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Evaluation of the *In Vitro* Activity of Eravacycline against a Broad Spectrum of Recent Clinical Anaerobic Isolates

David R. Snydman,[a] Laura A. McDermott,[a] Nilda V. Jacobus,[a] Kathryn Kerstein,[b] Trudy H. Grossman,[b]* Joyce A. Sutcliffe[b]*

[a]Department of Medicine, Tufts Medical Center, Boston, Massachusetts, USA

[b]Tetraphase Pharmaceuticals, Watertown, Massachusetts, USA

ABSTRACT The novel fluorocycline antibiotic eravacycline is in development for use in the treatment of serious infections caused by susceptible and multidrug-resistant (MDR) aerobic and anaerobic Gram-negative and Gram-positive pathogens. Eravacycline and 11 comparator antibiotics were tested against recent anaerobic clinical isolates, including MDR *Bacteroides* spp. and *Clostridium difficile*. Eravacycline was potent *in vitro* against all the isolates tested, including strains with tetracycline-specific resistance determinants and MDR anaerobic pathogens resistant to carbapenems and/or β-lactam–β-lactamase inhibitor combinations.

KEYWORDS eravacycline, anaerobes, *Bacteroides*, antimicrobial resistance

Increased resistance of anaerobic bacteria to standard antibiotics requires the development of new antibiotics for use in mixed aerobic-anaerobic organism infections (1–3). Eravacycline is a novel fluorocycline antibiotic in phase 3 clinical development for the treatment of serious infections due to Gram-negative and Gram-positive aerobic and anaerobic pathogens (4, 5). Eravacycline retains activity against commonly described tetracycline resistance mechanisms, such as tetracycline efflux pumps and ribosomal protection (6, 7), and is active *in vitro* against Gram-negative aerobic pathogens resistant to other classes of antibiotics, including *Acinetobacter baumannii* and *Enterobacteriaceae* expressing extended-spectrum β-lactamases, carbapenemases, and colistin/polymyxin resistance (8–14). The spectrum of eravacycline also includes potency against Gram-positive pathogens, such as methicillin-susceptible and -resistant staphylococci, vancomycin-susceptible and -resistant enterococci, and penicillin-susceptible and -resistant *Streptococcus pneumoniae* (10, 15). Eravacycline, however, has reduced activity against *Pseudomonas aeruginosa* and *Burkholderia cenocepacia* (10).

(These data were presented at the 55th Interscience Conference on Antimicrobial Agents and Chemotherapy, poster C-547, 17 to 21 September 2015, San Diego, CA [16].)

Earlier evaluations showed that eravacycline was potent against a wide variety of anaerobic pathogens *in vitro* (10, 17); however, the number of isolates in each species was limited and not as representative of antibiotic resistance as is currently seen. To expand and define the *in vitro* spectrum of eravacycline against anaerobic pathogens, particularly *Bacteroides fragilis* and *Clostridium difficile*, eravacycline and comparator antibiotics (tigecycline, minocycline, imipenem, meropenem, piperacillin-tazobactam, ampicillin-sulbactam, moxifloxacin, metronidazole, linezolid, clindamycin, and vancomycin [with Gram-positive isolates only]) were tested against 540 recent anaerobic clinical isolates, including MDR isolates, collected in the United States from 2012 to 2015 at Tufts Medical Center from hospitalized patient cultures and medical centers participating in *B. fragilis* and *C. difficile* surveillance studies (1, 3). Prior to testing, identification of the isolates was confirmed using API 20A methodology (bioMérieux, Inc., Durham, NC) and methods outlined in the Wadsworth-KTL anaerobic bacteriology manual (18), including plating on selective media and susceptibility to special potency

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Address correspondence to David R. Snydman, dsnydman@tuftsmedicalcenter.org.

* Present address: Trudy H. Grossman, Melinta Therapeutics, New Haven, Connecticut, USA; Joyce A. Sutcliffe, Consultant, Westbrook, Connecticut, USA.

antimicrobial disks. MIC assays were performed by an agar dilution method according to Clinical and Laboratory Standards Institute (CLSI) recommendations (19) using American Type Culture Collection strains *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, *Eggerthella lenta* ATCC 43055, and *Clostridium difficile* ATCC 700057 as controls. Percent resistance was calculated using applicable CLSI breakpoints (20), except for tigecycline, for which FDA breakpoints (21) were used, or EUCAST cutoff values (ECOFF) (22), which were used for *C. difficile*.

