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In Vitro Activity of Eravacycline against Carbapenem-Resistant *Enterobacteriaceae* and *Acinetobacter baumannii*

David M. Livermore,[a,b] Shazad Mushtaq,[a] Marina Warner,[a] Neil Woodford[a]

Antimicrobial Resistance and Healthcare Associated Infections Reference Unit, Public Health England, London, United Kingdom[a]; Norwich Medical School, University of East Anglia, Norwich, United Kingdom[b]

Eravacycline and comparators were tested against carbapenem- and tigecycline-resistant *Enterobacteriaceae* and *Acinetobacter* isolates received at the United Kingdom's national reference laboratory. Eravacycline MICs correlated closely with those of tigecycline but mostly were around 2-fold lower; both molecules retained full activity against isolates with high-level tetracycline and minocycline resistance. MIC_{90}s of eravacycline and tigecycline were raised ca. 2-fold for carbapenem-resistant *Enterobacteriaceae* compared with carbapenem-susceptible controls, probably reflecting subsets of isolates with increased efflux.

Carbapenemase-producing *Enterobacteriaceae* (CPE) present a growing challenge, as do strains that combine porin loss with AmpC or extended-spectrum β-lactamase (ESBL) activity. Many are susceptible only to tigecycline, colistin, and fosfomycin.

Tigecycline evades the Tet(A) to Tet(E) efflux pumps and ribosome protection mechanisms that cause most tetracycline resistance, but its utility as monotherapy is compromised by (i) disputed breakpoints for *Enterobacteriaceae* (U.S. Food and Drug Administration [FDA], susceptible [S], ≤2, intermediate [I], 4, and resistant [R], >4 [1]; European Committee on Antimicrobial Susceptibility Testing [EUCAST] [http://www.eucast.org], S, ≤1, I, 2, and R, >2; with no Clinical and Laboratory Standards Institute [CLSI] values), (ii) a lack of breakpoints for *Acinetobacter baumannii*, (iii) low serum drug peaks, and (iv) an FDA warning of excess mortality (1–5). Despite these concerns, case series suggest that patients with severe CPE infections respond better to colistin-tigecycline combinations than to colistin alone (6, 7).

Eravacycline (TP-434) is a new synthetic "fluorocycline" active against most Gram-negative species (8), again including those with acquired tetracycline efflux pumps and ribosomal protection. It is well tolerated, with simpler pharmacokinetics than tigecycline and higher serum drug levels (9). At 1 mg/kg of body weight intravenous (i.v.) every 12 h (q12h), eravacycline proved noninferior to ertapenem in a phase III trial for complicated intra-abdominal infection (9). A second phase III trial failed to establish eravacycline (1.5 mg/kg i.v. q24h, with step-down to 200 mg oral [p.o.] q12h from day 3) as noninferior to levofloxacin in complicated urinary tract infection, although revised regimens continue to merit study (10).

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Address correspondence to David M. Livermore, d.livermore@uea.ac.uk.

TABLE 1 Relevant phenotypic characteristics of the panel of isolates tested

Enzyme and/or characteristic	No. of isolates with characteristic						
	A. baumannii	*Citrobacter*	*E. coli*	*Enterobacter*	*Klebsiella*	*Proteeae*	*Serratia*
KPC		3	10	10	20[a]		2
VIM		4	10	10	20	1	
IMP				5	10		
NDM	5		10	10	20[b]	8	2
OXA-48		2	10	10	20[c]	1	2
Porin loss + AmpC[d]				10			
Porin loss + ESBL			10		20		
Tig[re] + carbapenemase				10	20		
OXA-23/40/51[d]/58[f]	39						
Tig[rg] + OXA-23	5						
Carbapenem susceptible	10	2	10	10	10	5	3

[a] Ten also with SHV ESBLs.
[b] One also with OXA-48.
[c] Eight also with ESBLs.
[d] Hyperproduced.
[e] Found tigecycline nonsusceptible (MIC of ≥2 μg/ml) by EUCAST criteria on previous BSAC agar dilution testing. Seven isolates had KPC, 9 had NDM, 7 had OXA-48, and 5 had VIM enzymes. All other groups were included without reference to prior tigecycline (or other tetracycline) results.
[f] Nine or ten representatives of each OXA-carbapenemase type listed.
[g] Found tigecycline nonsusceptible (MIC of ≥2 μg/ml) by EUCAST criteria for *Enterobacteriaceae* on previous BSAC agar dilution testing.

