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In Vitro Activity and Single-Step Mutational Analysis of Rifamycin SV Tested against Enteropathogens Associated with Traveler's Diarrhea and *Clostridium difficile*▽

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Rifamycin SV is a broad-spectrum, poorly absorbed antimicrobial agent that, when coupled with MMX technology, is being targeted for the oral treatment of traveler's diarrhea (TD) and *Clostridium difficile*-associated disease (CDAD). Rifamycin SV was tested for activity against 911 TD-associated enteropathogens and 30 *C. difficile* isolates collected from several global surveillance studies. Rifamycin SV demonstrated similar antimicrobial activity levels against the *Enterobacteriaceae*, with MIC_{50} values ranging from 32 to 128 μg/ml for all but one strain (an enterotoxigenic *Escherichia coli* at >512 μg/ml). For non-*Enterobacteriaceae* strains, MIC_{50} values ranged from 2 to 8 μg/ml, with the exception of *Campylobacter* spp., for which all strains had MIC values of >512 μg/ml. Rifamycin SV also demonstrated excellent activity (MIC_{50} of ≤0.03 μg/ml) against most *C. difficile* strains (including one hypervirulent NAP1 strain), and this activity was even superior to the potency observed for vancomycin, metronidazole, and rifaximin. In mutational passaging studies, rifamycin SV induced stable resistance and showed a mutation frequency in *E. coli* similar to that of rifampin. This study presents the potency of rifamycin SV for enteropathogens commonly recovered from patients with TD and CDAD. Additional *in vitro* and *in vivo* studies appear necessary to determine the utility of rifamycin SV as an oral agent for the prevention and treatment of TD and CDAD.

Rifamycin SV is a broad-spectrum semisynthetic antimicrobial agent of the rifamycin group with limited oral absorption that is active against Gram-positive bacteria and moderately active against Gram-negative organisms (18). Coupled with the proprietary drug delivery system MMX (Cosmo Technologies Ltd., Dublin, Ireland), designed to release the antibiotic in the colonic lumen, rifamycin SV has been formulated as a tablet for treatment of colonic bacterial infections, including traveler's diarrhea (TD) and *Clostridium difficile*-associated disease (CDAD). As with all members of the rifamycin group, rifamycin SV inhibits DNA transcription by interfering with bacterial RNA polymerases (2, 22).

TD is the most common gastrointestinal (GI) illness contracted by persons from developed countries when they visit resource-poor countries; annually, it is estimated that 100 million persons travel internationally, with an estimated 30 to 40% of them developing TD and approximately 3% progressing to postinfectious irritable bowel syndrome (15, 19). The most common bacterial etiologies of TD, accounting for nearly 60 to 80% of the cases, are pathogenic *Escherichia coli*, *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. although pathogen-specific prevalence rates do vary by geographic region (19).

Current travel medicine treatment guidelines recommend the use of antimicrobials for TD, in addition to fluid replacement and antimotility drugs, especially for severe disease (10). These guidelines recommend the use of a fluoroquinolone (FQ) or azithromycin for most travelers worldwide although regional pockets have significant levels of FQ-resistant (FQR) enteropathogens, especially in Southeast Asia and the Indian subcontinent. An alternative antimicrobial treatment option that is recommended for TD is rifaximin (12, 15), a compound closely related to rifamycin SV (18). Rifaximin is available in several countries and has been approved in the United States for the treatment of nondysenteric TD caused by noninvasive strains of *E. coli* in patients of ≥12 years of age (13). In clinical trials, rifaximin was shown to significantly shorten GI symptoms associated with TD compared to placebo and had similar outcomes compared to FQ treatment (13, 20). Since rifamycin SV is structurally closely related to rifaximin, it is possible that the biological activities of rifamycin SV will be similar to those of rifaximin. CDAD is the leading cause of nosocomial infectious diarrhea in developed countries, with a disease spectrum ranging from asymptomatic carriage to life-threatening pseudomembranous colitis (7). In addition, CDAD results in a very high economic burden for health care systems (8). Although antimicrobial resistance to standard treatment options of oral vancomycin and metronidazole is still rare, decreased susceptibility to these two antimicrobials has been reported along with increasing and geographically variable rates of resistance to other antimicrobials, such as the fluoroquinolones and macrolides (11). In addition, treatment has been compromised by the emergence of hypervirulent clones (such as NAP1) that cause outbreaks with both recurrent and refractory CDAD within groups at increased risk, such as children and peripartum women (7, 11). Poor response rates to current standard therapies have resulted in follow-up investigations into many alternative approaches (such as vaccines

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and stool transplantations, among others). Among several new antimicrobial treatment approaches, rifamycins have been investigated, and initial *in vitro* and animal and human studies support their use (7).

