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Comparative *In Vitro* Activities of SMT19969, a New Antimicrobial Agent, against 162 Strains from 35 Less Frequently Recovered Intestinal *Clostridium* Species: Implications for *Clostridium difficile* Recurrence

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We determined the comparative activity of SMT19969 (SMT) against 162 strains representing 35 well-characterized *Clostridium* species in clusters I to XIX and 13 *Clostridium* species that had no 16S rRNA match. SMT MICs ranged from 0.06 to >512 μg/ml and were not species related. SMT might have less impact on normal gut microbiota than other *Clostridium difficile* infection (CDI) antimicrobials.

Clostridium difficile infections (CDI) have increased in frequency and severity over the past decade and are a leading cause of hospital-acquired infections, contributing to increased hospital length of stay and costs, as well as associated increased mortality, especially among the elderly (1, 2). Standard therapy has been associated with 20 to 30% relapse rates (3, 4). Consequently, new CDI therapeutic approaches have emerged.

Recurrences of CDI are associated with disruption in the patient microbiome, with changes in richness, evenness, and diversity (5). This antibiotic-induced depletion of normal microbiota allows *C. difficile* to proliferate, produce toxin, and cause disease. Several investigators have suggested that a reduced impact by antimicrobials on normal flora might lower the risk of recurrent disease, especially on the *Bacteroides fragilis* group species and *Clostridium* species cluster XIVa and, to a lesser extent, cluster IV, which contain a large number of butyrate-producing anaerobes (6–8).

SMT19969 (SMT) is a novel, narrow-spectrum, nonabsorbable agent with previously shown activity against *C. difficile* but with poor activity against *B. fragilis* (9). Information about its effect on other gut organisms is limited, including data about its activity against the other *Clostridium* species/clusters. Consequently, we studied the comparative *in vitro* activity of SMT19969 against 162 strains of *Clostridium* representing 35 well-characterized species and 13 strains with no PCR species match within 8 different *Clostridium* clusters, especially those of cluster XIVa.

Isolates were recovered from clinical specimens from 1985 to 2013. They were identified by standard methods (10, 11) and by 16S RNA gene sequencing as previously published (12) and stored in 20% skim milk at −70°C. They were taken from the freezer and transferred at least twice on supplemented brucella agar to ensure purity and good growth. Inocula were prepared by direct suspensions of cells into brucella broth to achieve the turbidity of the 0.5 McFarland standard. The final inoculum was ~10^5 CFU/spot. Susceptibility to SMT19969, fidaxomicin, vancomycin, and metronidazole was determined using the agar dilution method according to the CLSI approved standard for anaerobes (M11-A8) (13).

The results of this study are shown in Table 1. SMT MICs were variable (range, 0.06 to >512 μg/ml). Resistance (MIC > 32 μg/ml) was not cluster or species related and occurred in *Clostridium ramosum* (10 of 10 samples), *Clostridium cadaveris* (2 of 6), *Clostridium colicanis* (1 of 2), *Clostridium glycolicum* (2 of 5), *Clostridium paraputrificum* (6 of 8), *Clostridium perfringens* (9 of 11), *Clostridium rectum* (3 of 3), *Clostridium sardiniense* (1 of 1), *Clostridium scindens* (1 of 5), *Clostridium sordellii* (1 of 6), *Clostridium sporogenes* (3 of 5), and 8 of 13 *Clostridium* species with no species match by 16S RNA gene sequencing. MICs of ≥32 μg/ml also occurred with fidaxomicin (MIC range of ≤0.03 to >128 μg/ml), but these species were different from the SMT-resistant species. The MIC range for vancomycin was 0.5 to >32 μg/ml and for metronidazole was ≤0.06 to 16 μg/ml, with one or more strains of the unidentifiable *Clostridium* species showing decreased susceptibility or resistance.

