

# Surotomycin Demonstrates Low *In Vitro* Frequency of Resistance and Rapid Bactericidal Activity in *Clostridium difficile*, *Enterococcus faecalis*, and *Enterococcus faecium*

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Surotomycin (CB-183,315) is an orally administered, minimally absorbed, selective bactericidal cyclic lipopeptide in phase 3 development for the treatment of *Clostridium difficile*-associated diarrhea. The aim of this study was to evaluate the emergence of resistance in *C. difficile* (ATCC 700057 and three recent clinical isolates from the restriction endonuclease analysis groups BI, BK, and K), vancomycin-susceptible (VS) *Enterococcus faecalis* (ATCC 49452), vancomycin-resistant (VR) *E. faecalis* (ATCC 700802), VS *Enterococcus faecium* (ATCC 6569), and VR *E. faecium* (ATCC 51559) under anaerobic conditions. The rate of spontaneous resistance was below the limit of detection ( $<10^{-8}$  to  $<10^{-9}$ ) for surotomycin at 16 and 32 $\times$  the MIC for all isolates tested. Under selective pressure by serial passage, *C. difficile* grew in a maximum of 4  $\mu\text{g/ml}$  surotomycin (final MICs of 2 to 8  $\mu\text{g/ml}$  [4- to 16-fold higher than those of the naive control]) at day 15, with the exception of the *C. difficile* BK strain, which grew in 16 to 32  $\mu\text{g/ml}$  (final MICs of 8 to 32  $\mu\text{g/ml}$  [16- to 64-fold higher than those of the naive control]). Enterococci remained relatively unchanged over 15 days, growing in a maximum of 8  $\mu\text{g/ml}$  surotomycin (final MICs of 2 to 16  $\mu\text{g/ml}$  [8- to 64-fold higher than those of the naive control]). Of the isolates tested, no cross-resistance to vancomycin, rifampin, ampicillin, metronidazole, or moxifloxacin was observed. Surotomycin at 20 $\times$  MIC demonstrated equally rapid bactericidal activity ( $\geq 3$ -log-unit reduction in CFU/ml in  $\leq 8$  h) against naive and reduced-susceptibility isolates of *C. difficile*, VS *Enterococcus* (VSE), and VR *Enterococcus* (VRE), except for *C. difficile* BK (2.6-log-unit reductions for both). These results suggest that emergence of resistance to surotomycin against *C. difficile*, *E. faecalis*, and *E. faecium* is likely to be rare.

First reported in the 1970s, *Clostridium difficile* infection is the most common cause of health care-related infectious diarrhea in developed countries, accounting for 20 to 30% of cases of antibiotic-associated diarrhea and nearly all cases of antibiotic-associated colitis (1, 2). *C. difficile*-associated diarrhea (CDAD) represents a significant clinical and economic burden and is associated with longer length of hospital stay, increased medical costs, and high rates of morbidity and mortality, especially among the elderly (3, 4). The frequency and severity of the disease have increased over the past decade, along with the emergence of particular epidemic strains (e.g., BI/NAP1/027) (5).

Vancomycin and metronidazole are the treatment options for the first and second episodes of CDAD. Each agent is equally effective among patients with mild disease, but vancomycin appears superior to metronidazole for the treatment of patients with severe CDAD (6). However, the greatest unmet need in patients with CDAD is disease recurrence, which may occur in up to 50% of treated patients (6–8). Fidaxomicin, a recently approved macrocyclic antibiotic, demonstrated improved recurrence rates in phase 3 studies (9), but even with the approval of fidaxomicin, additional therapies are required to address the increased prevalence of recurrent CDAD.

Surotomycin (CB-183,315) is an orally administered, minimally absorbed, selective bactericidal cyclic lipopeptide in phase 3 development for the treatment of CDAD (10). Potent activity against *C. difficile* (MIC<sub>90</sub> = 0.5  $\mu\text{g/ml}$ ) and other Gram-positive bacteria, with minimal impact on the Gram-negative organisms of the intestinal microbiota, has been demonstrated *in vitro* (11, 12). In a recent phase 2 clinical trial (NCT01085591), surotomycin doses of 125 mg and 250 mg twice daily (b.i.d.), compared with

oral vancomycin, were safe and well tolerated in patients with CDAD (13).

The aim of this study was to evaluate the emergence of spontaneous resistance and multistep resistance under selective pressure of recent clinical isolates of *C. difficile* (restriction endonuclease analysis groups BI, BK, and K), vancomycin-susceptible and vancomycin-resistant *Enterococcus faecalis* (VSEfs and VREfs, respectively), and vancomycin-susceptible and vancomycin-resistant *Enterococcus faecium* (VSEfm and VREfm, respectively), under anaerobic conditions. The activity of surotomycin against naive and reduced-susceptibility isolates was also evaluated, as was cross-resistance to various classes of antibiotics.