TABLE 2 MIC distributions of tetracycline analogues for *Enterobacteriaceae*, excluding *Proteeae*, in relation to carbapenem resistance types

Drug and characteristic(s) (*n*)	No. of isolates with characteristic(s) at MIC (μg/ml) of[a]:										
	≤0.06	0.13	0.25	0.5	1	2	4	8	16	32	≥64
Eravacycline											
KPC (45)		3	13	17	**9**	2	1				
VIM (44)			16	18	**8**	2					
IMP (15)		1	4	4	1	**5**					
NDM (42)		5	16	9	**9**	2	1				
OXA-48 (44)		2	18	15	**5**	2	2				
Porin loss + ESBL/AmpC (40)		1	13	10	8	**5**	3				
Susceptible controls (35)	2	9	10	**11**	3						
Carbapenemase positive chosen as Tig^r (30)			2	4	4	4	6	**9**	1		
Tigecycline											
KPC (45)			8	14	16	**4**	3				
VIM (44)			6	16	13	**8**	1				
IMP (15)			1	7	2		**5**				
NDM (42)		1	6	18	7	**9**	1				
OXA-48 (44)			7	22	10	**2**	2	1			
Porin loss + ESBL/AmpC (40)			5	11	12	5	**5**	2			
Susceptible controls (35)	1		10	15	**9**						
Carbapenemase-positive chosen as Tig^r (30)				2	4	6	6	**9**	3		
Minocycline											
KPC (45)				1	1	14	11	12	**3**	3	
VIM (44)					2	8	13	13	**4**	3	1
IMP (15)					1	5	2	1	3	**3**	
NDM (42)					1	8	8	8	9	**6**	2
OXA-48 (44)					2	14	13	7	3	**3**	2
Porin loss + ESBL/AmpC (40)					1	6	13	7	5	**6**	2
Susceptible controls (35)			2		11	14	**6**	2			
Carbapenemase positive chosen as Tig^r (30)						1	2	4	6	4	13
Tetracycline											
KPC (45)					5	8	10	2	2	**18**^{b}	
VIM (44)					1	8	3	1		**31**^{b}	
IMP (15)				1	3	2	2	1	4	**2**^{b}	
NDM (42)					4	4	7	1		**26**^{b}	
OXA-48 (44)					8	12	6	3	2	**13**^{b}	
Porin loss + ESBL/AmpC (40)				1		5	9	5	4	**16**^{b}	
Susceptible controls (35)				5	13	12	**2**	2		1^b	
Carbapenemase positive chosen as Tig^r (30)					1	2	2	3	5	**17**^{b}	

[a] MIC_{50}s are underlined, and MIC_{90}s are in boldface; in some cases, these values coincide.
[b] The MIC is greater than or equal to the indicated value.