Louie et al. (7) suggested that poor *in vitro* activity against aerobic and facultative Gram-negative bacteria, *Bacteroides* species, and other Gram-negative anaerobes would result in a "reduced ecological impact." They performed fecal quantitative counts of *Bacteroides* species on patients receiving either vancomycin or fidaxomicin in a phase II trial and noted that fidaxomicin's reduced activity was less suppressive. Tannock et al. (5) extended these observations on the fecal microbiota using temporal temperature gradient electrophoresis (TTGE) and quantification of phylogenetic groups using fluorescent *in situ* hybridization and flow cytometry (FISH/FC). In contrast to vancomycin, clostridial cluster XIVa and IV populations increased during and after fidaxomicin treatment. They postulated that this effect of these clusters and *Bifidobacterium* spp. might explain the reduced relapse rate of fidaxomicin in clinical trials.

Antharam et al. (6) studied the distal fecal flora of 39 patients with CDI and compared them to those of 36 *C. difficile*-colonized patients and 40 healthy controls. They found that there was a

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TABLE 1 Comparative *in vitro* activity of 162 *Clostridium* strains analyzed by cluster to SMT19969, fidaxomicin, metronidazole, and vancomycin

RMA no.[a]	Species	Clostridial cluster	MIC (μg/ml)			
			SMT19969[b]	Fidaxomicin	Metronidazole	Vancomycin
18328	*Clostridium baratii*	I	>512	≤0.03	1	2
6392	*C. baratii*-like	I	0.5	≤0.03	1	2
19025	*Clostridium butyricum*	I	0.25	0.06	0.5	0.5
19848	*C. butyricum*	I	0.25	≤0.03	0.5	0.5
21418	*C. butyricum*	I	0.25	0.06	1	0.5
22044	*C. butyricum*	I	0.25	0.06	1	0.5
22081	*C. butyricum*	I	0.5	0.125	1	0.5
14198	*Clostridium cadaveris*	I	1	≤0.03	0.125	2
16516	*C. cadaveris*	I	2	≤0.03	0.125	2
16863	*C. cadaveris*	I	1	0.06	0.125	4
18944	*C. cadaveris*	I	32	≤0.03	0.125	2
19962	*C. cadaveris*	I	256	0.06	0.125	2
20805	*C. cadaveris*	I	0.25	≤0.03	0.06	2
6433	*Clostridium colicanis*	I	0.5	≤0.03	2	>32
6786	*C. colicanis*	I	64	≤0.03	2	2
15999	*Clostridium disporicum*	I	0.06	≤0.03	1	0.5
21544	*C. disporicum*	I	0.25	≤0.03	0.25	0.25
12757	*Clostridium fallax*	I	0.125	≤0.03	0.5	0.5
21095	*C. fallax*	I	0.06	≤0.03	1	1
12522	*Clostridium novyi* A	I	0.25	≤0.03	1	0.5
15199	*Clostridium paraputrificum*	I	1	0.06	2	1
16518	*C. paraputrificum*	I	64	≤0.03	2	2
18947	*C. paraputrificum*	I	64	≤0.03	1	1
21627	*C. paraputrificum*	I	64	≤0.03	0.5	2
21630	*C. paraputrificum*	I	64	≤0.03	2	1
22852	*C. paraputrificum*	I	0.5	≤0.03	2	1
16521B	*C. paraputrificum*	I	64	≤0.03	1	1
16597A	*C. paraputrificum*	I	64	≤0.03	2	1
21966	*Clostridium perfringens*	I	256	≤0.03	1	1
22113	*C. perfringens*	I	>512	≤0.03	2	1
22244	*C. perfringens*	I	>512	≤0.03	0.5	1
22245	*C. perfringens*	I	>512	≤0.03	1	1
22509	*C. perfringens*	I	>512	0.06	4	1
22671	*C. perfringens*	I	>512	0.06	4	1
22722	*C. perfringens*	I	>512	≤0.03	2	1
22810	*C. perfringens*	I	64	≤0.03	1	1
22842	*C. perfringens*	I	256	≤0.03	2	1
22885	*C. perfringens*	I	1	≤0.03	0.5	1
23087	*C. perfringens*	I	8	≤0.03	4	1
21091	*Clostridium sardiniense*	I	>512	≤0.03	4	32
9638	*Clostridium sporogenes*	I	0.5	0.06	0.25	4
10379	*C. sporogenes*	I	64	0.06	0.25	2
10900	*C. sporogenes*	I	64	0.06	0.25	4
15061	*C. sporogenes*	I	64	0.06	0.25	2
16077	*C. sporogenes*	I	4	≤0.03	≤0.06	2
15329	*Clostridium subterminale* group	I	0.125	≤0.03	0.25	1
18693	*C. subterminale* group	I	2	≤0.03	0.5	1
19908	*C. subterminale* group	I	0.125	≤0.03	0.5	0.5
20775	*C. subterminale* group	I	0.5	≤0.03	0.25	1
8622B	*C. subterminale* group	I	≤0.03	≤0.03	0.5	1
14609	*Clostridium tertium*	I	0.5	≤0.03	1	2
16273	*C. tertium*	I	1	≤0.03	1	2
18836	*C. tertium*	I	4	≤0.03	2	2
19847	*C. tertium*	I	4	≤0.03	1	2
22841	*C. tertium*	I	0.5	0.06	2	2
18623	*Clostridium bartlettii*	XI	1	≤0.03	1	2
5262	*Clostridium bifermentans*	XI	0.125	≤0.03	0.5	0.5
5324	*C. bifermentans*	XI	0.5	≤0.03	0.5	0.5
9640	*C. bifermentans*	XI	0.5	≤0.03	0.5	0.5
9897	*C. bifermentans*	XI	0.5	≤0.03	0.5	0.5