The activities of eravacycline and comparators against Gram-negative anaerobic isolates are illustrated in Table 1. Versus comparator antibiotics, eravacycline showed the lowest MIC values against isolates of the *B. fragilis* group, including those resistant to tigecycline, minocycline, meropenem, piperacillin-tazobactam, ampicillin-sulbactam, moxifloxacin, and clindamycin. All isolates within the *B. fragilis* group ($n = 286$) were inhibited by ≤4 μg/ml eravacycline. The MIC values that inhibited 90% of the isolates (MIC_{90}) in a panel for eravacycline (MIC_{90}, 1 μg/ml) were 8- and 16-fold lower than those of tigecycline (MIC_{90}, 8 μg/ml) and minocycline (MIC_{90}, 16 μg/ml), respectively. Eravacycline was potent against *Prevotella* spp. ($n = 29$) and *Fusobacterium* spp. ($n = 20$); all isolates were inhibited by ≤0.5 μg/ml eravacycline.

The susceptibilities of the Gram-positive anaerobic isolates to eravacycline and comparator antibiotics are shown in Table 2. Eravacycline was potent against all isolates at concentrations of ≤1 μg/ml, including *C. difficile* strains resistant to moxifloxacin and clindamycin and with elevated MICs (4 μg/ml) to vancomycin and metronidazole. The eravacycline MIC_{90} values against *Clostridium perfringens* ($n = 15$), *C. difficile* ($n = 76$), other *Clostridium* spp. ($n = 22$), *Peptostreptococcus* spp. ($n = 53$), *Propionibacterium* species (including *P. acnes*, which was recently reclassified as *Cutibacterium acnes* spp.) ($n = 13$), and *Bifidobacterium* spp. ($n = 15$) were 1 μg/ml, 0.12 μg/ml, 0.12 μg/ml, 0.25 μg/ml, 0.25 μg/ml, and 0.5 μg/ml, respectively. The MIC ranges for *E. lenta* ($n = 6$) and *Lactobacillus* spp. ($n = 5$) were 0.03 to 0.12 μg/ml and 0.06 to 0.5 μg/ml, respectively.

The presence of the following tetracycline resistance genes previously reported in *B. fragilis* (http://faculty.washington.edu/marilynr/) was detected by standard PCR methodology for a set of 27 *B. fragilis* isolates covering the full range of minocycline MIC values (≤0.25 to 32 μg/ml): *tet*(Q) and *tet*(M), encoding ribosomal protection mechanisms (23); and *tet*(X), *tet*(X1), and *tet*(X2), encoding tetracycline-modifying flavin-dependent monooxygenases (24). The following primer sets were used in PCRs: for *tet*(Q), forward 5′-GTGCGTTTCGACAATGCATCTATTGTAG and reverse 5′-TGATGACATT GATTTTTGGAACATG primers (derived from GenBank accession no. Z21523) or forward 5′-ATCGGTATCAATGAGTTGTT and reverse 5′-GACTGATTCTGGAGGAAGTA primers (25); for *tet*(X), *tet*(X1), and *tet*(X2), forward 5′-CAGGAAGCAATGAAAAAAGCGG and reverse 5′-TAGCTTTTCTAAAGGAAATATCCG primers (derived from GenBank accession no. M37699); for *tet*(X) and *tet*(X2) only, forward 5′-TTAGCCTTACCAATGGGTGT and reverse 5′-CAAATCTGCTGTTTCACTCG primers (25); for *tet*(X1) only, forward 5′-TCAGG ACAAGAAGCAATGAA and reverse 5′-TATTTCGGGGTTGTCAAACT primers (25); and for *tet*(M), forward 5′-AACTCGAACAAGAGGAAAGC and reverse 5′-ATGGAAGCCCAGAAA GGAT primers (26). Plasmids carrying the *tet*(X), *tet*(Q), or *tet*(M) gene were used as positive PCR control templates, and sequencing reactions were performed by Genewiz (Cambridge, MA) to verify *tet*(X) alleles as well as to determine the *tet*(Q) sequences of two isolates with the lowest minocycline MIC values (≤2 μg/ml).

