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Comparative *In Vitro* Activities of LFF571 against *Clostridium difficile* and 630 Other Intestinal Strains of Aerobic and Anaerobic Bacteria

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The *in vitro* activities of LFF571, a novel analog of GE2270A that inhibits bacterial growth by binding with high affinity for protein synthesis elongation factor Tu, fidaxomicin, and 10 other antimicrobial agents were determined against 50 strains of *Clostridium difficile* and 630 other anaerobic and aerobic organisms of intestinal origin. LFF571 possesses potent activity against *C. difficile* and most other Gram-positive anaerobes (MIC_{90}, ≤0.25 μg/ml), with the exception of bifidobacteria and lactobacilli. The MIC_{90}s for aerobes, including enterococci, *Staphylococcus aureus* (as well as methicillin-resistant *S. aureus* [MRSA] isolates), *Streptococcus pyogenes*, and other streptococci were 0.06, 0.125, 2, and 8 μg/ml, respectively. Comparatively, fidaxomicin showed variable activity against Gram-positive organisms: MIC_{90}s against *C. difficile*, *Clostridium perfringens*, and *Bifidobacterium* spp. were 0.5, ≤0.015, and 0.125 μg/ml, respectively, but >32 μg/ml against *Clostridium ramosum* and *Clostridium innocuum*. MIC_{90} for *S. pyogenes* and other streptococci was 16 and >32 μg/ml, respectively. LFF571 and fidaxomicin were generally less active against Gram-negative anaerobes.

Toxigenic strains of *Clostridium difficile* are responsible for a spectrum of antibiotic-associated diarrheal diseases (*C. difficile* infection [CDI]) through elaboration of toxins A and B and other virulence factors (3, 9). In recent years, a hypervirulent strain (NAP-1, 027, BI) has emerged causing more severe disease and higher mortality, especially in more susceptible elderly patients. It is also seen increasingly in outpatients, including pregnant and postpartum women and people without previous antibiotic exposure (24, 25). Current antibiotic therapy for patients with CDI relies heavily on vancomycin or metronidazole, each of which has drawbacks, including treatment failure and frequent recurrence of disease. In addition, decreased susceptibility to metronidazole and vancomycin with emerging resistance to metronidazole (1, 2, 19) has potentiated the therapeutic dilemma. Only one new drug, fidaxomicin, has been developed during the past 30 years (17, 22). Therefore, there is an unmet need for other new drugs for this serious illness.

The current theory of CDI pathogenesis (15) is that the use of antimicrobials leads to unintended changes in the normal gastrointestinal microbiota that leave patients vulnerable to the effects of toxigenic *C. difficile*. Several strategies have emerged for the prevention and treatment of CDI, which include the use of probiotics (14), the restoration of the protective fecal microbiota (fecal biotherapy) (29), and the development of new agents that are less disruptive to the normal microbiota, especially the anaerobic component.

The thiopeptide LFF571 is a novel analog of the natural product GE2270 A, both of which inhibit bacterial growth by binding with high affinity for protein synthesis elongation factor Tu (10). GE2770 A has demonstrated excellent activity against a variety of Gram-positive organisms (16). In a study characterizing the mechanism of activity of LFF571, there was no evidence of inhibition of other biosynthetic pathways or disruption of bacterial membranes (20). LFF571 inhibits *C. difficile in vitro* and has proved more efficacious than vancomycin in an experimental hamster model of primary and relapsing *C. difficile* infection (26).

In order to fully assess LFF571's effect on fecal microbiota, we compared its *in vitro* activity with that of fidaxomicin, which has been shown to have a lesser effect on the levels of *Bacteroides* species and the gut microbiota than vancomycin and with 10 other antimicrobial agents against 50 strains of *C. difficile* and 630 other intestinal aerobic and anaerobic bacterial isolates representing 25 genera and 48 species.

MATERIALS AND METHODS

LFF571 was prepared by Novartis (Basel, Switzerland). Fidaxomicin (lipiarmycin A3) was prepared by fermentation of *Catellatospora* sp. Bp3323-81 at Novartis and supplied as a reference powder. Other laboratory reference powders were obtained from their manufacturer, USP or Sigma (St. Louis, MO), reconstituted according to the manufacturers' instructions, and stored at −70°C. On the day of testing, a tube of each stock solution was thawed and diluted according to the instructions in CLSI M7 and M11 documents (7, 8).

C. difficile strains were recovered from toxin-positive fecal specimens. The restriction endonuclease analysis (REA) groups included 16 BI, 6 Y, 4 J, 2 G, 2 CF, 1 BK, 1 Z, and 20 nonspecific strains. REA typing was conducted at Dale Gerding's laboratory using the method of Clabots (6). Other organisms representing 25 different genera and 48 species were cultured from clinical samples and identified by standard methods or by partial sequencing of the 16S rRNA gene and stored in 20% skim milk at −70°C (18, 23, 28). Strains were taken from the freezer and subcultured at least twice on supplemented brucella agar for anaerobes and on blood Trypticase soy agar for aerobes to ensure good growth. Anaerobes were incubated for 48 h and aerobes for 24 h prior to testing. Inocula were prepared by direct suspensions of cells into brucella broth for anaerobes or cation-adjusted Mueller-Hinton broth (CAMHB) for aerobes.

Quality control strains included *Clostridium difficile* ATCC 700057, *Bacteroides fragilis* ATCC 25285, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *Escherichia coli* ATCC 25922 (for the comparator drugs).