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TABLE 1 (Continued)

RMA no.[a]	Species	Clostridial cluster	MIC (μg/ml)			
			SMT19969[b]	Fidaxomicin	Metronidazole	Vancomycin
9948	*C. bifermentans*	XI	0.5	≤0.03	0.5	1
21658	*C. bifermentans*	XI	0.25	≤0.03	1	0.5
9388B	*C. bifermentans*	XI	0.25	≤0.03	2	0.5
8910	*Clostridium glycolicum*	XI	32	≤0.03	0.125	0.5
14467	*C. glycolicum*	XI	0.5	0.5	0.25	0.5
15023	*C. glycolicum*	XI	0.5	0.5	0.125	0.5
16312	*C. glycolicum*	XI	0.5	0.25	0.25	2
7121	*C. glycolicum*-like	XI	32	1	0.25	0.5
22811	*Clostridium mayombei*-like	XI	0.5	0.5	1	0.25
16782	*Clostridium sordellii*	XI	1	≤0.03	2	1
18788	*C. sordellii*	XI	64	≤0.03	4	1
21861	*C. sordellii*	XI	16	≤0.03	4	0.5
21976	*C. sordellii*	XI	1	≤0.03	8	1
22672	*C. sordellii*	XI	8	0.125	4	1
4634	*C. sordellii*-like	XI	2	≤0.03	1	1
16057	*Clostridium aldenense*	XIVa	0.5	64	≤0.06	1
18348	*C. aldenense*	XIVa	0.5	64	≤0.06	1
18939	*C. aldenense*	XIVa	0.5	64	≤0.06	1
23550	*C. aldenense*	XIVa	0.125	64	≤0.06	2
20918A	*Clostridium aminovalericum*	XIVa	0.25	2	0.25	8
10036	*Clostridium bolteae*	XIVa	0.25	128	≤0.06	1
18941	*C. bolteae*	XIVa	0.5	128	0.125	2
21972	*C. bolteae*	XIVa	0.125	64	0.125	1
22131	*C. bolteae*	XIVa	0.5	64	≤0.06	1
12934	*Clostridium celerecrescens*	XIVa	0.125	8	0.5	1
19024	*C. celerecrescens*	XIVa	0.25	32	0.5	1
19963	*C. celerecrescens*	XIVa	0.5	32	0.5	1
15980	*Clostridium citroniae*	XIVa	0.125	64	0.25	1
21971	*C. citroniae*	XIVa	0.125	128	0.125	1
23088	*C. citroniae*	XIVa	0.125	64	0.125	1
16102A	*C. citroniae*	XIVa	0.06	64	≤0.06	1
16521A	*C. citroniae*	XIVa	0.25	64	≤0.06	1
20713	*Clostridium clostridioforme*	XIVa	0.125	64	0.25	1
21282	*C. clostridioforme*	XIVa	0.25	128	≤0.06	1
21626	*C. clostridioforme*	XIVa	0.25	>128	≤0.06	2
22060	*C. clostridioforme*	XIVa	0.125	128	≤0.06	2
22084	*C. clostridioforme*	XIVa	0.25	128	≤0.06	2
18723	*Clostridium hathewayi*	XIVa	0.125	32	0.25	0.5
20145	*C. hathewayi*	XIVa	0.125	16	0.125	0.5
20647	*C. hathewayi*	XIVa	0.5	16	0.5	0.5
21975	*C. hathewayi*	XIVa	0.125	2	0.125	0.5
2489	*Clostridium hylemonae*	XIVa	0.06	≤0.03	0.5	1
13503	*C. hylemonae*	XIVa	0.5	0.25	0.25	2
15944	*C. hylemonae*	XIVa	0.5	0.25	0.125	2
16423	*C. hylemonae*	XIVa	0.5	0.25	0.125	2
16895	*C. hylemonae*	XIVa	0.5	0.25	0.125	2
18591	*C. hylemonae*	XIVa	0.5	0.25	0.25	2
22200	*C. hylemonae*	XIVa	0.5	0.25	0.25	2
15073A	*C. hylemonae*	XIVa	0.5	0.25	0.125	1
10628	*Clostridium lavalense*	XIVa	0.5	0.06	0.125	1
12736	*Clostridium scindens*	XIVa	0.125	0.02	0.125	0.5
21863	*C. scindens*	XIVa	0.06	0.06	0.125	0.5
21878	*C. scindens*	XIVa	64	1	0.5	0.5
22045	*C. scindens*	XIVa	0.125	0.06	0.25	0.5
22624	*C. scindens*	XIVa	0.25	1	0.25	0.5
20753	*Clostridium symbiosum*	XIVa	0.25	4	0.125	1
21214	*C. symbiosum*	XIVa	0.5	2	0.125	0.5
21868	*C. symbiosum*	XIVa	1	8	0.25	1
22082	*C. symbiosum*	XIVa	0.25	2	0.25	1
22366	*C. symbiosum*	XIVa	0.125	2	0.125	1