The first objective of this study was to evaluate the antimicrobial activity of rifamycin SV against bacterial pathogens commonly associated with TD and against *C. difficile* using reference *in vitro* assays. The second objective was to evaluate the ability of selected *E. coli* isolates to develop/acquire resistance following subinhibitory exposures (passaging) to rifamycin SV over 7 days, and the final objective was to establish the rates of single-step mutations at drug concentrations of 4×, 8×, and 16× MIC.

MATERIALS AND METHODS

Organism collection. A total of 941 enteropathogens were included in this study. Isolates with a known clinical source were collected from 33 countries (more than 100 medical centers) in five regions worldwide, including Latin America, North America, Europe, North Africa, and the Asia-Pacific region. The two primary sources for all enteropathogens were stool specimens (80.5%) and blood cultures (16.0%). All isolates were collected between 2000 and 2009, with 581 (63.8%) isolates collected between the years 2005 and 2009. Less frequently isolated species and those not within the scope of surveillance programs monitored by JMI Laboratories were collected from external sources dating from the year 2000.

The organism distribution was as follows: *E. coli*, 443 total isolates) including enterohemorrhagic *E. coli* ([EHEC]O157:H7; 105 isolates) enterotoxigenic *E. coli* ([ETEC] 201 isolates), enteropathogenic *E. coli* ([EPEC] 45 isolates), and enteroaggregative *E. coli* ([EAEC] 92 isolates); *Salmonella* spp. (102 isolates); *Shigella* spp. (105 isolates); *Aeromonas hydrophila* complex (101 isolates); *Campylobacter jejuni* and *Campylobacter coli* (102 isolates); *Vibrio parahaemolyticus* (42 isolates); *Plesiomonas shigelloides* (16 isolates); and *C. difficile* (30 isolates, including two NAP1 strains). For the passaging study, characterized *E. coli* strains were used, including the following: *E. coli* ATCC 25922 (control strain), *E. coli* 012-1222G (EHEC), *E. coli* 5347J (ETEC), *E. coli* 5753J (EAEC), and *E. coli* 915J (EPEC). All *E. coli* control strains used for the passaging experiments were susceptible to β-lactams, fluoroquinolones, aminoglycosides, tetracyclines, polymyxin B, and trimethoprim-sulfamethoxazole prior to and after passaging isolates over a 7-day interval (data not shown).

Antimicrobial susceptibility testing. MIC values were determined using the reference CLSI broth microdilution methods (3–5). Microdilution panels were produced by JMI Laboratories and utilized cation-adjusted Mueller-Hinton broth (CAMHB). Quality control (QC) ranges and interpretive criteria for an antimicrobial agent with established QC ranges were as published in CLSI document M100-S20 (6). QC strains used included *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Antimicrobial susceptibility testing of *C. jejuni* and *C. coli* isolates was determined using the reference CLSI broth microdilution method with 48 h of incubation at 36°C (5). Microdilution panels utilized CAMHB supplemented with 2.5 to 5% lysed horse blood. QC was confirmed by using *C. jejuni* ATCC 33560, and an antimicrobial agent with an established QC range was used for these determinations. Antimicrobial susceptibility testing of *C. difficile* was determined using the reference CLSI agar dilution method (4). Agar dilution plates were produced by JMI Laboratories utilizing brucella agar supplemented with laked sheep blood. QC and interpretive criteria for comparator compounds were those published by the CLSI (4), and tested QC strains included *C. difficile* ATCC 700057, *Bacteroides fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741. Rifamycin SV was provided by the sponsor in powder form and tested in a 12-$\log_2$ serial dilution schedule (0.25 to 512 μg/ml). Rifampin, obtained from Sigma-Aldrich (St. Louis, MO), was tested in parallel as a control agent.