(Portions of these data were previously presented at the 4th International *Clostridium difficile* Symposium [14] and at the 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy [15].)

## MATERIALS AND METHODS

**Strains, media, and antibiotics.** The study was performed using four *Clostridium difficile* isolates (ATCC 700057 [quality control {QC}] and three recent clinical isolates from the restriction endonuclease analysis groups BI, BK, and K, collected during the LCD-DR-09-03 phase 2 study),

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**TABLE 1** *C. difficile*, *E. faecalis*, and *E. faecium* strains and MICs used in this study

Organism	Strain	ATCC strain no.	MIC ( $\mu\text{g/ml}$ )		
			Surotomycin	Vancomycin	Rifampin
<i>C. difficile</i>	QC	700057	1	1	<0.001
	BI		0.5	0.5	<0.001
	BK		0.5	0.5	<0.001
	K		0.5	2	<0.001
<i>E. faecalis</i>	VSEfs	49452	0.5	1	0.125
	VREfs	700802	0.25	64	0.5
<i>E. faecium</i>	VSEfm	6569	0.5	0.5	16
	VREfm	51559	1	>64	4

VSEfs (ATCC 49452), VREfs (ATCC 700802), VSEfm (ATCC 6569), and VREfm (ATCC 51559). The starting MICs for surotomycin (Cubist Pharmaceuticals, Lexington, MA, USA), vancomycin, and rifampin against each organism tested are listed in Table 1. The MICs were determined according to methods described in Clinical and Laboratory Standards Institute approved standard M11-A7 (16), as previously reported (17). Besides surotomycin, all antibiotics were purchased from Sigma (St. Louis, MO, USA).

All work was performed in a Bactron I anaerobic chamber (Sheldon Manufacturing, Cornelius, OR, USA) infused with anaerobic mixed gas (5%  $\text{H}_2$ , 10%  $\text{CO}_2$ , 85%  $\text{N}_2$ ; Airgas, Salem, NH, USA) using reduced media. For *Enterococcus* studies, Mueller-Hinton broth and Mueller-Hinton agar (18 g/liter of BD Bacto agar added to Mueller-Hinton broth) were used (BD Diagnostic Systems, Sparks, MD, USA). For *C. difficile* studies, BBL brucella broth and BBL brucella agar from BD Diagnostic Systems (Sparks, MD, USA) were used. All assay media were prepared and sterilized according to the manufacturer's specifications and then supplemented to a final concentration of 50 mg/liter calcium. Additionally, brucella broth and agar were supplemented with hemin (5  $\mu\text{g/ml}$ ) and vitamin  $\text{K}_1$  (1  $\mu\text{g/ml}$ ). In order to grow frozen cultures, prepared brucella agar plates supplemented with 5% sheep blood, hemin, and vitamin  $\text{K}_1$  (BBHK) (BD Diagnostic Systems) and tryptic soy agar fortified with 5% sheep blood (TSAB) (bioMérieux, Durham, NC, USA) were used.

**Resistance incidence by direct plating.** Resistance incidence (RI) of *C. difficile* was determined in supplemented brucella broth and agar, while RIs of *E. faecalis* and *E. faecium* were determined in supplemented Mueller-Hinton broth and Mueller-Hinton agar. Cultures suspended to  $\sim 1$  McFarland (McF) and incubated at 37°C for  $\sim 4$  h were used as inocula (0.5 ml; between 3 to 4 McF) on selection plates containing surotomycin, vancomycin, or rifampin at 8, 16, or 32 $\times$  the MIC, in independent, duplicate assays. Rifampin was employed as an assay control given its known high RI. Plates were incubated at 37°C for 48 h. Viable CFU/ml of each inoculum was determined, and the RI was calculated as the colony frequency (equal to the number of colonies on drug-containing plates divided by the total number of colonies plated). Colonies recovered on drug-containing plates were all evaluated by broth MIC after three transfers on drug-free media to confirm elevated MIC values compared with the MICs of naive controls.

**Selection for resistance by serial passage.** Serial passages of *C. difficile*, *E. faecalis*, and *E. faecium* were performed in the presence of increasing concentrations of surotomycin, vancomycin, or rifampin, with the exception of VREfs and VREfm which were passaged against only surotomycin, as previously described (18). The *C. difficile* serial passages were performed in supplemented brucella broth, while the *E. faecalis* and *E. faecium* serial passages were performed in calcium-supplemented Mueller-Hinton broth. Cultures suspended in media to equal  $\sim 1$  McF, incubated at 37°C for  $\sim 3$  h, were inoculated into media containing various concentrations of drug above and below the MIC and grown overnight at 37°C

**TABLE 2** Resistance incidence of *C. difficile* BI, BK, K, and QC strains against surotomycin, vancomycin, and rifampin