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TABLE 1 (Continued)

RMA no.[a]	Species	Clostridial cluster	MIC (μg/ml)			
			SMT19969[b]	Fidaxomicin	Metronidazole	Vancomycin
20132	*Clostridium xylanolyticum*	XIVa	0.125	16	1	0.5
15167	*Clostridium lactatifermentans*	XIVb	0.06	0.06	0.25	>32
5491	*Clostridium innocuum*	XVI	0.25	>128	2	8
5615	*C. innocuum*	XVI	0.25	>128	1	16
20638	*C. innocuum*	XVI	1	256	0.5	16
20645	*C. innocuum*	XVI	0.25	256	0.5	16
20648	*C. innocuum*	XVI	0.25	128	0.5	16
20913	*C. innocuum*	XVI	0.25	256	1	16
21213	*C. innocuum*	XVI	0.06	256	1	16
21737	*C. innocuum*	XVI	1	256	1	16
21860	*C. innocuum*	XVI	0.25	256	1	16
21903	*C. innocuum*	XVI	0.25	256	16	16
22441	*C. innocuum*	XVI	0.25	512	0.5	16
23130	*C. innocuum*	XVI	0.125	128	2	16
10072	*Clostridium rectum*-like	XIX	>512	>128	0.5	>32
14707	*C. rectum*-like	XIX	>512	>128	1	>32
16549	*C. rectum*-like	XIX	32	>128	0.125	>32
20917	*Clostridium ramosum*	XVIII	>512	>512	0.5	4
21212	*C. ramosum*	XVIII	>512	>512	0.5	4
21215	*C. ramosum*	XVIII	>512	>512	0.5	4
21414	*C. ramosum*	XVIII	>512	>512	8	4
21738	*C. ramosum*	XVIII	128	>512	0.5	4
21862	*C. ramosum*	XVIII	>512	>512	0.5	4
21902	*C. ramosum*	XVIII	512	>512	0.5	4
21974	*C. ramosum*	XVIII	512	>512	1	4
22193	*C. ramosum*	XVIII	>512	>512	1	4
22623	*C. ramosum*	XVIII	>512	>512	1	4
705	*Clostridium* species		0.5	≤0.03	0.125	1
9906	*Clostridium* species		>512	129	0.125	32
10271	*Clostridium* species		1	≤0.03	2	0.5
14157	*Clostridium* species		32	>128	2	>32
16187	*Clostridium* species		>512	>128	2	>32
16338	*Clostridium* species		>512	>128	2	>32
19909	*Clostridium* species		0.25	>128	16	16
21472	*Clostridium* species		32	>128	4	>32
21876	*Clostridium* species		0.25	≤0.03	1	8
22256	*Clostridium* species		32	≤0.03	0.25	1
22279	*Clostridium* species		32	≤0.03	0.25	1
15596B	*Clostridium* species		0.06	≤0.03	0.05	1
18576W	*Clostridium* species		32	0.06	0.125	2
16034	*Flavonifractor plautii*		0.06	≤0.03	0.25	8
22112	*Robinsoniella* species		0.06	2	2	2