As shown in Table 3, 23 of 27 isolates were positive for *tet*(Q), with minocycline, tigecycline, and eravacycline MIC values ranging from 0.5 to 32 μg/ml, 0.25 to 16 μg/ml, and 0.06 to 4 μg/ml, respectively, while the 4 isolates negative for *tet*(Q) had minocycline, tigecycline, and eravacycline MIC values ranging from ≤0.25 μg/ml, 0.5 to 2 μg/ml, and 0.12 to 0.5 μg/ml, respectively. The *tet*(Q) genes from two positive isolates with relatively lower minocycline MIC values of 0.5 and 2 μg/ml were sequenced; each encoded amino acid sequences with 100% identity to 74 Tet(Q) proteins in the UniProtKB database (http://www.uniprot.org/help/uniprotkb, released 15 March 2017), indicating that the Tet(Q) protein in these two isolates was a common variant. In addition to being positive for *tet*(Q), two isolates were positive for *tet*(X), and three

TABLE 1 *In vitro* activities of eravacycline and comparator antibiotics against 335 Gram-negative anaerobic clinical isolates

Organism (*n*)	Antimicrobial agent	MIC (μg/ml)			% resistant by guideline[a]	
		Range	MIC_{50}	MIC_{90}	CLSI/FDA	EUCAST
Bacteroides fragilis group (286)	Eravacycline	0.03 to 4	0.25	1	NA	NA
	Tigecycline	0.12 to 16	1	8	3.1	NA
	Minocycline	≤0.25 to 64	8	16	NA	NA
	Imipenem	≤0.12 to 4	0.25	1	0	0
	Meropenem	≤0.12 to 16	0.25	2	1.0	1.0
	Piperacillin-tazobactam	≤0.5 to 256	4	32	0.7	10.8
	Ampicillin-sulbactam	≤0.5 to >128	2	16	4.9	22.7
	Moxifloxacin	≤0.5 to 64	2	32	38.8	NA
	Linezolid	≤1 to >16	2	4	NA	NA
	Clindamycin	≤0.5 to >128	4	>128	43.7	43.7
	Metronidazole	≤1 to 2	≤1	2	0	0
Bacteroides fragilis (110)	Eravacycline	0.03 to 4	0.25	1	NA	NA
	Tigecycline	0.12 to 16	1	8	4.5	NA
	Minocycline	≤0.25 to 64	8	16	NA	NA
	Imipenem	≤0.12 to 4	0.25	1	0	0
	Meropenem	≤0.12 to 16	0.25	2	1.8	1.8
	Piperacillin-tazobactam	≤0.5 to 128	1	8	0.9	1.8
	Ampicillin-sulbactam	1 to 32	2	16	1.8	17.3