For anaerobic organisms, supplemented brucella agar deeps were ob-

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TABLE 1 Summary of the *in vitro* activities (μg/ml) of LFF571 and fidaxomicin against diverse species of anaerobic and aerobic bacteria[a]

Organism/group	No. tested	LFF571		Fidaxomicin	
		Range	$MIC_{50/90}$	Range	$MIC_{50/90}$
Gram-positive anaerobes					
C. difficile	50	0.125–0.5	0.25/0.25	0.03–0.5	0.25/0.5
C. perfringens	20	≤0.015–0.25	0.03/0.03	≤0.015–0.03	<0.015/<0.015
C. innocuum	20	0.125–0.25	0.125–0.25	>32	>32–>32
C. ramosum	20	≤0.015–0.03	≤0.015/<0.015	>32	>32–>32
Lactobacillus species[b]	24	0.06–>32	2/>32	≤0.015–>32	8/32
Bifidobacterium species[c]	22	>32	>32/>32	≤0.015–0.125	0.06/0.125
Eggerthella lenta	20	≤0.015–0.06	0.03/0.06	≤0.015–0.25	<0.015/0.125
Eubacterium limosum	20	0.06–0.25	0.125/0.25	16–>32	32/>32
Eubacterium group[d]	28	≤0.015–1	0.125/0.5	0.125–>32	2/>32
Finegoldia magna	20	≤0.015–0.25	0.125–0.125	0.5–2	1/2
Parvimonas micra	20	≤0.015–0.25	0.125–0.125	≤0.015–2	0.06–0.06
Peptostreptococcus anaerobius group[e]	20	≤0.03–0.06	0.06/0.06	≤0.015–0.03	<0.015/<0.015
Gram-negative anaerobes					
B. fragilis	21	4–8	4/8	>32	>32/>32
B. ovatus	20	4–>32	16/>32	>32	>32/>32
B. thetaiotaomicron	20	4–>32	32/>32	>32	>32/>32
B. vulgatus	20	4–>32	16/32	>32	>32/>32
P. distasonis	20	4–>32	16/32	>32	>32/>32
P. bivia	20	0.5–>32	4/>32	>32	>32/>32
P. melaninogenica group[f]	21	8–>32	>32–>32	>32	>32/>32
Porphyromonas uenonis	20	0.06–0.25	0.125/0.25	8–>32	>32/>32
Porphyromonas asaccharolytica	20	0.06–0.25	0.125/0.25	8–>32	32/>32
Fusobacterium nucleatum	22	2–>32	16/32	0.125–>32	>32/>32
F. mortiferum-varium group[g]	20	>32	>32/>32	>32	>32/>32
Veillonella species	20	>32	>32/>32	16–>32	32/>32
Aerobic organisms					
Aerococcus species[h]	10	0.06–1	0.5/1	0.5–16	2/2
E. faecalis	22	≤0.015–0.06	0.03/0.03	0.5–4	2/4
E. faecium	20	≤0.015–0.06	0.03/0.06	2–8	4/4
S. pyogenes	21	0.5–4	1/2	4–16	8/8
Streptococcus anginosus	21	1–16	2/8	4–>32	32/>32
Streptococcus constellatus/intermedius[i]	26	1–32	2/8	4–>32	32/>32
Staphylococcus aureus	20	0.125–0.25	0.125/0.125	4–8	8/8
Unusual Gram-positive cocci[j]	12	8–>32	32/>32	2–>32	>32/>32

[a] Anaerobic organisms were tested by the agar dilution method; aerobic organisms were tested by broth microdilution (7, 8).
[b] *Lactobacillus antri* (1), *L. casei* (6), *L. catenaformis* (4), *L. crispatus* (1), *L. gasseri* (4), *L. reuteri* (1), *L. rhamnosus* (6), and *L. salivarius* (1).
[c] *Bifidobacterium adolescentis* (2), *B. bifidum* (4), *B. breve* (4), *B. dentium* (5), *B. longum* (5), and *B. pseudocatenulatum* (2).
[d] *Collinsella aerofaciens* (6), *Pseudoramibacter alactolyticus* (8), *Eubacterium cylindroides* (1), *Slackia exigua* (5), *Solobacterium moorei* (5), *Olsenella uli* (2), and *Eubacterium* species (1).
[e] *Peptostreptococcus anaerobius* (12), *P. stomatis* (8).
[f] *Prevotella melaninogenica* (15), *P. denticola* (6).
[g] *Fusobacterium mortiferum* (10), *F. varium* (10).
[h] *Aerococcus sanguinicola* (2), *A. viridans* (8).
[i] *Streptococcus constellatus* (16), *S. intermedius* (10).
[j] *Lactococcus* sp. (3), *Leuconostoc* sp. (5), *Pediococcus* sp. (3), and *Weissella cibaria* (1).

tained from Anaerobe Systems (Morgan Hill, CA). Defibrinated sheep blood (Hardy Diagnostics, Santa Maria, CA) was frozen and thawed to produce laked blood. On the day of testing, laked blood and the antimicrobial agents were added to the tubes of molten agar before pouring the agar dilution plates. The strains were applied to the plates using a Steers multipronged inoculator for a final concentration of approximately 10^5 CFU/spot. After 44 h of incubation at 36°C in the anaerobic chamber incubator, the plates were examined for growth and the MICs interpreted (7).

MIC panels for testing aerobic organisms were prepared in-house using the Quick-Spense apparatus (Sandy Spring Instrument Co. Inc., Germantown, MD) at double antimicrobial strength, 50 μl/well, using CAMHB, and stored at −70°C until used. Tests for *Streptococcus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Aerococcus* strains were supplemented with 2.5% lysed horse blood (LHB; Hardy Diagnostics) by adding 5% LHB to the inoculum tube and then adding 50 μl of inoculum to each well for a final concentration of ~5×10^5 CFU/ml. Panels were incubated for 20 h at 35°C before reading and interpreting the MICs (8).

RESULTS AND DISCUSSION

Table 1 summarizes and compares the activities of LFF571 and fidaxomicin against the major groups of organisms. Table 2 shows

TABLE 2 *In vitro* activities (μg/ml) of LFF571 and comparator antimicrobial agents against *C. difficile* and other intestinal organisms[a]