<i>C. difficile</i> strain	Antibiotic	Concn(s) tested ( $\times$ MIC)	RI <sup>a</sup>	
			Sample A	Sample B
BI	Surotomycin	8	4.4E-09	<5.4E-09
		16, 32	<4.4E-09	<5.4E-09
		8, 16, 32	<4.4E-09	<5.4E-09
	Vancomycin	8	<4.4E-09	3.2E-08
		16	1.3E-08	5.4E-09
		8, 16, 32	<4.4E-09	<5.4E-09
BK	Surotomycin	8	<4.2E-08	<6.0E-08
		16	<4.2E-08	6.0E-08
		32	<4.2E-08	<6.0E-08
	Vancomycin	8, 16, 32	<4.2E-08	<6.0E-08
		8	<4.2E-08	1.2E-07
		16	4.2E-08	<6.0E-08
K	Surotomycin	8, 16, 32	<5.8E-09	<4.9E-09
		8, 16, 32	<5.8E-09	<4.9E-09
		8	<5.8E-09	9.9E-09
	Rifampin	16	1.7E-08	9.9E-09
		8, 16, 32	<6.5E-09	<5.5E-09
		8, 16, 32	<6.5E-09	<5.5E-09
QC	Surotomycin	8	1.3E-08	1.1E-08
		16	2.6E-08	5.5E-09
		8, 16, 32	<6.5E-09	<5.5E-09
	Vancomycin	8, 16, 32	<6.5E-09	<5.5E-09
		8	1.3E-08	1.1E-08
		16	2.6E-08	5.5E-09

<sup>a</sup> RI, resistance incidence. The average numbers of CFU/ml plated for the *C. difficile* strains were as follows: 1.9E+08 to 2.3E+08 for strain BI, 1.7E+07 to 2.4E+07 for strain BK, 1.7E+08 to 2.0E+08 for strain K, and 1.6E+08 to 1.8E+08 for strain QC.

for 20 to 24 h. Each day, wells with the highest concentration of drug (in micrograms per milliliter) permitting growth equal to  $\sim 1$  McF (equal to  $10^7$  CFU/ml) were used to inoculate the next day's drug series; this process was repeated for 15 days with each drug/bacterium combination tested in duplicate. Colonies recovered from day 15 were all evaluated by broth MIC after three drug-free transfers to confirm elevated MIC values compared with those of naive controls.

**Time-kill analysis.** Individual *C. difficile*, *E. faecalis*, and *E. faecium* colonies were isolated by streaking onto rich, nonselective media (BBHK agar for *C. difficile*, TSAB for enterococci) with anaerobic incubation at 35 to 37°C for 48 h. On the day of testing, cultures were scraped off plates, suspended in reduced media ( $>1$  McF), and incubated for  $\sim 2$  h. At the start of the assay, cultures were diluted to equal  $\sim 1$  McF ( $10^7$  CFU/ml), either left untreated (no-drug control) or treated separately with 20 $\times$  the MIC of surotomycin, vancomycin, or metronidazole, and incubated at 35 to 37°C. The concentrations of vancomycin, metronidazole and surotomycin in fecal samples in CDAD patients have all been reported to be  $\geq 20\times$  the MIC of the isolates used in this study (vancomycin [VAN] [19], metronidazole [MET] [20], and surotomycin [SUR] [data on file at Cubist Pharmaceuticals, Lexington, MA, USA]). Cell viability was determined at 0, 2, 4, 8, and 24 h by serial dilution and plating for quantification of CFU/ml.

## RESULTS

**Spontaneous resistance.** The spontaneous resistance incidence (RI) to surotomycin, vancomycin, and rifampin was determined under anaerobic conditions at 8, 16, and 32 $\times$  the MIC. The observed RI values of surotomycin, vancomycin, and rifampin against *C. difficile* strains are shown in Table 2. The rate of spontaneous resistance was below the limit of detection ( $<10^{-8}$  to  $<10^{-9}$ ) for surotomycin at 16 and 32 $\times$  the MIC for all isolates tested. A single reduced-susceptibility isolate was identified for strains BI (final MIC of 1  $\mu\text{g/ml}$ ; 2-fold higher than that of the

**TABLE 3** Resistance incidence of vancomycin-susceptible and -resistant *E. faecalis* and *E. faecium* strains against surotomycin, vancomycin, and rifampin