	Moxifloxacin	≤0.5 to 32	2	16	36.4	NA
	Linezolid	2 to >16	2	4	NA	NA
	Clindamycin	≤0.5 to >128	1	>128	30.0	30.0
	Metronidazole	≤1 to 2	≤1	2	0	0
Non-*B. fragilis* (176)	Eravacycline	0.06 to 4	0.25	1	NA	NA
	Tigecycline	0.12 to 16	1	8	2.3	NA
	Minocycline	≤0.25 to 32	4	16	NA	NA
	Imipenem	≤0.12 to 4	0.5	1	0	0
	Meropenem	≤0.12 to 16	0.25	2	0.6	0.6
	Piperacillin-tazobactam	≤0.5 to 256	8	32	0.6	16.5
	Ampicillin-sulbactam	≤0.5 to >128	4	16	6.8	26.1
	Moxifloxacin	≤0.5 to 64	4	32	40.3	NA
	Linezolid	≤1 to >16	2	4	NA	NA
	Clindamycin	≤0.5 to >128	8	>128	52.3	52.3
	Metronidazole	≤1 to 2	≤1	2	0	0
Bacteroides caccae (10)	Eravacycline	0.12 to 0.5	0.5	0.5	NA	NA
	Tigecycline	0.5 to 4	1	4	0	NA
	Minocycline	4 to 16	8	16	NA	NA
	Imipenem	≤0.12 to 1	0.25	0.5	0	0
	Meropenem	≤0.12 to 4	0.25	1	0	0
	Piperacillin-tazobactam	≤0.5 to 4	2	4	0	0
	Ampicillin-sulbactam	1 to 8	2	8	0	0
	Moxifloxacin	≤0.5 to 64	4	32	50.0	NA
	Linezolid	All 2	2	2	NA	NA
	Clindamycin	4 to >128	>128	>128	90.0	90.0
	Metronidazole	≤1 to 2	≤1	2	0	0
Bacteroides ovatus (30)	Eravacycline	0.06 to 1	0.25	1	NA	NA
	Tigecycline	0.25 to 8	1	8	0	NA
	Minocycline	≤0.25 to 16	4	8	NA	NA
	Imipenem	≤0.12 to 2	0.25	0.5	0	0
	Meropenem	≤0.12 to 8	0.25	2	0	0
	Piperacillin-tazobactam	≤0.5 to 32	4	32	0	13.3
	Ampicillin-sulbactam	≤0.5 to 32	2	16	3.3	20.0
	Moxifloxacin	≤0.5 to 32	4	8	20.0	NA
	Linezolid	≤1 to 8	2	4	NA	NA
	Clindamycin	≤0.5 to >128	8	>128	53.3	53.3
	Metronidazole	≤1 to 2	≤1	2	0	0
Bacteroides thetaiotaomicron (70)	Eravacycline	0.06 to 4	0.25	1	NA	NA
	Tigecycline	0.12 to 16	1	8	4.3	NA
	Minocycline	≤0.25 to 16	4	8	NA	NA
	Imipenem	≤0.12 to 1	0.5	1	0	0
	Meropenem	≤0.12 to 2	0.25	1	0	0
	Piperacillin-tazobactam	1 to 64	16	32	0	20.0
	Ampicillin-sulbactam	1 to 64	2	16	4.3	18.6
	Moxifloxacin	≤0.5 to 64	2	32	35.7	NA
	Linezolid	2 to 4	4	4	NA	NA
	Clindamycin	≤0.5 to >128	8	>128	51.4	51.4
	Metronidazole	≤1 to 2	≤1	2	0	0

