut can reach levels of 8000 mg/mL [9], but it is unlikely that this mechanism of rifampin resistance can be overcome by increased concentrations. The majority of our resistant strains had accessory *rpoB* mutations (in addition to R505K), which O'Connor et al. [11] showed to be found in isolates viable at the highest rifampin concentrations used in the agar dilution method (MIC, >256 μg/mL). The observation of multiple RpoB polymorphisms within BI/NAP1 isolates suggests that secondary point mutations in *rpoB* occurred during circulation of the epidemic strain at our institution. Alternatively, isolates with multiple RpoB amino acid

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substitutions may represent unique BI/NAP1 circulating strains, some of which may have been selected for by rifampin pressure.

Despite the association between rifampin resistance and the epidemic clone, 18.5% of BI/NAP1 isolates were susceptible to rifampin. This could reflect reversion of the epidemic clone to a wild-type *rpoB* in the relative absence of rifampin pressure, because 34 of the 38 rifampin-susceptible epidemic clone isolates in this study were obtained during 2005. Alternatively, *rpoB* mutant isolates may have a competitive disadvantage over wild-type isolates, as has been demonstrated by Maughan et al. [16] for sporulation efficiency in *Bacillus cereus*. The decrease in rifamycin exposure during our second study period and the increased infection control efforts, may have been largely responsible for the re-emergence of rifampin-susceptible epidemic clone isolates at UPMC.

The association of rifampin resistance with the BI/NAP1 clone may be specific to our institution and may merely reflect clonal expansion. Additional studies will be required to determine the prevalence of rifampin resistance in *C. difficile* at other institutions and to determine whether the epidemic BI/NAP1 clone and/or other strains of *C. difficile* are associated with rifampin resistance. The number of individuals exposed to rifampin may have been underestimated in the present study, because electronic pharmacy records did not include outpatient exposures to rifamycins, although these exposures are presumably rare, given the rarity of rifamycin indications for outpatients in southwestern Pennsylvania. In addition, the true prevalence of rifampin-resistant *C. difficile* may be underestimated in this study because of our focus on isolates from patients with primary CDAD, as opposed to patients with relapsed CDAD, for which rifampin use is more common.

In summary, the present study demonstrates widespread primary rifampin resistance in *C. difficile* isolates from a cohort of patients with CDAD. Sequence analysis of the *rpoB* gene identified amino acid substitutions that are associated with rifampin resistance. These mutations are associated with rifaximin MICs that are not likely overcome even by the gut levels achieved after rifaximin administration [9]. Although this study raises questions about the use of rifaximin for treatment of CDAD at our institution, rifaximin may hold promise as an adjunct therapy for patients experiencing CDAD relapse. Additional studies are required to determine the prevalence of rifampin-resistant *C. difficile* at other institutions and to characterize the effect of *rpoB* mutations on *C. difficile* fitness, sporulation, germination, and toxin production.

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References

1. Dallal RM, Harbrecht BG, Boujoukas AJ, et al. Fulminant *Clostridium difficile:* an underappreciated and increasing cause of death and complications. Ann Surg 2002;235:363–72. [PubMed: 11882758]
2. Muto CA, Pokrywka M, Shutt K, et al. A large outbreak of *Clostridium difficile*–associated disease with an unexpected proportion of deaths and colectomies at a teaching hospital following increased fluoroquinolone use. Infect Control Hosp Epidemiol 2005;26:273–80. [PubMed: 15796280]
3. Loo VG, Poirier L, Miller MA, et al. A predominantly clonal multiinstitutional outbreak of *Clostridium difficile*–associated diarrhea with high morbidity and mortality. N Engl J Med 2005;353:2442–9. [PubMed: 16322602]