Against this background, we tested eravacycline *in vitro* against circulating carbapenem-resistant *Enterobacteriaceae* and *A. baumannii* isolates from the United Kingdom and sought to define the interrelationship between eravacycline and tigecycline MICs. The test organisms ($n = 369$) (Table 1) were recent submissions from United Kingdom clinical diagnostic laboratories to the national reference laboratory. For *Enterobacteriaceae*, "carbapenem resistant" was defined as resistant at least to ertapenem, as tested by British Society for Antimicrobial Chemotherapy (BSAC) agar dilution methodology (11). Carbapenemase genes were identified by PCR (12). Carbapenem resistance contingent on porin loss plus AmpC or ESBL activity was inferred from the absence of carbapenemase genes together with appropriate cefotaxime-cloxacillin or oxyimino-cephalosporin-clavulanate synergy. Isolates included specifically for tigecycline nonsusceptibility (Tig^r) (Table 1) were chosen based on MICs of ≥2 μg/ml by BSAC agar dilution; other organisms were chosen without reference to previous tigecycline MICs. Controls were chosen as carbapenem and tigecycline susceptible and as lacking ESBLs or copious AmpC. MICs were determined by CLSI broth microdilution (13) using plates (Thermofisher, Oakwood Village, OH) containing eravacycline and tigecycline (both 0.06 to 16 μg/ml), minocycline (0.12 to 64 μg/ml), and tetracycline (0.25 to 16 μg/ml). Results were reviewed against EUCAST breakpoints (http://www.eucast.org [values as of the end of 2015]) since EUCAST, unlike CLSI, has values for tigecycline as the major comparator.

Meropenem (0.03 to 128 μg/ml), amikacin (0.25 to 128 μg/ml), levofloxacin (0.03 to 32 μg/ml), colistin (0.12 to 32 μg/ml),

TABLE 3 MIC distributions of eravacycline and tigecycline in relation to species and genus, excluding isolates chosen specifically for tigecycline resistance

Drug and organism (*n*)	No. of isolates with MIC (μg/ml) of[a]:									
	0.06	0.13	0.25	0.5	1	2	4	8	16	>16
Eravacycline										
A. baumannii (55)	7	2	6	15	**23**	2				
Citrobacter (11)			3	1	5	**1**	1			
E. coli (60)	2	16	35	**6**	1					
Enterobacter (65)			9	40	**13**	3				
Klebsiella (120)		5	43	35	21	**13**	3			
Proteeae (15)				1	4	3	5	**1**	1	
Serratia (9)				2	3	1	3			
Tigecycline										
A. baumannii (55)		3	4	6	7	25	**10**			
Citrobacter (11)				2	3	4	**1**	1		
E. coli (60)	1	1	33	**23**	2					
Enterobacter (65)			1	30	27	**4**	3			
Klebsiella (120)			9	48	33	**19**	9	2		
Proteeae (15)					2	4	4	**4**		1
Serratia (9)					4	1	4			

[a] MIC_{50}s are underlined, and MIC_{90}s are in boldface.

and fosfomycin (8 to 64 μg/ml) were included as additional comparators, and the proportions of the carbapenem-resistant *Enterobacteriaceae* that were nonsusceptible (intermediate plus resistant) were as follows: amikacin, 27.7%; colistin, 10.6% (excluding *Proteeae* and *Serratia* spp.); fosfomycin, 43.7%; levofloxacin, 58.9%; and meropenem, 69.3%. Isolates with NDM carbapenemases were the most multiresistant, with the following proportions nonsusceptible: amikacin, 65.3%; colistin, 7.7% (excluding *Proteeae* and *Serratia* spp.); fosfomycin, 36.7%; levofloxacin, 75.5%; and meropenem, 93.9%. Among carbapenem-resistant *A. baumannii* strains, the proportions nonsusceptible were as follows: amikacin, 66%; colistin, 8%; levofloxacin, 96%; and meropenem, 100%; *A. baumannii* is inherently resistant to fosfomycin. All of the control *Enterobacteriaceae* were susceptible to comparators, except (i) *Proteeae* and *Serratia* spp. were inherently resistant to colistin, (ii) one *Escherichia coli* isolate was resistant to colistin at EUCAST's 2-μg/ml breakpoint, and (iii) a few isolates were resistant to fosfomycin. Two of the 10 carbapenem-susceptible *A. baumannii* controls were nonsusceptible to amikacin, and one was nonsusceptible to levofloxacin.