Enterococcal strain	Antibiotic	Concn(s) tested (× MIC)	RI <sup>a</sup>	
			Sample A	Sample B
VSEfs	Surotomycin	8, 16, 32	<1.33E-09	<2.34E-09
	Vancomycin	8, 16, 32	<1.33E-09	<2.34E-09
	Rifampin	8, 16	>1.3E-06	>2.3E-07
VSEfm	Surotomycin	8, 16, 32	<2.9E-09	<1.97E-09
	Vancomycin	8, 16, 32	<2.9E-09	<1.97E-09
VREfs	Surotomycin	8	3.2E-09	<9.09E-10
		16	<1.6E-09	9.1E-10
		32	<1.6E-09	<9.09E-10
	Rifampin	8	>8.0E-07	>1.6E-07
		16	1.4E-08	7.8E-08
VREfm	Surotomycin	8, 16, 32	<3.39E-09	<4.04E-09

<sup>a</sup> The average numbers of CFU/ml plated for vancomycin-susceptible and -resistant *E. faecalis* and *E. faecium* strains were as follows: 4.3E+08 to 7.5E+08 for VSEfs, 3.5E+08 to 5.1E+08 for VSEfm, 6.3E+08 to 1.1E+09 for VREfs, and 2.4E+08 to 3.0E+08 for VREfm.

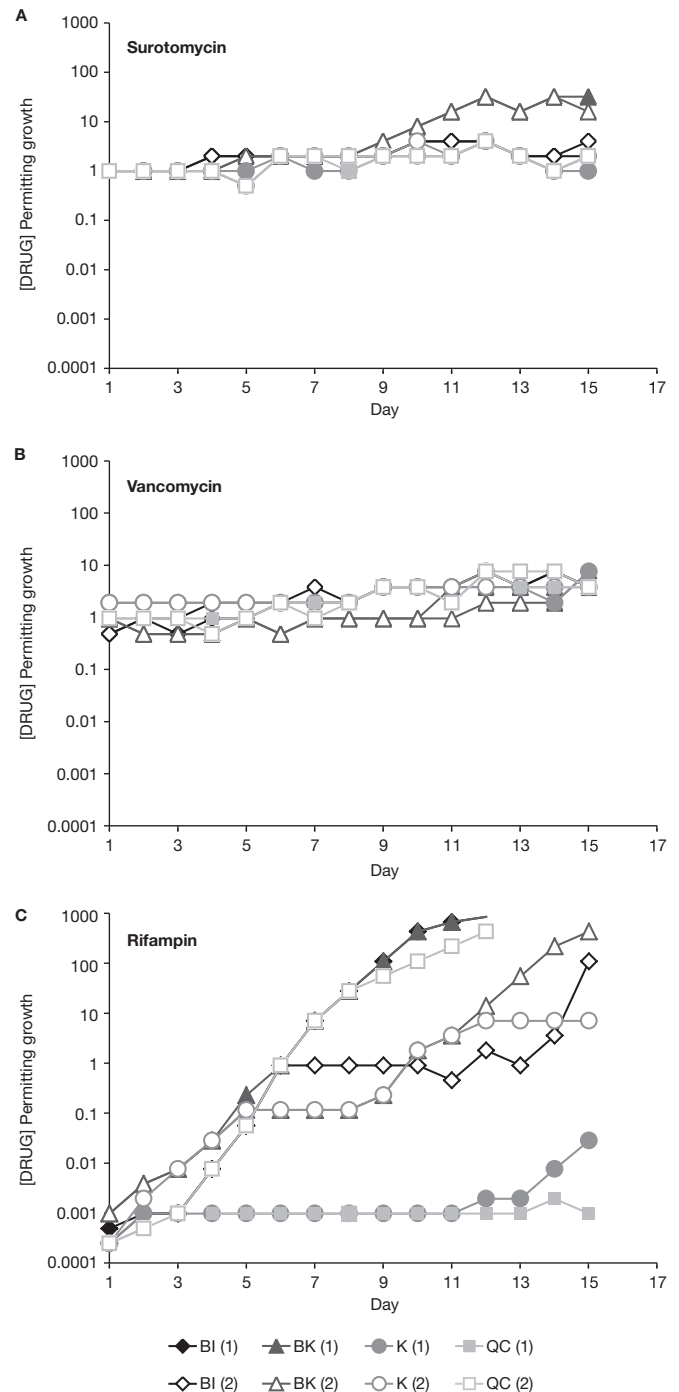
naive control) and BK (final MIC of 2 µg/ml; 4-fold higher than that of the naive control) at 8× the MIC. Vancomycin RI was below the limit of detection at 8, 16, and 32× the MIC for all isolates tested. Rifampin, used as a positive assay control, had RI frequencies of 10<sup>-7</sup> to 10<sup>-9</sup> against *C. difficile*. Resistant isolates had MICs 30- to 1,000-fold higher than those of naive controls.