(Continued on next page)

TABLE 1 (Continued)

Organism (*n*)	Antimicrobial agent	MIC (μg/ml)			% resistant by guideline[a]	
		Range	MIC_{50}	MIC_{90}	CLSI/FDA	EUCAST
Parabacteroides distasonis (26)	Eravacycline	0.12 to 4	0.25	1	NA	NA
	Tigecycline	0.5 to 16	1	8	3.8	NA
	Minocycline	≤0.25 to 32	4	16	NA	NA
	Imipenem	0.25 to 2	0.5	2	0	0
	Meropenem	≤0.12 to 4	0.5	4	0	0
	Piperacillin-tazobactam	1 to 64	8	32	0	26.9
	Ampicillin-sulbactam	2 to 64	8	32	15.4	38.5
	Moxifloxacin	≤0.5 to 64	2	32	46.2	NA
	Linezolid	2 to 4	2	4	NA	NA
	Clindamycin	≤0.5 to >128	4	>128	38.5	38.5
	Metronidazole	≤1 to 2	≤1	2	0	0
Bacteroides uniformis (15)	Eravacycline	0.06 to 1	0.25	1	NA	NA
	Tigecycline	0.25 to 8	1	4	0	NA
	Minocycline	≤0.25 to 32	8	16	NA	NA
	Imipenem	≤0.12 to 2	0.25	1	0	0
	Meropenem	≤0.12 to 4	0.25	2	0	0
	Piperacillin-tazobactam	≤0.5 to 32	4	32	0	13.3
	Ampicillin-sulbactam	≤0.5 to 16	2	16	0	20.0
	Moxifloxacin	≤0.5 to 64	8	32	53.3	NA
	Linezolid	2 to 4	2	4	NA	NA
	Clindamycin	≤0.5 to >128	>128	>128	60.0	60.0
	Metronidazole	≤1 to 2	≤1	2	0	0
Bacteroides vulgatus (18)	Eravacycline	0.06 to 0.5	0.12	0.5	NA	NA
	Tigecycline	0.12 to 8	0.5	8	0	NA
	Minocycline	≤0.25 to 16	8	16	NA	NA
	Imipenem	0.25 to 4	0.5	2	0	0
	Meropenem	0.25 to 16	0.5	4	5.6	5.6
	Piperacillin-tazobactam	2 to 256	4	32	5.6	11.1
	Ampicillin-sulbactam	2 to 64	16	64	16.7	61.1
	Moxifloxacin	1 to 64	32	64	72.2	NA
	Linezolid	2 to >16	2	4	NA	NA
	Clindamycin	≤0.5 to >128	>128	>128	55.6	55.6
	Metronidazole	≤1 to 2	≤1	≤1	0	0
Other *Parabacteroides/Bacteroides* spp. (7)[b]	Eravacycline	0.06 to 1				
	Tigecycline	0.5 to 8				
	Minocycline	≤0.25 to 32				
	Imipenem	≤0.12 to 1				
	Meropenem	0.25 to 1				
	Piperacillin-tazobactam	≤0.5 to 16				
	Ampicillin-sulbactam	2 to >128				
	Moxifloxacin	≤0.5 to 8				
	Linezolid	2 to 4				
	Clindamycin	≤0.5 to >128				
	Metronidazole	≤1 to 2				
Prevotella spp. (29)	Eravacycline	≤0.015 to 0.5	0.25	0.5	NA	NA
	Tigecycline	≤0.06 to 1	0.5	1	0	NA
	Minocycline	≤0.25 to 32	4	32	NA	NA
	Imipenem	≤0.12 to 2	≤0.12	2	0	0
	Meropenem	≤0.12 to 8	≤0.12	2	0	0
	Piperacillin-tazobactam	≤0.5 to 128	≤0.5	8	3.4	3.4
	Ampicillin-sulbactam	≤0.5 to 32	1	4	3.4	3.4
	Moxifloxacin	≤0.5 to >64	1	16	10.3	NA
	Linezolid	≤1 to 4	2	4	NA	NA
	Clindamycin	≤0.5 to >128	≤0.5	>128	27.6	27.6
	Metronidazole	≤1 to >16	≤1	2	3.4	3.4
Fusobacterium spp. (20)	Eravacycline	≤0.015 to 0.5	0.03	0.12	NA	NA
	Tigecycline	≤0.06 to 1	0.12	0.5	0	NA
	Minocycline	≤0.25 to 0.5	≤0.25	0.5	NA	NA
	Imipenem	≤0.12 to 0.5	≤0.12	0.5	0	0
	Meropenem	≤0.12 to 0.5	0.25	0.25	0	0
	Piperacillin-tazobactam	≤0.5 to 2	1	2	0	0
	Ampicillin-sulbactam	≤0.5 to 2	1	2	0	0
	Moxifloxacin	≤0.5 to 2	≤0.5	1	0	NA
	Linezolid	≤1 to 2	≤1	≤1	NA	NA
	Clindamycin	All ≤0.5	≤0.5	≤0.5	0	0
	Metronidazole	All ≤1	≤1	≤1	0	0

[a]The FDA breakpoint applicable only to tigecycline. EUCAST epidemiological cutoff (ECOFF) breakpoints were used for *C. difficile*. NA, not applicable.
[b]Only the MIC range is indicated when the total number of isolates was <10.

TABLE 2 *In vitro* activities of eravacycline and comparator antibiotics against 205 Gram-positive anaerobic clinical isolates