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4. McDonald LC, Killgore GE, Thompson A, et al. An epidemic, toxin genevariant strain of *Clostridium difficile*. N Engl J Med 2005;353:2433–41. [PubMed: 16322603]
5. Pepin J, Valiquette L, Cossette B. Mortality attributable to nosocomial *Clostridium difficile*–associated disease during an epidemic caused by a hypervirulent strain in Quebec. CMAJ 2005;173:1037–42. [PubMed: 16179431]
6. Killgore G, Thompson A, Johnson S, et al. Comparison of seven techniques for typing international epidemic strains of *Clostridium difficile:* restriction endonuclease analysis, pulsed-field gel electrophoresis, PCR-ribotyping, multilocus sequence typing, multilocus variable-number tandem-repeat analysis, amplified fragment length polymorphism, and surface layer protein A gene sequence typing. J Clin Microbiol 2008;46:431–7. [PubMed: 18039796]
7. Hecht DW, Galang MA, Sambol SP, Osmolski JR, Johnson S, Gerding DN. In vitro activities of 15 antimicrobial agents against 110 toxigenic *Clostridium difficile* clinical isolates collected from 1983 to 2004. Antimicrob Agents Chemother 2007;51:2716–9. [PubMed: 17517836]
8. Marchese A, Salerno A, Pesce A, Debbia EA, Schito GC. In vitro activity of rifaximin, metronidazole and vancomycin against *Clostridium difficile* and the rate of selection of spontaneously resistant mutants against representative anaerobic and aerobic bacteria, including ammonia-producing species. Chemotherapy 2000;46:253–66. [PubMed: 10859431]
9. Jiang ZD, Ke S, Palazzini E, Riopel L, Dupont H. In vitro activity and fecal concentration of rifaximin after oral administration. Antimicrob Agents Chemother 2000;44:2205–6. [PubMed: 10898704]
10. Johnson S, Schriever C, Galang M, Kelly CP, Gerding DN. Interruption of recurrent *Clostridium difficile*–associated diarrhea episodes by serial therapy with vancomycin and rifaximin. Clin Infect Dis 2007;44:846–8. [PubMed: 17304459]
11. O'Connor JR, Galang MA, Sambol SP, et al. Rifampin and rifaximin resistance in clinical isolates of *Clostridium difficile*. Antimicrob Agents Chemother 2008;52:2813–7. [PubMed: 18559647]
12. Muto CA, Blank MK, Marsh JW, et al. Control of an outbreak of infection with the hypervirulent *Clostridium difficile* BI strain in a university hospital using a comprehensive "bundle" approach. Clin Infect Dis 2007;45:1266–73. [PubMed: 17968819]
13. Curry SR, Marsh JW, Muto CA, O'Leary MM, Pasculle AW, Harrison LH. tcdC genotypes associated with severe TcdC truncation in an epidemic clone and other strains of *Clostridium difficile*. J Clin Microbiol 2007;45:215–21. [PubMed: 17035492]
14. Sebaihia M, Wren BW, Mullany P, et al. The multidrug-resistant human pathogen *Clostridium difficile* has a highly mobile, mosaic genome. Nat Genet 2006;38:779–86. [PubMed: 16804543]
15. Marsh JW, O'Leary MM, Shutt KA, et al. Multilocus variable-number tandem-repeat analysis for investigation of *Clostridium difficile* transmission in hospitals. J Clin Microbiol 2006;44:2558–66. [PubMed: 16825380]
16. Maughan H, Galeano B, Nicholson WL. Novel rpoB mutations conferring rifampin resistance on *Bacillus subtilis:* global effects on growth, competence, sporulation, and germination. J Bacteriol 2004;186:2481–6. [PubMed: 15060052]

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Table 1

Rifampin MICs in *Clostridium difficile* isolates from University of Pittsburgh Medical Center–Presbyterian Hospital, 2001–2002 and 2005

	No. (%) of isolates, by rifampin MIC			
Clone type	**≤0.002 μg/mL**	**0.003–32 μg/mL**	**>32 μg/mL**	**Total**
BI/NAP1	38 (18.5)	0 (0.0)	167 (81.5)	205
Non-BI/NAP1	253 (95.4)	6 (2.3)	6 (2.3)	265

NOTE. $P < .001$, by Fisher’s exact test.

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Table 2

Amino acid substitutions in RpoB associated with rifampin resistance of *Clostridium difficile*

	No. of isolates, by rifampin MICs			
RpoB amino acid substitution	**≤0.002 μg/mL**	**0.003–32 μg/mL**	**>32 μg/mL**	**Total**
Wild type	18	0	0	18
R505K	0	0	38	38
S488T R505K	0	0	8	8
S498T R505K	0	0	5	5
H502N R505K	0	0	8	8
H502N	0	4	0	4
R505K I548M	0	0	21	21
Total	18	4	80	102

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Table 3

Rifamycin class exposure in patients with *Clostridium difficile*–associated disease (CDAD) at University of Pittsburgh Medical Center–Presbyterian Hospital, 2001–2002 and 2005

Patient	Year	Rifamycin class	No. of defined daily doses before CDAD onset	Time from last dose to CDAD onset, days	Indication	Rifampin susceptibility (RpoB amino acid substitutions)
1	2001	Rifabutin	384	0	Disseminated MAI	R (R505K)
2	2001	Rifampin	5	22	Suspected *Staphylococcus aureus* pneumonia	S (WT)
3	2001	Rifampin	10.5	23	*S. aureus* septic arthritis	R (R505K/I548M)
4[a]	2001	Rifampin	0.5	93	Relapsed CDAD	R (S488T/R505K)[a]
5	2001	Rifampin	8	0	MRSA vertebral osteomyelitis and bone graft infection	No isolate
6	2001	Rifampin	2	3	Empirical TB therapy	R (R505K)
7	2001	Rifampin	54.5	69	MRSA endocarditis with osteomyelitis	R (R505K/I548M)
8	2001	Rifampin	14	20	*Legionella* pneumonia	R (R505K)
9	2001	Rifampin	18.5	0	MRSA osteomyelitis with hardware infection	R (R505K/I548M)
10	2005	Rifampin	34	25	CoNS catheter exit site infection	R (R505K)
11	2001	Rifabutin and rifampin	52 and 45	0	Culture-proven TB therapy	No isolate

NOTE. CoNS, coagulase-negative *Staphylococcus* species; DDD, defined daily dose (600 mg for rifampin and 150 mg for rifabutin), MAI, *Mycobacterium avium-intracellulare* complex; MRSA, methicillin-resistant *S. aureus;* TB, pulmonary *Mycobacterium tuberculosis*.

[a]The isolate from the first episode in patient 4 was not recovered, but an isolate from a relapse 50 days later was recovered. Data on this isolate are not included in tables 1 and 2.