[a] RMA, R.M. Alden Research Laboratory number.
[b] SMT19969, Summit 19969.

"paucity of *Firmicutes* sequences in the aggregate gut microbiota" in the CDI and *C. difficile*-colonized patients compared to in controls. The majority (68.4%) of *Firmicutes* were clostridia, and "strikingly members of *Clostridium* cluster XIVa and to a lesser extent cluster IV" were depleted in those CDI and colonized patients. They suggested that "mechanistic studies focusing on the functional roles of these organisms in diarrheal diseases and *C. difficile* colonization resistance" be performed.

Previously, Goldstein et al. (9) studied the comparative *in vitro* activity of SMT19969 against 174 Gram-positive and 136 Gram-negative intestinal anaerobes and 40 Gram-positive aerobes. SMT19969 was generally less active against Gram-negative anaerobes, especially the *Bacteroides fragilis* group species, than vancomycin and metronidazole, suggesting a lesser impact on the normal intestinal microbiota that maintain colonization resistance. SMT19969 showed limited activity against other Gram-positive anaerobes, including *Bifidobacterium* species, *Eggerthella lenta*, *Finegoldia magna*, and *Peptostreptococcus anaerobius*, with MIC_{90} values of >512, >512, 64, and 64 μg/ml, respectively. This suggested that SMT19969's selective activity makes it an excellent candidate for therapy of CDI.

Our current study extends these observations to 162 *Clostridium* strains representing 35 species within 8 clusters. *Clostridium* species showed varied susceptibility to SMT19969. *Clostridium innocuum* (cluster XVII) was susceptible (MIC_{90} of 1 μg/ml) and *C. ramosum* (cluster XVI) and *C. perfringens* (cluster I) were nonsusceptible (MIC_{90} of >512 μg/ml) to SMT19969. Against *Clostridium* cluster XIVa, the MICs ranged from 0.125 to 64 μg/ml and

were species specific. Comparatively, XIVa isolates, except for *Clostridium hylemonae*, *Clostridium lavalense*, and some *C. scindens* isolates, had higher MICs to fidaxomicin (0.006 to >128 μg/ml) and vancomycin (0.5 to 2 μg/ml) and lower MICs to metronidazole (0.05 to 1 μg/ml). SMT19969 had higher MICs than fidaxomicin against *C. paraputrificum* (cluster I) and *C. sordellii* (cluster XI).

These data show that SMT199969's activity was variable according to *Clostridium* species and strains within species. Coupled with its lack of activity against *B. fragilis* and aerobic enteric flora, it might have a lesser impact than other antimicrobials used for CDI therapy on the normal gut microbiota that maintains colonization resistance. Further evaluation by clinical trials seems warranted.

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