Passaging and selection of resistant strains. From the MIC panel, the entire contents of the last well with growth was removed from both the rifamycin SV and rifampin panels and placed into tubes of broth medium. Tubes were placed in an ambient air incubator to allow growth to reach a 0.5 McFarland standard (1.5 to 3 h). Within 15 min of preparing a 0.5 McFarland, the appropriate amount of bacterial suspension was transferred to testing medium and vortexed. MIC panels were then inoculated using the appropriate volume and concentration, and this process was repeated through seven passage days. The strains were tested against numerous selected antimicrobial agents prior to resistance selection and upon completion of passaging to evaluate the emergence of cross-resistance. Reversion to the original rifamycin SV and rifampin MIC values was assessed by three passages performed on drug-free Mueller-Hinton (MH) medium, with final retesting by the broth microdilution method panel containing these agents. Passaging of each strain was performed in duplicate.

TABLE 1. Antimicrobial activity of rifamycin SV against a collection of 911 TD-associated enteropathogens

Organism (no. of isolates tested)	Rifamycin SV MIC (μg/ml)		
	50%	90%	Range
E. coli (443)[a]	32	128	2–>512
EHEC (105)	32	64	8–256
ETEC (201)	64	128	2–>512
EPEC (45)	64	128	16–128
EAEC (92)	32	128	4–256
Salmonella spp. (102)[b]	128	256	16–256
Shigella spp. (105)[c]	32	64	8–128
Campylobacter spp. (102)[d]	>512	>512	>512
A. hydrophila (101)	4	16	2–512
P. shigelloides (16)	8	8	4–8
V. parahaemolyticus (42)	2	2	2–4

[a] EHEC, enterohemorrhagic *E. coli*; ETEC, enterotoxigenic *E. coli*; EPEC, enteropathogenic *E. coli*; EAEC, enteroaggregative *E. coli*.

[b] *Salmonella enterica* serovar Choleraesuis (1 strain), *S. enterica* serovar Derby (1 strain), *S. enterica* serovar Dublin (1 strain), *S. enterica* (1 strain), *S. enterica* serovar Enteritidis (10 strains), *S. enterica* serovar Hadar (1 strain), *S. enterica* serovar Heidelberg (three strains), *S. enterica* serovar Java (1 strain), *S. enterica* serovar Panama (2 strains), *S. enterica* serovar Paratyphi (12 strains), *S. enterica* serovar Reading (1 strain), *S. enterica* serovar Stanley (1 strain), *S. enterica* serovar Typhi (19 strains), *S. enterica* serovar Typhimurium (five strains), *S. enterica* serovar Virchow (1 strain), group B *Salmonella* (12 strains), group C *Salmonella* (2 strains), group D *Salmonella* (6 strains), and unspeciated *Salmonella* (22 strains).

[c] *Shigella boydii* (3 strains), *Shigella dysenteriae* (five strains), *Shigella flexneri* (33 strains), *Shigella sonnei* (51 strains), and unspeciated *Shigella* (13 strains).

[d] *C. coli* (16 strains) and *C. jejuni* (86 strains).

Single-step mutation rates. Fresh colonies from an agar plate were emulsified in sterile broth or saline until at least a 4 McFarland standard (1.9×10^8 to 4.6×10^9 CFU/ml) was achieved. Final volumes of 0.1 and 1 ml of the inoculum suspension were plated onto agar plates containing 4×, 8×, and 16× MIC for both rifamycin SV and rifampin. Serial dilutions of the inoculum suspensions were plated onto antimicrobial-free MH agar plates to quantify the colony count (CFU/ml) at each exposure concentration. Each strain count was performed in triplicate, and the mutant counts were averaged for calculation of the mutation frequency. The mutant frequency was determined to be the ratio of the number of mutants to the total number of bacteria in the population.

Molecular methods. Pulsed-field gel electrophoresis (PFGE) analysis of parent strain and mutant strains was assessed upon completion of the experiment. Digestion with the appropriate restriction enzyme (SpeI) was used to produce DNA fragments of a number and size that yielded useful patterns for strain typing (21).