Organism (no. tested)/antimicrobial agents	MIC (μg/ml)			Percent resistant
	Range	50%	90%	
Gram-positive anaerobes				
Clostridium difficile (50)				
LFF571	0.125–0.5	0.25	0.25	NA[k]
Fidaxomicin	0.03–0.5	0.25	0.5	NA
Vancomycin	1–4	1	2	0
Imipenem	4–16	8	16	18
Piperacillin-tazobactam	2–16	8	16	0
Ampicillin	1–2	2	2	0
Ampicillin-sulbactam	0.5–2	2	2	0
Cefoxitin	>64	>64	>64	100
Ceftriaxone	32–>64	64	64	50
Metronidazole	0.25–4	1	2	0
Clindamycin	1–>128	8	8	66
Moxifloxacin	1–>32	2	16	26
Clostridium perfringens (20)				
LFF571	≤0.015–0.25	0.03	0.03	NA
Fidaxomicin	≤0.015–0.03	≤0.015	≤0.015	NA
Vancomycin	0.5–1	0.5	1	0
Imipenem	0.06–0.5	0.125	0.5	0
Piperacillin-tazobactam	≤0.06–1	≤0.06	0.5	0
Ampicillin	≤0.06–1	0.125	0.5	0
Ampicillin-sulbactam	≤0.06–1	0.125	0.5	0
Cefoxitin	0.5–2	1	2	0
Ceftriaxone	≤0.06–4	0.25	1	0
Metronidazole	0.5–2	1	2	0
Clindamycin	≤0.06–2	1	2	0
Moxifloxacin	0.25–8	0.5	0.5	5
Clostridium innocuum (20)				
LFF571	0.125–0.25	0.125	0.25	NA
Fidaxomicin	>32	>32	>32	NA
Vancomycin	4–16	16	16	55
Imipenem	1–8	4	8	0
Piperacillin-tazobactam	0.5–4	1	2	0
Ampicillin	≤0.06–1	0.25	1	0
Ampicillin-sulbactam	≤0.06–1	0.25	1	0
Cefoxitin	64–>64	>64	>64	100
Ceftriaxone	8–32	16	32	0
Metronidazole	0.125–2	1	1	0
Clindamycin	0.125–>128	1	>128	15
Moxifloxacin	1–>32	2	8	20
Clostridium ramosum (20)				
LFF571	≤0.015–0.03	≤0.015	≤0.015	NA
Fidaxomicin	>32	>32	>32	NA
Vancomycin	2–4	4	4	0
Imipenem	0.25–1	1	1	0
Piperacillin-tazobactam	≤0.06–2	0.125	0.25	0
Ampicillin	0.125–2	0.25	1	0
Ampicillin-sulbactam	0.125–2	0.5	1	0
Cefoxitin	1–>64	16	16	5
Ceftriaxone	0.25–0.5	0.25	0.5	0
Metronidazole	0.25–1	0.5	1	0
Clindamycin	2–>128	4	8	10
Moxifloxacin	1–16	4	4	5
Lactobacillus species[b] (24)				
LFF571	0.06–>32	2	>32	NA
Fidaxomicin	≤0.015 > 32	8	32	NA
Vancomycin	≤0.25–>64	>64	>64	63
Imipenem	≤0.25–4	<0.25	2	0
Piperacillin-tazobactam	≤0.06–16	1	4	0
Ampicillin	≤0.06–4	0.5	2	0

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TABLE 2 (Continued)

Organism (no. tested)/antimicrobial agents	MIC (μg/ml)			Percent resistant
	Range	50%	90%	
Ampicillin-sulbactam	≤0.06–4	0.5	2	0
Cefoxitin	0.25–>32	>32	>32	67
Ceftriaxone	≤0.06–>32	8	>32	50
Metronidazole	0.125–>64	>64	>64	83
Clindamycin	≤0.06–4	0.125	2	0
Moxifloxacin	0.125–8	0.25	1	4
Bifidobacterium species[c] (22)				
LFF571	>32	>32	>32	NA
Fidaxomicin	≤0.015–0.125	0.06	0.125	NA
Vancomycin	0.25–1	0.5	1	0
Imipenem	≤0.03–0.25	≤0.03	0.25	0
Piperacillin-tazobactam	≤0.06–1	≤0.06	0.125	0
Ampicillin	≤0.06–1	≤0.06	0.25	0
Ampicillin-sulbactam	≤0.06–1	≤0.06	0.125	0
Cefoxitin	≤0.06–32	1	4	0
Ceftriaxone	≤0.06–4	0.125	0.5	0
Metronidazole	1–>64	8	>64	36
Clindamycin	≤0.06–0.125	≤0.06	≤0.06	0
Moxifloxacin	0.25–8	0.5	1	5
Eggerthella lenta (20)				
LFF571	≤0.015–0.06	0.03	0.06	NA
Fidaxomicin	≤0.015–0.25	≤0.015	0.125	NA
Vancomycin	0.5–2	2	2	0
Imipenem	≤0.03–1	1	1	0
Piperacillin-tazobactam	0.5–32	16	32	0
Ampicillin	0.5–8	4	4	75
Ampicillin-sulbactam	0.5–8	4	4	80
Cefoxitin	8–32	16	16	0
Ceftriaxone	0.5–>64	>64	>64	95
Metronidazole	0.25–1	0.5	0.5	0
Clindamycin	≤0.06–0.5	0.25	0.5	0
Moxifloxacin	0.125–8	0.5	2	5
Eubacterium limosum (20)				
LFF571	0.06–0.25	0.125	0.25	NA
Fidaxomicin	16–>32	32	>32	NA
Vancomycin	2–4	2	2	0
Imipenem	0.06–0.125	0.06	0.06	0
Piperacillin-tazobactam	≤0.06–0.5	0.25	0.5	0
Ampicillin	0.125–0.25	0.25	0.25	0
Ampicillin-sulbactam	≤0.06–0.5	0.25	0.5	0
Cefoxitin	0.5–4	1	4	0
Ceftriaxone	≤0.06–1	0.5	1	0
Metronidazole	0.125–1	0.25	1	0
Clindamycin	≤0.06–64	1	2	7
Moxifloxacin	1–2	1	2	0
Eubacterium group[d] (28)				
LFF571	≤0.015–1	0.125	0.5	NA
Fidaxomicin	0.125–>32	2	>32	NA
Vancomycin	0.25–4	0.5	1	0
Imipenem	≤0.03–0.5	≤0.03	0.06	0
Piperacillin-tazobactam	≤0.06–2	0.25	1	0
Ampicillin	≤0.06–1	≤0.06	0.5	0
Ampicillin-sulbactam	≤0.06-1	0.125	1	0
Cefoxitin	≤0.06–16	4	16	0
Ceftriaxone	≤0.06–8	0.5	4	0
Metronidazole	≤0.06–2	0.5	2	0
Clindamycin	≤0.06–>128	≤0.06	1	4
Moxifloxacin	0.125–1	0.25	1	0

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TABLE 2 (Continued)