Table 3 demonstrates observed RI values of surotomycin, vancomycin, and rifampin against *E. faecalis* and *E. faecium* strains. The RI for surotomycin was below the limit of detection for three of the four strains tested at all three concentrations. Three resistant mutants were isolated using the VREfs strain (RI of 3.2<sup>-9</sup> to <9.09<sup>-10</sup>) with MICs 4-fold higher than that of the naive control (final MIC of 1 µg/ml). The RIs for VSEfs and VSEfm were below the limit of detection for vancomycin at 8, 16, and 32× the MIC; the RI for vancomycin was not tested for the VRE. Rifampin had RI frequencies of 10<sup>-6</sup> to 10<sup>-8</sup> against VSEfs and VREfs where all rifampin isolates had MICs 32- to >64-fold higher than those of the naive controls. No cross-resistance to vancomycin, rifampin, ampicillin, metronidazole, or moxifloxacin was observed in the *C. difficile*, *E. faecalis*, or *E. faecium* RI isolates.

**Multistep resistance.** Multistep resistance under selective pressure was evaluated under anaerobic conditions by serial passage over 15 days. The highest concentration of drug permitting growth was determined daily for each sample. Figure 1 displays serial passage results of *C. difficile* BI, BK, K, and QC strains against surotomycin, vancomycin, and rifampin. *C. difficile* BI, K, and QC grew in a maximum of 4 µg/ml surotomycin during passage, while the BK strain grew in 16 to 32 µg/ml surotomycin by day 15 (Fig. 1A). After three daily drug-free transfers, broth MIC values were 2 to 4 µg/ml (4- to 8-fold higher than those of naive controls) for *C. difficile* K, 4 to 8 µg/ml (8- to 16-fold higher than those of naive controls) for *C. difficile* BI and QC, and 8 to >16 µg/ml (16- to 32-fold higher than those of naive controls) for *C. difficile* BK. *C. difficile* BI, BK, K, and QC grew in a maximum of 8 µg/ml vancomycin (Fig. 1B); broth MIC values were 2 to 8 µg/ml (4- to 16-fold higher than those of naive controls). Rapid generation of rifampin

resistance was observed, with the exception of *C. difficile* K (sample 1) and QC (sample 1) (Fig. 1C).

Serial passage results of vancomycin-susceptible and -resistant *E. faecalis* and *E. faecium* against surotomycin, vancomycin, and rifampin are shown in Fig. 2. VSEfs, VSEfm, VREfs, and VREfm grew in a maximum of 8 µg/ml surotomycin (Fig. 2A). Broth MIC values were 2 µg/ml (4-fold higher than those of naive controls)



**FIG 1** Serial passage of *C. difficile* BI, BK, K, and QC strains against surotomycin, vancomycin, and rifampin. The concentration of drug permitting growth is shown on the y axes, and time (in days) is shown on the x axes.