RESULTS

Antimicrobial susceptibility study. Similar rifamycin SV antimicrobial activity levels were observed for all *Enterobacteriaceae*, with MIC_{50} values ranging from 32 to 128 μg/ml (Tables 1 and 2). Rifamycin SV inhibited all tested *E. coli* strains (MIC_{50} and MIC_{90} [$MIC_{50/90}$], 32/128 μg/ml), with the exception of one ETEC strain having a MIC value of >512 μg/ml (Table 1). Similar antimicrobial activities were noted among the pathogenic *E. coli* subgroups, with rifamycin SV being slightly more potent against EHEC and EAEC (MIC_{50}, 32 μg/ml for both) than against ETEC and EPEC (MIC_{50}, 64 μg/ml for both). Similar MIC values were observed for *Shigella* spp. ($MIC_{50/90}$, 32/64 μg/ml), with 100.0% of the *Shigella* spp.

TABLE 2. *In vitro* activity of rifamycin SV in comparison to selected antimicrobial agents tested against *C. difficile* (30 strains)

Antimicrobial agent	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)
Rifamycin SV	0.03	256	≤0.015–512
Rifaximin	0.12	>512	≤0.06–>512
Metronidazole	0.25	0.5	0.12–0.5
Vancomycin	0.5	1	0.5–8

strains having MIC values at ≤128 μg/ml (Tables 1 and 2). Rifamycin SV was slightly less potent against *Salmonella* spp. ($MIC_{50/90}$, 128/256 μg/ml), with the vast majority of isolates (93.1%) exhibiting MIC values at 128 μg/ml (55.9%) and 256 μg/ml (38.2%).

Among the non-*Enterobacteriaceae* species tested, rifamycin SV lacked measurable activity against *C. coli* and *C. jejuni*, with all strains having a MIC value of >512 μg/ml (Table 1). Against *A. hydrophila*, rifamycin SV MIC values ranged from 2 to 512 μg/ml ($MIC_{50/90}$, 4/16 μg/ml), but all *P. shigelloides* strains were inhibited by a rifamycin SV MIC value of ≤8 μg/ml. The most susceptible organism species among the non-*Enterobacteriaceae* was *Vibrio parahaemolyticus* (MIC_{50} and MIC_{90}, 2 μg/ml), which demonstrated a very narrow MIC distribution, with 95.2% of strains inhibited at 2 μg/ml and the remaining two isolates at 4 μg/ml (Table 1). Rifamycin SV was very active (MIC_{50}, ≤0.03 μg/ml) against 26/30 (86.6%) *C. difficile* strains (including one hypervirulent NAP1 strain), but high MIC values (256 to 512 μg/ml) were observed against the remaining four *C. difficile* strains (also including one hypervirulent NAP1 strain). Rifamycin SV (MIC_{50}, 0.03 μg/ml) was 4-fold more active than rifaximin (MIC_{50}, 0.12 μg/ml), 8-fold more active than metronidazole (MIC_{50}, 0.25 μg/ml), and 16-fold more active than vancomycin (MIC_{50}, 0.5 μg/ml) against *C. difficile* (Table 2).

Passaging study. Table 3 lists the passaging results for all tested strains. The MIC for *E. coli* ATCC 25922, the designated wild-type isolate, increased only 2-fold with rifamycin SV (32 μg/ml to 64 μg/ml) and rifampin (8 μg/ml to 16 μg/ml) after seven passage days, with slight MIC variations observed in the daily replicate assays. The *E. coli* 012-1222G (EHEC O157:H7) MIC of rifamycin SV increased 4- to 64-fold and that of rifampin increased 2- to 4-fold at day 7. For this strain, significant variation was observed between the daily replicated assays, with up to a 32-fold difference between replicates. The MICS of both rifamycin SV and rifampin for *E. coli* 5347J (ETEC) and *E. coli* 5753J (EAEC) increased 8- to 16-fold. The *E. coli* 915J (EPEC) MIC remained consistent at 32 μg/ml throughout the experiment for both replicates; however, rifampin MIC values increased 4- to 64-fold for this isolate, with significant variability between the two replicates in the last day of passaging. After passage on antimicrobial-free medium, most strains maintained the MIC value obtained after passaging for 7 days, and none of the isolates reverted back to the original (baseline) MIC value. For each strain, PFGE patterns of the parent strain (day 0) and matched day 7 strains with three additional days passaging on antimicrobial-free medium were considered to be identical (data not shown).