Organism (no. tested)/antimicrobial agents	MIC (μg/ml)			Percent resistant
	Range	50%	90%	
Finegoldia magna (20)				
LFF571	≤0.015–0.25	0.125	0.125	NA
Fidaxomicin	0.5–2	1	2	NA
Vancomycin	0.125–1	0.25	0.5	0
Imipenem	≤0.03–0.25	0.06	0.125	0
Piperacillin-tazobactam	≤0.06–1	0.125	0.25	0
Ampicillin	≤0.06–0.5	0.25	0.5	0
Ampicillin-sulbactam	≤0.06–0.5	0.25	0.25	0
Cefoxitin	≤0.06–1	0.5	1	0
Ceftriaxone	0.125–16	4	8	0
Metronidazole	≤0.06–2	0.5	1	0
Clindamycin	≤0.06–>128	0.5	>128	25
Moxifloxacin	0.06–16	2	4	5
Parvimonas micra (20)				
LFF571	≤0.015–0.25	0.125	0.125	NA
Fidaxomicin	≤0.015–2	0.06	0.06	NA
Vancomycin	0.5–1	1	1	0
Imipenem	≤0.03–0.25	≤0.03	0.06	0
Piperacillin-tazobactam	≤0.06–1	≤0.06	0.125	0
Ampicillin	≤0.06–2	0.125	1	0
Ampicillin-sulbactam	≤0.06–1	0.125	0.5	0
Cefoxitin	0.5–8	1	2	0
Ceftriaxone	0.125–2	0.25	1	0
Metronidazole	0.125–1	0.25	0.5	0
Clindamycin	≤0.06–32	0.25	16	15
Moxifloxacin	0.125–32	0.25	16	15
P. anaerobius/stomatis group[e] (20)				
LFF571	≤0.015–0.06	0.06	0.06	NA
Fidaxomicin	≤0.015–0.03	≤0.015	≤0.015	NA
Vancomycin	0.125–0.5	0.25	0.5	0
Imipenem	≤0.03–2	0.06	1	0
Piperacillin-tazobactam	0.25–16	0.25	16	0
Ampicillin	≤0.06–32	0.125	8	20
Ampicillin-sulbactam	≤0.06–32	0.125	16	20
Cefoxitin	0.25–16	0.5	16	0
Ceftriaxone	0.5–16	2	16	0
Metronidazole	≤0.06–1	0.25	1	0
Clindamycin	≤0.06–0.5	≤0.06	0.25	0
Moxifloxacin	0.125–8	0.125	0.25	0
Gram-negative anaerobes				
Bacteroides fragilis (21)				
LFF571	4–8	4	8	NA
Fidaxomicin	>32	>32	>32	NA
Vancomycin	32–>32	32	32	100
Imipenem	≤0.03–>32	0.06	4	5
Piperacillin-tazobactam	0.25–>64	1	64	10
Ampicillin	16–>64	32	>64	100
Ampicillin-sulbactam	1–>64	2	32	19
Cefoxitin	8–>64	16	32	10
Ceftriaxone	4–>64	64	>64	62
Metronidazole	0.25–>64	1	1	5
Clindamycin	≤0.06–>128	0.5	>128	14
Moxifloxacin	0.5–8	1	8	38
B. ovatus (20)				
LFF571	4–>32	16	>32	NA
Fidaxomicin	>32	>32	>32	NA
Vancomycin	>32	>32	>32	100
Imipenem	0.06–2	0.125	0.25	0
Piperacillin-tazobactam	2–>64	4	16	5
Ampicillin	8–>64	32	>64	100

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TABLE 2 (Continued)

Organism (no. tested)/antimicrobial agents	MIC (μg/ml)			Percent resistant
	Range	50%	90%	
Ampicillin-sulbactam	0.5–32	1	8	5
Cefoxitin	8–>64	32	32	10
Ceftriaxone	4–>64	>64	>64	95
Metronidazole	0.25–2	1	1	0
Clindamycin	1–>128	2	>128	25
Moxifloxacin	1–>32	2	32	20
B. thetaiotaomicron (20)				
LFF571	4–>32	>32	>32	NA
Fidaxomicin	>32	>32	>32	NA
Vancomycin	32–>32	>32	>32	100
Imipenem	0.125–2	0.25	0.5	0
Piperacillin-tazobactam	8–>64	16	32	5
Ampicillin	16–>64	>32	>64	100
Ampicillin-sulbactam	1–16	2	8	0
Cefoxitin	16–>64	32	64	5
Ceftriaxone	>64	>64	>64	100
Metronidazole	0.5–2	1	2	0
Clindamycin	0.5–>128	4	>128	45
Moxifloxacin	1–>32	2	32	20
B. vulgatus (20)				
LFF571	4–>32	16	>32	NA
Fidaxomicin	>32	>32	>32	NA
Vancomycin	16–>32	32	>32	100
Imipenem	0.06–8	0.25	1	0
Piperacillin-tazobactam	≤0.06–32	4	16	0
Ampicillin	4–>64	>64	>64	100
Ampicillin-sulbactam	1–32	8	16	5
Cefoxitin	4–>64	8	32	5
Ceftriaxone	1–>64	64	>64	50
Metronidazole	0.25–4	0.5	1	0
Clindamycin	≤0.06–>128	1	>128	45
Moxifloxacin	0.5–>32	1	32	45
Parabacteroides distasonis (20)				
LFF571	4–>32	16	32	NA
Fidaxomicin	>32	>32	>32	NA
Vancomycin	32–>32	>32	>32	100
Imipenem	0.125–1	0.5	0.5	0
Piperacillin-tazobactam	2–32	4	32	0
Ampicillin	2–>64	4	>64	100
Ampicillin-sulbactam	2–32	4	32	15
Cefoxitin	8–64	16	32	10
Ceftriaxone	0.25–>64	8	>64	45
Metronidazole	0.5–8	2	4	0
Clindamycin	≤0.06–>128	4	>128	45
Moxifloxacin	0.125–32	0.5	16	30
Prevotella bivia (20)				
LFF571	0.5–>32	4	>32	NA
Fidaxomicin	>32	>32	>32	NA
Vancomycin	>64	>64	>64	100
Imipenem	≤0.03–0.06	≤0.03	0.06	0
Piperacillin-tazobactam	≤0.06	≤0.06	≤0.06	0
Ampicillin	≤0.06–>64	8	32	60
Ampicillin-sulbactam	≤0.06–4	1	2	0
Cefoxitin	0.25–2	0.5	1	0
Ceftriaxone	≤0.06–64	8	32	10
Metronidazole	0.25–4	1	4	0
Clindamycin	≤0.06–>128	≤0.06	>128	30
Moxifloxacin	1–>32	2	32	15