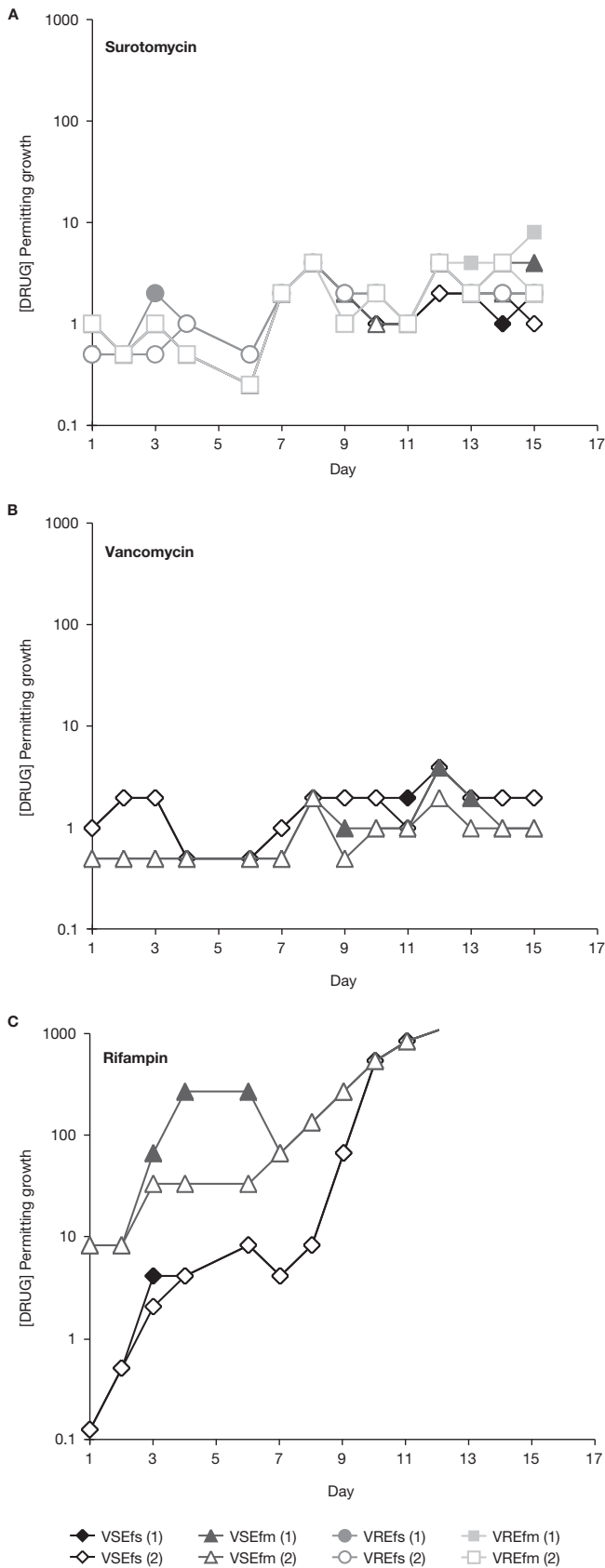


FIG 2 Serial passage of vancomycin-susceptible and -resistant *E. faecalis* and *E. faecium* against surotomycin, vancomycin, and rifampin.

for VSEfs, 4 to 8 µg/ml (8- to 16-fold higher than those of naive controls) for VSEfm, 4 µg/ml (16-fold higher than those of naive controls) for VREfs, and 4 to 16 µg/ml (4- to 16-fold higher than those of naive controls) for VREfm. VSEfs and VSEfm grew in a maximum of 4 µg/ml vancomycin (Fig. 2B); broth MIC values were 1 µg/ml (1- to 2-fold compared to those of naive controls). Consistent with *C. difficile*, rapid generation of rifampin resistance was observed in VSEfs and VSEfm (Fig. 2C); MIC values confirmed resistance (>64 µg/ml [ $>2$ - to  $>128$ -fold higher than those of naive controls]).

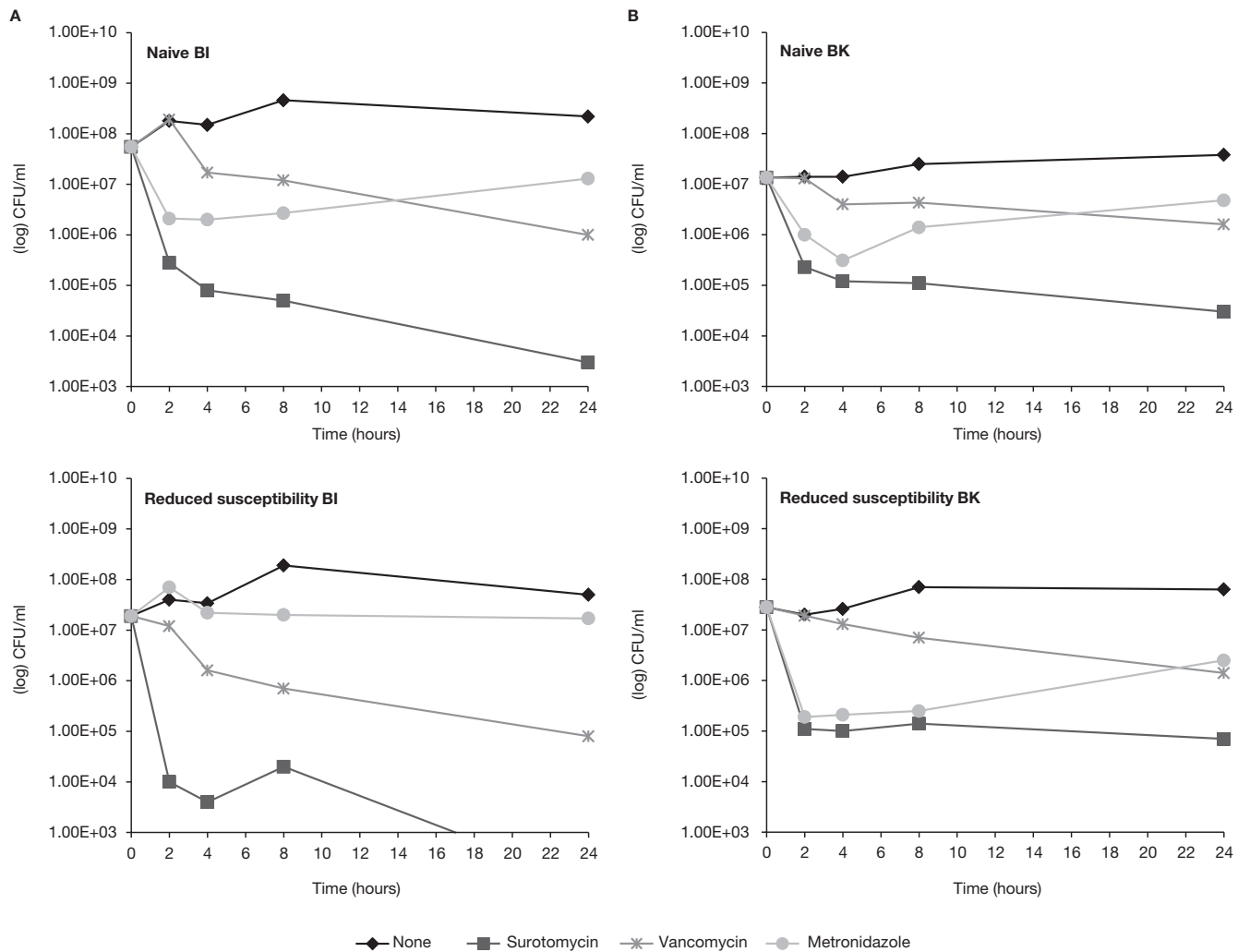
The isolates selected in the surotomycin serial passage were not cross-resistant to vancomycin, rifampin, ampicillin, metronidazole, or moxifloxacin. Following serial passage in surotomycin, VREfm became susceptible to vancomycin (MIC shifted from >64 µg/ml at day 1 to 0.5 µg/ml at day 15).