TABLE 3. MIC results of serial passaging experiments with rifamycin SV and rifampin tested against five *E. coli* strains

E. coli strain	Passage day or period	MIC(μg/ml)			
		Rifamycin SV		Rifampin	
		Assay 1	Assay 2	Assay 1	Assay 2
ATCC 25922	0	32	32	8	8
	1	32	32	8	16
	2	32	32	8	16
	3	64	32	16	16
	4	64	32	16	16
	5	64	64	16	16
	6	128	64	16	16
	7	64	64	16	16
	R[a]	64	64	16	16
012-1222G	0	32	32	8	8
	1	32	64	16	8
	2	64	64	16	8
	3	128	64	16	16
	4	1,024	64	16	16
	5	1,024	128	16	16
	6	2,048	128	16	8
	7	2,048	128	32	16
	R	2,048	64	16	16
5753J	0	8	8	4	8
	1	32	32	16	8
	2	32	32	32	16
	3	64	64	16	16
	4	64	64	16	64
	5	64	64	32	64
	6	128	128	32	128
	7	128	128	32	128
	R	64	64	16	64
5347J	0	8	8	4	4
	1	16	16	8	8
	2	16	32	16	16
	3	64	64	32	32
	4	64	64	32	32
	5	64	64	32	32
	6	128	128	32	32
	7	64	64	32	32
	R	64	64	32	32
915J	0	32	32	8	8
	1	32	32	8	8
	2	32	32	8	8
	3	32	32	8	8
	4	32	32	8	8
	5	32	32	16	16
	6	32	32	32	32
	7	32	32	1,024	16
	R	32	32	1,024	16

[a] Postpassaging reversion subcultures on drug-free medium.

Mutation frequency determination study. Rifamycin SV and rifampin mutants were observed when the strains were exposed to 4×, 8×, and 16× the MIC value for all five *E. coli* strains tested; however, the mutation frequency was independent of the antimicrobial concentration. Among the *E coli* strains tested, the mutation frequencies for rifamycin SV ranged from 1.4×10^{-6} to $<5.0 \times 10^{-10}$, and for rifampin, they ranged from 3.3×10^{-7} to $<5.0 \times 10^{-10}$ (Table 4). PFGE patterns of rifamycin SV for the single-step mutants were identical to

TABLE 4. Mutation frequency with a single exposure to rifamycin SV and rifampin in five *E. coli* strains

Drug and strain	Mutation frequency at the indicated drug concn[a]		
	4× MIC	8× MIC	16× MIC
Rifamycin SV			
E. coli ATCC 25922	5.9×10^{-8}	5.9×10^{-8}	8.8×10^{-8}
E. coli 012-1222G	1.4×10^{-7}	1.4×10^{-7}	$<5.0 \times 10^{-10}$
E. coli 5347J	7.8×10^{-7}	8.7×10^{-8}	1.7×10^{-7}
E. coli 915J	1.4×10^{-6}	6.3×10^{-7}	1.6×10^{-7}
E. coli 5753J	8.7×10^{-7}	2.0×10^{-7}	2.0×10^{-7}
Rifampin			
E. coli ATCC 25922	1.3×10^{-8}	8.5×10^{-9}	4.8×10^{-9}
E. coli 012-1222G	3.5×10^{-8}	3.9×10^{-8}	3.3×10^{-8}
E. coli 5347J	4.7×10^{-9}	2.9×10^{-9}	1.8×10^{-9}
E. coli 915J	4.0×10^{-9}	$<5.0 \times 10^{-10}$	$<5.0 \times 10^{-10}$
E. coli 5753J	1.4×10^{-8}	5.0×10^{-9}	6.7×10^{-8}

[a] Drug concentration is based on the MIC for each individual strain. The mutation frequency was determined to be the ratio of the number of mutants to the total number of bacteria in the population.

those of the initial (parent) isolate for all five strains at each of the tested concentrations (data not shown).