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TABLE 2 (Continued)

Organism (no. tested)/antimicrobial agents	MIC (μg/ml)			Percent resistant
	Range	50%	90%	
P. melaninogenica group[f] (21)				
LFF571	8–>32	>32	>32	NA
Fidaxomicin	>32	>32	>32	NA
Vancomycin	>64	>64	>64	100
Imipenem	≤0.03–0.06	≤0.03	0.06	0
Piperacillin-tazobactam	≤0.06–0.125	≤0.06	≤0.06	0
Ampicillin	≤0.06–64	1	32	38
Ampicillin-sulbactam	≤0.06–2	0.25	2	0
Cefoxitin	≤0.06–4	0.5	2	0
Ceftriaxone	≤0.06–64	2	64	14
Metronidazole	≤0.06–2	0.25	1	0
Clindamycin	≤0.06–>128	≤0.06	≤0.06	10
Moxifloxacin	0.5–32	0.5	32	19
Porphyromonas asaccharolytica (20)				
LFF571	0.06–0.25	0.125	0.25	NA
Fidaxomicin	8–>32	>32	>32	NA
Vancomycin	0.5–2	1	2	0
Imipenem	≤0.03	≤0.03	≤0.03	0
Piperacillin-tazobactam	≤0.06–0.125	≤0.06	≤0.06	0
Ampicillin	≤0.06–0.25	≤0.06	≤0.06	0
Ampicillin-sulbactam	≤0.06	≤0.06	≤0.06	0
Cefoxitin	≤0.06–0.5	0.125	0.25	0
Ceftriaxone	≤0.06–0.25	≤0.06	≤0.06	0
Metronidazole	≤0.06–0.125	≤0.06	≤0.06	0
Clindamycin	≤0.06–64	≤0.06	64	25
Moxifloxacin	0.125–8	0.25	1	5
P. uenonis (20)				
LFF571	0.06–0.25	0.125	0.25	NA
Fidaxomicin	8–>32	>32	>32	NA
Vancomycin	0.5–4	2	2	0
Imipenem	≤0.03	≤0.03	≤0.03	0
Piperacillin-tazobactam	≤0.06	≤0.06	≤0.06	0
Ampicillin	≤0.06–2	≤0.06	≤0.06	5
Ampicillin-sulbactam	≤0.06–0.25	≤0.06	≤0.06	0
Cefoxitin	≤0.06–2	0.125	0.25	0
Ceftriaxone	≤0.06–8	≤0.06	0.25	0
Metronidazole	≤0.06	≤0.06	≤0.06	0
Clindamycin	≤0.06	≤0.06	≤0.06	0
Moxifloxacin	0.125–0.5	0.5	0.5	0
Fusobacterium nucleatum (22)				
LFF571	2–>32	16	32	NA
Fidaxomicin	0.125–>32	>32	>32	NA
Vancomycin	32–>32	>32	>32	100
Imipenem	≤0.03–≤0.03	≤0.03	≤0.03	0
Piperacillin-tazobactam	≤0.06	≤0.06	≤0.06	0
Ampicillin	≤0.06	≤0.06	≤0.06	0
Ampicillin-sulbactam	≤0.06	≤0.06	≤0.06	0
Cefoxitin	≤0.06–0.25	0.125	0.25	0
Ceftriaxone	≤0.06–0.125	≤0.06	0.125	0
Metronidazole	≤0.06–1	≤0.06	0.5	0
Clindamycin	≤0.06–0.25	≤0.06	0.125	0
Moxifloxacin	≤0.06–16	0.125	2	11
F. mortiferum/varium group[g] (20)				
LFF571	>32	>32	>32	NA
Fidaxomicin	>32	>32	>32	NA
Vancomycin	>32	>32	>32	100
Imipenem	0.06–1	1	1	0
Piperacillin-tazobactam	0.125–4	0.25	4	0
Ampicillin	0.125–4	1	4	25
Ampicillin-sulbactam	0.125–4	1	4	0

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TABLE 2 (Continued)

Organism (no. tested)/antimicrobial agents	MIC (μg/ml)			Percent resistant
	Range	50%	90%	
Cefoxitin	0.25–8	4	8	0
Ceftriaxone	≤0.06–>64	16	>64	50
Metronidazole	≤0.06–2	1	2	0
Clindamycin	≤0.06–32	0.125	4	5
Moxifloxacin	0.5–>32	0.5	16	20
Veillonella species (20)				
LFF571	>32	>32	>32	NA
Fidaxomicin	16–>32	32	>32	NA
Vancomycin	64–>64	>64	>64	100
Imipenem	0.06–1	0.5	1	0
Piperacillin-tazobactam	4–>64	64	>64	35
Ampicillin	0.25–2	0.5	2	25
Ampicillin-sulbactam	0.125–2	0.5	2	0
Cefoxitin	0.5–16	4	16	0
Ceftriaxone	0.25–8	4	8	0
Metronidazole	0.25–8	2	4	0
Clindamycin	≤0.06–128	≤0.06	0.25	5
Moxifloxacin	0.06–>32	0.5	4	10
Gram-positive aerobes				
Aerococcus species[h] (10)				
LFF571	0.06–1	0.5	1	NA
Fidaxomicin	0.5–16	2	2	NA
Vancomycin	≤0.25–0.5	≤0.25	≤0.25	100
Imipenem	≤0.25–<0.25	≤0.25	≤0.25	100
Piperacillin-tazobactam	≤0.25–4	2	4	100
Ampicillin	≤0.125–<0.125	≤0.125	≤0.125	100
Ampicillin-sulbactam	≤0.125–<0.125	≤0.125	≤0.125	100
Cefoxitin	≤0.25–32	4	16	100
Ceftriaxone	≤0.25–8	2	8	100
Clindamycin	≤0.125–0.5	≤0.125	0.25	100
Moxifloxacin	≤0.06–>8	0.25	0.5	100
Enterococcus faecalis (22)				
LFF571	≤0.015–0.06	0.03	0.03	NA
Fidaxomicin	0.5–4	2	4	NA
Vancomycin	0.5–>16	>16	>16	64
Imipenem	0.5–16	2	16	0
Piperacillin-tazobactam	2–16	8	16	0
Ampicillin	0.25–4	1	4	0
Ampicillin-sulbactam	0.25–4	1	4	0
Cefoxitin	>32	>32	>32	100
Ceftriaxone	>32	>32	>32	100
Clindamycin	2–>16	>16	>16	NA
Moxifloxacin	0.125–>8	4	8	27
Enterococcus faecium (20)				
LFF571	≤0.015–0.06	0.03	0.06	NA
Fidaxomicin	2–8	4	4	NA
Vancomycin	0.5–>16	>16	>16	90
Imipenem	>32	>32	>32	100
Piperacillin-tazobactam	>32	>32	>32	100
Ampicillin	16–>16	>16	>16	100
Ampicillin-sulbactam	16–>16	>16	>16	100
Cefoxitin	>32	>32	>32	100
Ceftriaxone	>32	>32	>32	100
Clindamycin	0.25–>16	>16	>16	NA
Moxifloxacin	2–>8	>8	>8	75
Streptococcus pyogenes (21)				
LFF571	0.5–4	1	2	NA
Fidaxomicin	4–16	8	8	NA
Vancomycin	≤0.25	≤0.25	≤0.25	0
Imipenem	≤0.25	≤0.25	≤0.25	0