**In vitro bactericidal activity.** The *in vitro* bactericidal activity of surotomycin, vancomycin, and metronidazole against naive and reduced-susceptibility isolates, selected by serial passage over 15 days in the presence of increasing concentrations of surotomycin, was evaluated. Surotomycin at 20× the MIC demonstrated equally rapid bactericidal activity ( $\geq 3$ -log-unit reduction in CFU/ml in  $\leq 8$  h) against naive and reduced-susceptibility isolates of *C. difficile* (BI, K, and QC), except for *C. difficile* BK, which demonstrated 2.6-log-unit reductions for both naive and reduced-susceptibility isolates (Fig. 3; time-kill analysis of *C. difficile* BI and BK presented). At 20× the MIC, surotomycin demonstrated a  $\geq 4$ -log-unit reduction in CFU/ml in  $\leq 4$  h in both naive and reduced-susceptibility isolates of enterococci, including VRE isolates (Fig. 4; time-kill analysis of VSEfs presented). In contrast, neither vancomycin nor metronidazole at 20× the MIC displayed bactericidal activity (defined by achieving a 3-log-unit reduction in 24 h) against naive or strains with reduced susceptibility to surotomycin.

## DISCUSSION

Despite current treatments, morbidity and mortality rates in patients with *C. difficile*-associated diarrhea (CDAD) remain high. The first-line treatment options for CDAD, vancomycin and metronidazole, are equally effective among patients with mild disease with clinical response rates of  $\sim 90\%$ , while vancomycin appears superior to metronidazole (97% versus 76% [ $P = 0.02$ ]) for the treatment of patients with severe CDAD (6). However, unacceptable recurrence rates and emergence of *C. difficile* isolates with elevated MICs to vancomycin and metronidazole, suggesting potential reduced susceptibility (21, 22), highlight the need for new agents.

Surotomycin is a novel, cyclic lipopeptide with Gram-positive activity against *C. difficile* and limited activity against Gram-negative pathogens (11, 12). In a recent phase 2 clinical trial comparing surotomycin (125 mg or 250 mg b.i.d.) with oral vancomycin, clinical cure rates at end of treatment were similar across treatment groups: 92.4% and 86.6% for the 125-mg b.i.d. and 250-mg b.i.d. surotomycin groups, respectively, and 89.4% for the oral vancomycin group. However, lower rates of recurrence were observed with 125 mg of surotomycin b.i.d. (27.9%) and 250 mg of surotomycin b.i.d. (17.2%) compared with oral vancomycin (35.6%). Furthermore, the rates of sustained cure, defined as cure at the end of the 10-day treatment period with no recurrence during 4-week follow-up, were improved with surotomycin at 125 mg b.i.d. and 250 mg b.i.d. (66.7% and 70.1%, respectively) com-



**FIG 3** Time-kill analysis of surotomycin, vancomycin, and metronidazole at 20 $\times$  the MIC against naive and reduced-susceptibility isolates of *C. difficile* BI (A) and BK (B). The surotomycin MICs for naive and reduced-susceptibility isolates of *C. difficile* BI were 0.5 and 8  $\mu$ g/ml, respectively, and the surotomycin MICs for naive and reduced-susceptibility isolates of *C. difficile* BK were 0.5 and 32  $\mu$ g/ml, respectively.

pared with oral vancomycin (56.1%) (13, 23). Development of resistance to surotomycin in *C. difficile* is likely to be a rare event, as previously demonstrated in an earlier *in vitro* serial passage study (10).