Species (*n*)	Antimicrobial agent	MIC (μg/ml)			% resistant by guideline[a]	
		Range	MIC_{50}	MIC_{90}	CLSI/FDA	EUCAST
Clostridium perfringens (15)	Eravacycline	0.03 to 1	0.12	1	NA	NA
	Tigecycline	0.12 to 2	0.5	2	0	NA
	Minocycline	≤0.25 to 8	0.5	8	NA	NA
	Imipenem	≤0.12 to 1	0.25	0.5	0	0
	Meropenem	≤0.12 to 1	≤0.12	0.25	0	0
	Piperacillin-tazobactam	≤0.5 to 1	≤0.5	1	0	0
	Ampicillin-sulbactam	≤0.5 to 2	≤0.5	1	0	0
	Moxifloxacin	≤0.5 to 4	1	4	0	NA
	Linezolid	≤1 to 2	2	2	NA	NA
	Clindamycin	≤0.5 to 128	1	64	13.3	13.3
	Metronidazole	≤1 to 2	≤1	2	0	0
	Vancomycin	0.5 to 64	1	32	NA	20.0
Clostridium difficile (76)	Eravacycline	≤0.015 to 0.25	0.06	0.12	NA	NA
	Tigecycline	≤0.06 to 1	0.12	0.25	0	6.6
	Minocycline	≤0.25 to 16	≤0.25	4	NA	NA
	Imipenem	≤0.12 to 16	4	8	6.6	NA
	Meropenem	≤0.12 to 8	2	4	0	NA
	Piperacillin-tazobactam	≤0.5 to 64	8	16	0	NA
	Ampicillin-sulbactam	≤0.5 to 16	2	4	0	NA
	Moxifloxacin	≤0.5 to 32	2	16	18.4	18.4
	Linezolid	≤1 to 8	2	2	NA	NA
	Clindamycin	≤0.5 to >128	4	128	28.9	NA
	Metronidazole	≤1 to 4	≤1	≤1	0	1.3
	Vancomycin	≤0.5 to 4	1	2	NA	2.6
Other *Clostridium* spp. (22)	Eravacycline	≤0.015 to 0.5	0.03	0.12	NA	NA
	Tigecycline	≤0.06 to 1	0.12	0.5	0	NA
	Minocycline	≤0.25 to 16	≤0.25	4	NA	NA
	Imipenem	≤0.12 to 4	1	4	0	0
	Meropenem	≤0.12 to 2	1	2	0	0
	Piperacillin-tazobactam	≤0.5 to 32	2	16	0	9.1
	Ampicillin-sulbactam	≤0.5 to 2	≤0.5	2	0	0
	Moxifloxacin	≤0.5 to >64	2	4	9.1	NA
	Linezolid	≤1 to 8	2	4	NA	NA
	Clindamycin	≤0.5 to >128	4	128	40.9	40.9
	Metronidazole	≤1 to >16	≤1	≤1	4.5	4.5
	Vancomycin	≤0.25 to 16	2	16	NA	31.8
Peptostreptococcus spp. (53)	Eravacycline	0.03 to 0.25	0.12	0.25	NA	NA
	Tigecycline	≤0.06 to 1	0.25	0.25	0	NA
	Minocycline	≤0.25 to 16	0.5	8	NA	NA
	Imipenem	≤0.12 to 0.5	≤0.12	≤0.12	0	0
	Meropenem	≤0.12 to 0.5	≤0.12	≤0.12	0	0
	Piperacillin-tazobactam	≤0.5 to 4	≤0.5	≤0.5	0	0
	Ampicillin-sulbactam	≤0.5 to 2	≤0.5	1	0	0
	Moxifloxacin	≤0.5 to 16	≤0.5	4	9.4	NA
	Linezolid	≤1 to 2	≤1	2	NA	NA
	Clindamycin	≤0.5 to >128	≤0.5	64	11.3	11.3
	Metronidazole	≤1 to >16	≤1	≤1	5.7	5.7
	Vancomycin	≤0.25 to 4	0.5	1	NA	5.7
Propionibacterium spp./*Cutibacterium acnes* (13)	Eravacycline	≤0.015 to 1	0.06	0.25	NA	NA
	Tigecycline	0.12 to 2	0.12	0.5	0	NA
	Minocycline	≤0.25 to 8	0.5	2	NA	NA
	Imipenem	≤0.12 to 0.5	≤0.12	≤0.12	0	0
	Meropenem	≤0.12 to 0.25	≤0.12	0.25	0	0
	Piperacillin-tazobactam	≤0.5 to 1	≤0.5	≤0.5	0	0
	Ampicillin-sulbactam	≤0.5 to 4	≤0.5	1	0	0
	Moxifloxacin	≤0.5 to 4	≤0.5	1	0	NA
	Linezolid	≤1 to 2	≤1	≤1	NA	NA
	Clindamycin	≤0.5 to 64	1	64	23.1	23.1
	Metronidazole	≤1 to >16	>16	>16	92.3	92.3
	Vancomycin	0.5 to 32	1	8	NA	23.1

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TABLE 2 (Continued)