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TABLE 2 (Continued)

Organism (no. tested)/antimicrobial agents	MIC (μg/ml)			Percent resistant
	Range	50%	90%	
Piperacillin-tazobactam	≤0.25	≤0.25	≤0.25	0
Ampicillin	≤0.125	≤0.125	≤0.125	0
Ampicillin-sulbactam	≤0.125	≤0.125	≤0.125	0
Cefoxitin	≤0.25–2	1	1	0
Ceftriaxone	≤0.25	≤0.25	≤0.25	0
Clindamycin	≤0.125–1	≤0.125	≤0.25	0
Moxifloxacin	≤0.06–0.25	0.125	0.25	0
S. anginosus (22)				
LFF571	1–16	2	8	NA
Fidaxomicin	4–>32	32.	>32	NA
Vancomycin	≤0.25–1	0.5	0.5	0
Imipenem	≤0.25	≤0.25	≤0.25	0
Piperacillin-tazobactam	≤0.25	≤0.25	≤0.25	0
Ampicillin	≤0.125	≤0.125	≤0.125	0
Ampicillin-sulbactam	≤0.125	≤0.125	≤0.125	0
Cefoxitin	2–8	4	8	0
Ceftriaxone	≤0.25–0.5	≤0.25	≤0.25	0
Clindamycin	≤0.125–>16	≤0.125	>16	15
Moxifloxacin	≤0.06–0.5	0.125	0.25	0
S. constellatus/intermedius[i] (26)				
LFF571	1–32	2	8	NA
Fidaxomicin	4–>32	32	>32	NA
Vancomycin	≤0.25–1	0.5	0.5	0
Imipenem	≤0.25	≤0.25	≤0.25	0
Piperacillin-tazobactam	≤0.25–0.5	≤0.25	0.5	0
Ampicillin	≤0.125–4	≤0.125	2	0
Ampicillin-sulbactam	≤0.125–0.25	≤0.125	≤0.25	0
Cefoxitin	≤0.25–16	4	8	0
Ceftriaxone	≤0.25–0.5	≤0.25	0.5	0
Clindamycin	≤0.125–>16	≤0.125	0.5	5
Moxifloxacin	≤0.06–0.5	0.125	0.25	0
Staphylococcus aureus (20)				
LFF571	0.125–0.25	0.125	0.125	NA
Fidaxomicin	4–8	8	8	NA
Vancomycin	0.5–1	0.5	1	0
Imipenem	≤0.25–4	≤0.25	2	0
Piperacillin-tazobactam	1–>32	32	>32	55
Ampicillin	0.25–>16	16	>16	95
Ampicillin-sulbactam	0.25–16	2	16	0
Cefoxitin	4–>32	16	>32	50
Ceftriaxone	2–>32	32	>32	55
Clindamycin	≤0.125–>16	≤0.125	0.25	10
Moxifloxacin	≤0.06–>8	0.125	4	10
Unusual Gram-positive cocci[j] (12)				
LFF571	8–>32	32	>32	NA
Fidaxomicin	2–>32	>32	>32	NA
Vancomycin	0.5–>16	>16	>16	75
Imipenem	≤0.25–4	≤0.25	4	0
Piperacillin-tazobactam	2–8	2	4	0
Ampicillin	≤0.125–2	0.25	1	0
Ampicillin-sulbactam	≤0.125–2	0.25	1	0
Cefoxitin	>32	>32	>32	100
Ceftriaxone	≤0.25–>32	32	>32	58
Clindamycin	≤0.125–0.25	≤0.125	0.25	0
Moxifloxacin	0.25–2	0.5	2	0

[a] Anaerobic organisms were tested by the agar dilution method; aerobic organisms were tested by broth microdilution (7, 8).
[b] *Lactobacillus antri* (1), *L. casei* (6), *L. catenaformis* (4), *L. crispatus* (1), *L. gasseri* (4), *L. reuteri* (1), *L. rhamnosus* (6), and *L. salivarius* (1).
[c] *Bifidobacterium adolescentis* (2), *B. bifidum* (4), *B. breve* (4), *B. dentium* (5), *B. longum* (5), and *B. pseudocatenulatum* (2).
[d] *Collinsella aerofaciens* (6), *Pseudoramibacter alactolyticus* (8), *Eubacterium cylindroides* (1), *Slackia exigua* (5), *Solobacterium moorei* (5), *Olsenella uli* (2), and *Eubacterium* species (1).
[e] *Peptostreptococcus anaerobius* (12), *P. stomatis* (8).
[f] *Prevotella melaninogenica* (15), *P. denticola* (6).
[g] *Fusobacterium mortiferum* (10), *F. varium* (10).
[h] *Aerococcus sanguinicola* (2), *A. viridans* (8).
[i] *Streptococcus constellatus* (16), *S. intermedius* (10).
[j] *Lactococcus* sp. (3), *Leuconostoc* sp. (5), *Pediococcus* sp. (3), and *Weissella cibaria* (1).
[k] NA, not available.

the ranges, $MIC_{50/0.90}$, and percent resistance for all antimicrobial agents. Overall, LFF571 had excellent activity against the 50 *C. difficile* strains studied (MIC_{90}, 0.25 μg/ml), which was one dilution lower than that of fidaxomicin (MIC_{90}, 0.5 μg/ml) and three dilutions lower than both vancomycin and metronidazole (MIC_{90}s, 2 μg/ml).