In this *in vitro* study, emergence of spontaneous resistance and potential multistep resistance in *C. difficile*, *E. faecalis*, and *E. faecium* was evaluated. Enterococci are common gastrointestinal commensal organisms in humans, known for their high level of intrinsic antibiotic resistance and propensity to acquire and disseminate resistance mechanisms (24), therefore providing a rationale for evaluation of VSE and VRE. The rate of spontaneous resistance to surotomycin at 8, 16, and 32 $\times$  the MIC was either low or below the limit of detection in *C. difficile* and enterococci, including VRE. Under selective pressure with surotomycin, *C. difficile* BI, K, and QC remained relatively unchanged over 15 days of serial passage, while *C. difficile* BK remained unchanged until approximately day 9. Strain BK cells grew in 16 to 32  $\mu$ g/ml at the end of day 15 (MIC values of 8 to >16  $\mu$ g/ml [8- to 16-fold increase than those of the naive controls]). VSEfs, VSEfm, VREfs, and VREfm remained relatively unchanged over 15 days of serial

passage, growing in a maximum of 8  $\mu$ g/ml surotomycin (MIC values 2 to 16  $\mu$ g/ml [2- to 16-fold increase than those of the naive controls]).

During a recent phase 2 clinical trial of patients with CDAD, stool samples were collected from 26 patients treated with either surotomycin (125 mg b.i.d. or 250 mg b.i.d.) or oral vancomycin. Surotomycin concentrations in fecal samples were >1,000  $\mu$ g/g (data on file at Cubist Pharmaceuticals, Lexington, MA, USA), which is significantly higher than the highest MIC recorded in any of the follow-up isolates during this study. Therefore, the emergence of *C. difficile* resistance to surotomycin during treatment of CDAD is likely to be infrequent.

Surotomycin maintained potent MIC activity and bactericidal activity against both naive and reduced-susceptibility isolates of *C. difficile*, *E. faecalis*, and *E. faecium*, selected by 15-day serial passage. Furthermore, of the isolates tested, no cross-resistance was observed to various classes of antibiotics. These findings are consistent with clinical observations in phase 1 and phase 2 trials (data on file at Cubist Pharmaceuticals, Lexington, MA, USA), in which surotomycin did not select for *C. difficile* resistance to other cyclic

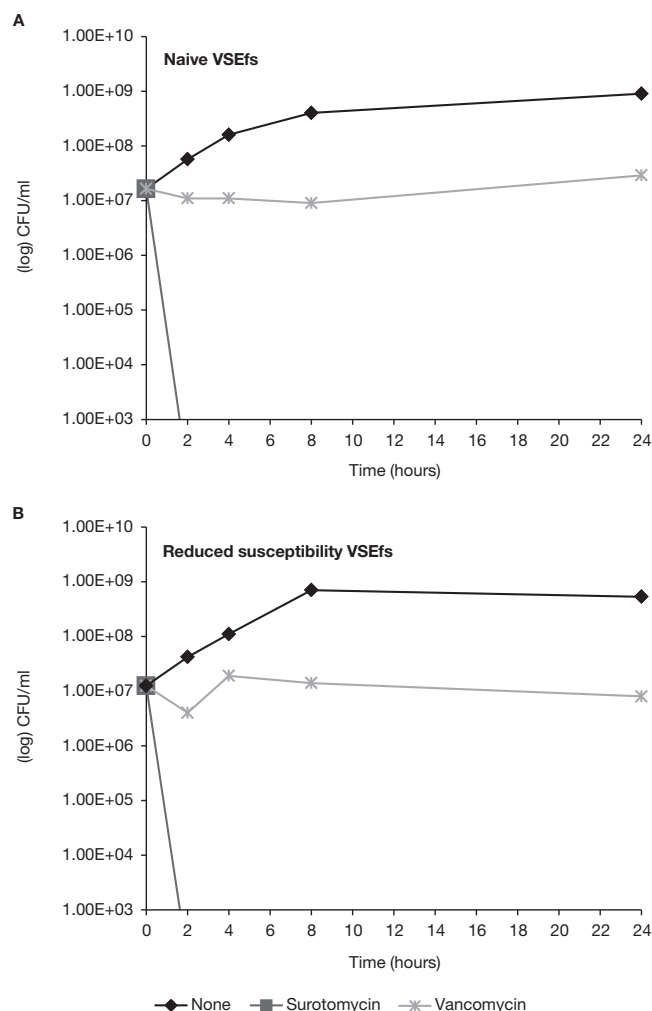


FIG 4 Time-kill analysis of surotomycin and vancomycin at 20× the MIC against naive and reduced-susceptibility isolates of VSEfs. The surotomycin MICs for naive and reduced-susceptibility isolates of VSEfs were 0.5 and 2 µg/ml, respectively.

lipopeptides or other antibiotic drug classes. Taken together, these *in vitro* data suggest that the emergence of resistance to surotomycin against *C. difficile*, *E. faecalis*, and *E. faecium* (VSE and VRE) is likely to be rare, supporting continued clinical development of this agent for the treatment of CDAD.

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