DISCUSSION

Rifamycin SV is a semisynthetic antimicrobial agent closely related to another rifamycin-derived antimicrobial agent, rifaximin, which is licensed for the treatment of TD in the United States and is widely available in several countries. Rifaximin, a poorly absorbed oral antimicrobial agent, achieves luminal concentration up to 8,000 μg/gram of feces after a 3-day treatment regimen (12), which is manyfold higher than measured MIC values obtained from *in vitro* testing for several enteropathogens. Likewise, rifamycin SV has been shown to have very limited absorption in humans after oral administration (1). When rifamycin SV was administered in tablets to be delivered directly to the colon, with little or no loss of antimicrobial activity while transiting the gastrointestinal tract, the total recovery in the feces was >80% of the administered dose, which represents in the colonic environment a concentration far exceeding the MIC values for the isolates tested (data on file; Cosmo Technologies Ltd., Dublin, Ireland).

In our study, rifamycin SV showed consistent potency among the groupings of enteropathogens, both *Enterobacteriaceae* and non-*Enterobacteriaceae*, with the exception of *Campylobacter* spp. Compared to rifaximin *in vitro* MIC studies (9, 16), rifamycin SV was slightly less potent against *Enterobacteriaceae* (rifaximin MIC_{90} of 8 to 64 μg/ml versus a rifamycin SV MIC_{90} of 64 to 256 μg/ml), had similar potency for non-*Enterobacteriaceae* (rifaximin MIC_{90} of 4 μg/ml versus a rifamycin SV MIC_{90} of 2 to 16 μg/ml), and was much less potent for *Campylobacter* spp. (rifaximin MIC_{90} of 32 μg/ml versus a rifamycin SV MIC_{90} of >512 μg/ml). Nevertheless, these differences of microbiological activity between the two antibiotics should not have a great clinical impact considering the high concentrations of rifamycin SV reached in the lumen of the colon due to the MMX formulation, which is specifically designed for colonic release.

Our data also show that rifamycin SV was very active against most *C. difficile* strains tested, including one hypervirulent NAP1 clone, resulting in 4-, 8-, and 16-fold more potency than rifaximin, metronidazole, and vancomycin, respectively. This new and interesting activity could be regarded as a promising option for a possible role of rifamycin SV in the treatment of *C. difficile*-related enteritis.

For an antimicrobial agent to remain effective, the selection of resistance among targeted species should be minimized during antimicrobial exposure, and then any increase in resistance should revert back to baseline once the exposure is removed. Previous studies have noted that rifamycins, including rifaximin and rifampin, are prone to inducing resistant mutants in *E. coli* at approximately the rate of 10^{-8}, and these mutants are then stable, maintaining increased MIC values even after successive passage on antibiotic-free medium. The results of our passaging experiments (4- to 64-fold stable increases in MIC values) and the single-step mutation experiments (approximately 10^{-7} for rifamycin SV and approximately 10^{-9} for rifampin) were consistent with these previously published studies referring to rifaximin and rifampin resistance (14, 17, 18, 22). In Ruiz et al. (17), the authors suggest that sustained rifaximin resistance was likely due to the presence of chromosomal mutation in the *rpoB* gene or a stable deregulated efflux pump. Specific molecular studies will be required of our rifamycin SV-resistant mutants to determine the underlying mechanism(s) of resistance, as no obvious genetic changes between parent and progeny strains were observed in the PFGE results.

In conclusion, the data presented here quantitate the baseline potency of rifamycin SV when it is tested against enteropathogens causative TD and CDAD. The results of our study should be interpreted in the context of the high concentrations of rifamycin SV achieved in the intestinal lumen and the promising results of initial clinical trials. Antimicrobial agents with bioavailability restricted to the gastrointestinal tract (high intraluminal and fecal concentrations) that largely exceed the pathogen MIC values are needed against a wide range of organisms for the treatment of bacterial diarrheal disease. Pending further clinical trials examining clinical outcomes and microbial eradication, rifamycin SV may prove to be a valuable oral agent for the prevention and treatment of acute gastroenteritis for several enteropathogens associated with TD and CDAD.