LFF571 demonstrated consistently excellent activity against all anaerobic Gram-positive rods and cocci ($MIC_{50/90}$, 0.125/0.25 μg/ml for 284 strains), with the exception of bifidobacteria and some species of lactobacilli. Activity against lactobacilli was species dependent with all strains of *Lactobacillus catenaformis* susceptible to ≤0.125 μg/ml, while MICs for the other species ranged from 2 to 16 μg/ml for the vancomycin-resistant *Lactobacillus casei-rhamnosus* group but >32 μg/ml for the vancomycin-susceptible *Lactobacillus gasseri* strains. Against the Gram-negative anaerobes, the 40 strains of *Porphyromonas* spp. were susceptible to ≤0.25 μg/ml of LFF571, similar to their relatively unusual susceptibility to vancomycin (MIC 0.5 to 4 μg/ml). MICs for *Bacteroides fragilis* were 4 and 8 μg/ml, although the other species in the *B. fragilis* group, including *Bacteroides thetaiotaomicron*, *Bacteroides ovatus*, and *Parabacteroides* (*Bacteroides*) *distasonis*, were less susceptible, with an overall MIC_{90} of >32 μg/ml. There was no apparent difference in the range of MICs for the individual *Bacteroides* species. Similarly, *Prevotella bivia*, *Prevotella melaninogenica/denticola*, and *Veillonella* spp. also displayed MIC_{90} of >32 μg/ml, although some of the *P. bivia* strains had MICs as low as 0.5 μg/ml. Similar to fidaxomicin, the relatively poor activity against Gram-negative anaerobes suggests that LFF571 might have a lesser impact on the normal gut microbiota that maintain colonization resistance (21, 27).

Fidaxomicin results for the Gram-positive organisms were more variable. While activity against *C. difficile* and *Clostridium perfringens* was excellent (MIC_{90}, 0.5 and ≤0.015 μg/ml, respectively), MICs for *Clostridium ramosum* and *Clostridium innocuum* were all >32 μg/ml. Unlike LFF571, fidaxomicin inhibited all strains of *Bifidobacterium* species with MIC_{90} at 0.125 μg/ml, but similar to LFF571, activity against lactobacilli was species dependent. While *Eggerthella lenta* strains were inhibited by ≤0.25 μg/ml of fidaxomicin, *Eubacterium limosum* strains required 16 to >32 μg/ml for inhibition. All anaerobic Gram-positive coccus strains were very susceptible with fidaxomicin MICs ranging from ≤0.015 to 2 μg/ml. Against the anaerobic Gram-negative organisms, fidaxomicin showed poor activity with $MIC_{50/90}$ of 32/>32 μg/ml for all strains, including *Veillonella* spp.

Among the aerobic strains, LFF571 was most active against vancomycin-resistant and -susceptible strains of *Enterococcus faecalis* and *Enterococcus faecium* with MIC_{90} at 0.03 and 0.06 μg/ml, respectively. It was equally active against methicillin-susceptible and -resistant strains of staphylococci with MIC_{90} of 0.125 μg/ml. Against the streptococci, LFF571 was slightly less active: the MIC_{90} for *Streptococcus pyogenes* was 2 μg/ml and for the *S. milleri* group, 8 μg/ml. *Aerococcus* strains were inhibited by 0.06 to 1 μg/ml, although other unusual cocci such as *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Weissella* were less susceptible with MICs ranging from 8 to >32 μg/ml. There was no relationship in resistance by other classes of antimicrobial agents and LFF571. Fidaxomicin was less active than LFF571 against the aerobic strains. The MIC_{90} against enterococci was 4 μg/ml, with no apparent difference between vancomycin-resistant and -susceptible strains. The MIC_{90} for *Aerococcus* species was 2 μg/ml, for *S. pyogenes*, 16 μg/ml, and for the *S. milleri* group, >32 μg/ml.

Susceptibilities for the comparator agents were typical for what has been reported in other surveys of anaerobic intestinal organisms (4, 5, 12, 13, 11). *C. difficile* resistance to cefoxitin, imipenem, clindamycin, and moxifloxacin was present in 100, 18, 66, and 26% of our isolates, respectively, while elevated MICs of 4 μg/ml were found in two strains for vancomycin and in one for metronidazole. Moxifloxacin resistance was present in 10 of 14 (71%) REA-BI (027, NAP1) strains, 1 of 4 type J, the single type Z strain, and 4 of 20 (20%) nonspecific type strains. All strains of *C. innocuum* were also resistant to cefoxitin and 55% to vancomycin. *Eggerthella lenta* displayed resistance to ampicillin (75%) and ceftriaxone (95%) while the other nonsporeforming Gram-positive rods were mostly susceptible to these drugs. Among the Gram-positive cocci, 25% of *Finegoldia magna* and 15% of *Parvimonas micra* strains were resistant to clindamycin while 5% and 15%, respectively, were also resistant to moxifloxacin. Ampicillin resistance was present in 4 of 12 (33%) *Peptostreptococcus anaerobius* strains, although all of the phenotypically similar *Peptostreptococcus stomatis* strains were susceptible.

Through a novel mechanism, LFF571 shows excellent activity against *C. difficile* and good activity against other Gram-positive anaerobes but little activity against the anaerobic Gram-negative organisms. All strains of enterococci, regardless of vancomycin susceptibility, were inhibited. With this relatively narrow spectrum of activity, LFF571 shows promise as a new drug for treating CDI. It is currently in phase II clinical trials.