Species (*n*)	Antimicrobial agent	MIC (μg/ml)			% resistant by guideline[a]	
		Range	MIC_{50}	MIC_{90}	CLSI/FDA	EUCAST
Bifidobacterium spp. (15)	Eravacycline	0.12 to 0.5	0.25	0.5	NA	NA
	Tigecycline	0.12 to 1	0.5	1	0	NA
	Minocycline	0.5 to 64	1	32	NA	NA
	Imipenem	≤0.12 to 2	0.5	2	0	0
	Meropenem	≤0.12 to 8	0.5	4	0	0
	Piperacillin-tazobactam	1 to 16	2	8	0	0
	Ampicillin-sulbactam	1 to 16	1	2	0	6.7
	Moxifloxacin	1 to 64	1	32	13.3	NA
	Linezolid	≤1 to 2	≤1	2	NA	NA
	Clindamycin	≤0.5 to >128	≤0.5	>128	13.3	13.3
	Metronidazole	4 to >16	>16	>16	93.3	93.3
	Vancomycin	0.5 to 32	1	4	NA	13.3
Eggerthella lenta (6)[b]	Eravacycline	0.03 to 0.12				
	Tigecycline	≤0.06 to 0.5				
	Minocycline	1 to 32				
	Imipenem	≤0.12 to 0.5				
	Meropenem	≤0.12 to 0.5				
	Piperacillin-tazobactam	≤0.5 to 64				
	Ampicillin-sulbactam	≤0.5 to 8				
	Moxifloxacin	≤0.5 to >64				
	Linezolid	≤1 to 4				
	Clindamycin	≤0.5 to 16				
	Metronidazole	All ≤1				
	Vancomycin	1 to 4				
Lactobacillus spp. (5)[b]	Eravacycline	0.06 to 0.5				
	Tigecycline	0.25 to 1				
	Minocycline	≤0.25 to 4				
	Imipenem	≤0.12 to 2				
	Meropenem	≤0.12 to 8				
	Piperacillin-tazobactam	≤0.5 to 4				
	Ampicillin-sulbactam	≤0.5 to 2				
	Moxifloxacin	≤0.5 to 2				
	Linezolid	≤1 to 4				
	Clindamycin	All ≤0.5				
	Metronidazole	All >16				
	Vancomycin	0.5 to >64				

[a]The FDA breakpoint is applicable only to tigecycline. EUCAST epidemiological cutoff (ECOFF) breakpoints used for *C. difficile*. NA, not applicable.
[b]Only the MIC range is indicated when the total number of isolates is <10.

isolates were positive for both *tet*(X1) and *tet*(X2); this set of isolates showed eravacycline, tigecycline, and minocycline MIC values ranging from 0.25 to 1 μg/ml, 2 to 8 μg/ml, and 2 to 32 μg/ml, respectively. No isolates were positive for *tet*(M).

In conclusion, this evaluation showed that eravacycline exhibited potent activity *in*

TABLE 3 *In vitro* activities of eravacycline and comparator tetracyclines against 27 *Bacteroides fragilis* clinical isolates characterized for tetracycline resistance determinants

No. of strains	Presence of tetracycline resistance determinant[a]					MIC range (μg/ml)[b]		
	tet(Q)	*tet*(X)[2]	*tet*(X2)[2]	*tet*(X1)[3]	*tet*(M)	ERV	TIG	MIN
4	−	−	−	−	−	0.12–0.5	0.5–2	≤0.25
18	+[4]	−	−	−	−	0.06–4	0.25–16	0.5–32
3	+[4]	−	+	+	−	0.25–1	2–8	2–16
2	+	+	−	−	−	0.5–1	2–8	4–32

[a]*tet*(X) was distinguished from *tet*(X2) by generating PCR products with universal *tet*(X) primers, sequencing with primers specific to both *tet*(X) and *tet*(X2), and identifying sequences specific to either *tet*(X) or *tet*(X2). *tet*(X1) was identified by generating PCR products with primers specific to *tet*(X1) and sequencing with the same primer set to verify *tet*(X1)-specific sequences. The *tet*(Q) gene in special studies laboratory strain numbers 28441 and 27741, with MIN MICs of 0.5 and 2 μg/ml, respectively, were sequenced and shown to encode amino acid sequences identical to each other and to 74 Tet(Q) proteins in the UniProtKB database (http://www.uniprot.org/help/uniprotkb, released on 15 March 2017), indicating that the genes encoded a common variant of Tet(Q).
[b]MIC determined by agar dilution method; ERV, eravacycline; TIG, tigecycline; MIN, minocycline.

vitro against MDR *B. fragilis* and other Gram-negative species, as well as Gram-positive anaerobic species, including isolates containing tetracycline-specific resistance determinants and isolates resistant to commonly used antibiotics, including carbapenems, fluoroquinolones, clindamycin, and β-lactam–β-lactamase inhibitor combinations. Based on its activity *in vitro*, eravacycline shows promise for the treatment of mixed aerobic-anaerobic infections, such as intra-abdominal infections.

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