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REFERENCES

1. **Bergamini, N., and G. Fowst.** 1965. Rifamycin SV, a review. Arzneimittelforschung **15**(Suppl.)**:**953–1002.
2. **Calvori, C., L. Frontali, L. Leoni, and G. Tecce.** 1965. Effect of rifamycin on protein synthesis. Nature **207:**417–418.
3. **CLSI.** 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard M07-A8, 8th ed. Clinical and Laboratory Standards Institute, Wayne, PA.
4. **CLSI.** 2008. Methods for antimicrobial susceptibility testing of anaerobic bacteria; approved standard M11-A7, 7th ed. Clinical and Laboratory Standards Institute, Wayne, PA.

5. **CLSI.** 2006. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; approved standard M45-A. Clinical and Laboratory Standards Institute, Wayne, PA.
6. **CLSI.** 2010. Performance standards for antimicrobial susceptibility testing; 20th informational supplement. CLSI document M100-S20. Clinical and Laboratory Standards Institute, Wayne, PA.
7. **Garey, K. W., M. Salazar, D. Shah, R. Rodrigue, and H. L. DuPont.** 2008. Rifamycin antibiotics for treatment of *Clostridium difficile*-associated diarrhea. Ann. Pharmacother. **42:**827–835.
8. **Ghantoji, S. S., K. Sail, D. R. Lairson, H. L. Dupont, and K. W. Garey.** 2010. Economic healthcare costs of *Clostridium difficile* infection: a systematic review. J. Hosp. Infect. **74:**309–319.
9. **Gomi, H., et al.** 2001. In vitro antimicrobial susceptibility testing of bacterial enteropathogens causing traveler's diarrhea in four geographic regions. Antimicrob. Agents Chemother. **45:**212–216.
10. **Hill, D. R., et al.** 2006. The practice of travel medicine: guidelines by the Infectious Diseases Society of America. Clin. Infect. Dis. **43:**1499–1539.
11. **Huang, H., A. Weintraub, H. Fang, and C. E. Nord.** 2009. Antimicrobial resistance in *Clostridium difficile*. Int. J. Antimicrob. Agents **34:**516–522.
12. **Jiang, Z. D., S. Ke, E. Palazzini, L. Riopel, and H. Dupont.** 2000. In vitro activity and fecal concentration of rifaximin after oral administration. Antimicrob. Agents Chemother. **44:**2205–2206.
13. **Koo, H. L., H. L. Dupont, and D. B. Huang.** 2009. The role of rifaximin in the treatment and chemoprophylaxis of travelers' diarrhea. Ther. Clin. Risk Manag. **5:**841–848.
14. **Miller, K., A. J. O'Neill, and I. Chopra.** 2002. Response of *Escherichia coli* hypermutators to selection pressure with antimicrobial agents from different classes. J. Antimicrob. Chemother. **49:**925–934.
15. **Pimentel, M., and M. S. Riddle.** 2008. Prevention of traveler's diarrhea: a call to reconvene. Clin. Infect. Dis. **46:**151–152.
16. **Ruiz, J., et al.** 2007. In vitro antimicrobial activity of rifaximin against enteropathogens causing travelers' diarrhea. Diagn. Microbiol. Infect. Dis. **59:**473–475.
17. **Ruiz, J., L. Mensa, M. J. Pons, J. Vila, and J. Gascon.** 2008. Development of *Escherichia coli* rifaximin-resistant mutants: frequency of selection and stability. J. Antimicrob. Chemother. **61:**1016–1019.
18. **Sensi, P.** 1983. History of the development of rifampin. Rev. Infect. Dis. **5**(Suppl. 3)**:**S402–S406.
19. **Shah, N., H. L. DuPont, and D. J. Ramsey.** 2009. Global etiology of travelers' diarrhea: systematic review from 1973 to the present. Am. J. Trop. Med. Hyg. **80:**609–614.
20. **Taylor, D. N., et al.** 2006. A randomized, double-blind, multicenter study of rifaximin compared with placebo and with ciprofloxacin in the treatment of travelers' diarrhea. Am. J. Trop. Med. Hyg. **74:**1060–1066.
21. **Tenover, F. C., et al.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
22. **Wehrli, W.** 1983. Rifampin: mechanisms of action and resistance. Rev. Infect. Dis. **5**(Suppl. 3)**:**S407–S411.