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REFERENCES

1. **Al-Nassir WN, et al.** 2008. Comparison of clinical and microbiological response to treatment of *Clostridium difficile*-associated disease with metronidazole and vancomycin. Clin. Infect. Dis. **47**:56–62.
2. **Baines SD, et al.** 2008. Emergence of reduced susceptibility to metronidazole in *Clostridium difficile*. J. Antimicrob. Chemother. **62**:1046–1052.
3. **Carroll KC, Bartlett JG.** 2011. Biology of *Clostridium difficile*: implications for epidemiology and diagnosis. Annu. Rev. Microbiol. **65**:501–521.
4. **Citron DM, et al.** 2003. In vitro activities of ramoplanin, teicoplanin, vancomycin, linezolid, bacitracin, and four other antimicrobials against intestinal anaerobic bacteria. Antimicrob. Agents Chemother. **47**:2334–2338.
5. **Citron DM, Warren YA, Tyrrell KL, Merriam V, Goldstein EJ.** 2009. Comparative in vitro activity of REP3123 against *Clostridium difficile* and other anaerobic intestinal bacteria. J. Antimicrob. Chemother. **63**:972–976.
6. **Clabots CR, et al.** 1993. Development of a rapid and efficient restriction endonuclease analysis typing system for *Clostridium difficile* and correlation with other typing systems. J. Clin. Microbiol. **31**:1870–1875.
7. **Clinical and Laboratory Standards Institute.** 2007. Methods for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard, 7th ed. CLSI document M11-A7. Clinical and Laboratory Standards Institute, Wayne, PA.
8. **Clinical and Laboratory Standards Institute.** 2009. Methods for dilution antimicrobial susceptibility testing of bacteria that grow aerobically. Approved standard, 8th ed. CLSI document M7-A8. Clinical and Laboratory Standards Institute, Wayne, PA.
9. **Deneve C, Janoir C, Poilane I, Fantinato C, Collignon A.** 2009. New trends in *Clostridium difficile* virulence and pathogenesis. Int. J. Antimicrob. Agents **33**(Suppl 1):S24–S28.
10. **Deng G, et al.** 2011. Investigation of mode of binding of elongation factor Tu inhibitor, LFF571. 51st Intersci. Conf. Antimicrob. Agents Chemother., abstr F1-1859.

11. **Dzink-Fox JL, et al.** 2011. Antimicrobial activity of the novel elongation factor Tu inhibitor, LFF571. 51st Intersci. Conf. Antimicrob. Agents Chemother., abstr F1-1346.
12. **Finegold SM, et al.** 2004. In vitro activity of ramoplanin and comparator drugs against anaerobic intestinal bacteria from the perspective of potential utility in pathology involving bowel flora. Anaerobe **10**:205–211.
13. **Finegold SM, et al.** 2004. In vitro activities of OPT-80 and comparator drugs against intestinal bacteria. Antimicrob. Agents Chemother. **48**: 4898–4902.
14. **Gao XW, Mubasher M, Fang CY, Reifer C, Miller LE.** 2010. Dose-response efficacy of a proprietary probiotic formula of *Lactobacillus acidophilus* CL1285 and *Lactobacillus casei* LBC80R for antibiotic-associated diarrhea and *Clostridium difficile*-associated diarrhea prophylaxis in adult patients. Am. J. Gastroenterol. **105**:1636–1641.
15. **Gerding DN, Johnson S.** 2010. Management of *Clostridium difficile* infection: thinking inside and outside the box. Clin. Infect. Dis. **51**:1306–1313.
16. **Goldstein BP, et al.** 1993. In vitro antimicrobial activity of a new antibiotic, MDL 62,879 (GE2270 A). Antimicrob. Agents Chemother. **37**:741–745.
17. **Goldstein EJ, et al.** 2011. Comparative susceptibilities to fidaxomicin (OPT-80) of isolates collected at baseline, recurrence, and failure from patients in two phase III trials of fidaxomicin against *Clostridium difficile* infection. Antimicrob. Agents Chemother. **55**:5194–5199.
18. **Jousimies-Somer HR, et al.** 2002. Wadsworth-KTL anaerobic bacteriology manual. Star Publishing, Belmont, CA.
19. **Kuijper EJ, Wilcox MH.** 2008. Decreased effectiveness of metronidazole for the treatment of *Clostridium difficile* infection? Clin. Infect. Dis. **47**:63–65.
20. **Leeds JA, et al.** 2011. Antibacterial mechanism of action of LFF571. 51st Intersci. Conf. Antimicrob. Agents Chemother., abstr F1-1347.
21. **Louie TJ, Emery J, Krulicki W, Byrne B, Mah M.** 2009. OPT-80 eliminates *Clostridium difficile* and is sparing of Bacteroides species during treatment of *C. difficile* infection. Antimicrob. Agents Chemother. **53**: 261–263.
22. **Miller M.** 2010. Fidaxomicin (OPT-80) for the treatment of *Clostridium difficile* infection. Expert Opin. Pharmacother. **11**:1569–1578.
23. **Murray PR, Baron EJ.** 2007. Manual of clinical microbiology. ASM Press, Washington, DC.
24. **Rouphael NG, et al.** 2008. *Clostridium difficile*-associated diarrhea: an emerging threat to pregnant women. Am. J. Obstet. Gynecol. **198**:635–636.
25. **Rupnik M, Wilcox MH, Gerding DN.** 2009. *Clostridium difficile* infection: new developments in epidemiology and pathogenesis. Nat. Rev. Microbiol. **7**:526–536.
26. **Trzasko A, et al.** 2011. Efficacy of LFF571: a novel semi-synthetic thiopeptide in a hamster model of *C. difficile* infection. 51st Intersci. Conf. Antimicrob. Agents Chemother., abstr B-1189.
27. **Vollaard EJ, Clasener HA.** 1994. Colonization resistance. Antimicrob. Agents Chemother. **38**:409–414.
28. **Warren YA, Tyrrell KL, Citron DM, Goldstein EJ.** 2006. *Clostridium aldenense* sp. nov. and *Clostridium citroniae* sp. nov. isolated from human clinical infections. J. Clin. Microbiol. **44**:2416–2422.
29. **Yoon SS, Brandt LJ.** 2010. Treatment of refractory/recurrent *C. difficile*-associated disease by donated stool transplanted via colonoscopy: a case series of 12 patients. J. Clin. Gastroenterol. **44**:562–566.