TABLE 4 Eravacycline MICs for *A. baumannii* by carbapenem resistance mechanism

Drug and characteristic (*n*)	No. of isolates with MIC (μg/ml) of[a]:									
	≤0.06	0.13	0.25	0.5	1	2	4	8	16	32
Eravacycline										
NDM (5)		1	4							
OXA-23/40/51/58 (39)	1[b]		2	12	**22**	2				
Susceptible controls (10)	6[b]	1		**2**	1					
OXA-23, selected as Tig^r (5)					1		2	2		
Tigecycline										
NDM (5)				4	1					
OXA-23/40/51/58 (39)			1	1	5	22	**10**			
Susceptible controls (10)		3	3	1	1	**2**				
OXA-23, selected as Tig^r (5)						1		2	2	
Minocycline										
NDM (5)		1		3	1					
OXA-23/40/51/58 (39)		1	2	2	6	7	7	7	**6**	1[c]
Susceptible controls (10)		7	1		**1**	1				
OXA-23, selected as Tig^r (5)						1		1	1	2[c]
Tetracycline										
NDM (5)						1		1		3[c]
OXA-23/40/51/58 (39)							4	0	5	**30**[c]
Susceptible controls (10)					6	2	**1**		1	
OXA-23, selected as Tig^r (5)										5[c]

[a] MIC_{50}s are underlined, and MIC_{90}s are in boldface.
[b] The MIC is less than or equal to the indicated value.
[c] The MIC is greater than or equal to the indicated value.

MIC eravacycline (μg/ml)	MIC tigecycline (μg/ml)									
	0.06	0.13	0.25	0.5	1	2	4	8	16	>16
0.06	1	**3**	5							
0.13		1	16	6						
0.25			**25**	**68**	5					
0.5			1	36	**56**	10				
1				1	21	**45**	8			
2						8	**19**			
4							9	**10**	1	
8							1	8	**3**	
16									1	1
>16										

FIG 1 Interrelationship between eravacycline and tigecycline MICs for the full panel of 369 isolates. Gray boxes represent the line of equivalence. Numbers above this line indicate eravacycline is more active, and numbers below indicate tigecycline is more active. Boldface indicates the modal MIC of eravacycline for each tigecycline MIC value.

Eravacycline MICs for the *Enterobacteriaceae* series (excluding *Proteeae*, discussed below) were unimodally distributed, as were those of tigecycline (Table 2). Minocycline distributions were unimodal, but with more positive skew (i.e., a wider spread of MICs above than below the mode) than for eravacycline and tigecycline and with a few highly resistant isolates. MIC distributions of tetracycline were bimodal. Although their distributions overlapped considerably, the MICs of eravacycline were mostly 2-fold lower than those of tigecycline, with modes at 0.25 to 0.5 μg/ml, according to the species and resistance group, versus 0.5 to 1 μg/ml. MIC_{50}s of eravacycline (underlined in Table 2) for the carbapenem-resistant *Enterobacteriaceae* groups mostly were 2-fold higher than those for carbapenem-susceptible control strains, while MIC_{90}s (boldface in Table 2) were 2- or 4-fold higher—a differential also evident for tigecycline MIC_{90}s (not MIC_{50}s). These raised summary MICs partly reflected a larger proportion of *Klebsiella* versus *E. coli* isolates among the carbapenem-resistant isolates than the controls (Table 1), coupled with a general trend for *Klebsiella* to be less susceptible to eravacycline and tigecycline than *E. coli* (Table 3). Nevertheless, the pattern persisted if only *Klebsiella* spp. were considered, indicating that a subset of the carbapenem-resistant *K. pneumoniae* isolates had reduced eravacycline and tigecycline susceptibility.

Among the 30 carbapenemase-producing *Enterobacteriaceae* specifically included as tigecycline nonsusceptible based on prior BSAC agar testing, 18 were confirmed resistant, with MICs of 4 to 16 μg/ml, and another 6 as intermediate, with MICs of 2 μg/ml. MICs of eravacycline remained below those of tigecycline, but with 16 values in the range 4 to 16 μg/ml (Table 2).

The 15 *Proteeae* isolates (Table 1) comprised 6 *Morganella morganii*, 5 *Providencia rettgeri*, and 3 *P. stuartii* isolates and 1 *Proteus mirabilis* isolate: 10 isolates had carbapenemases, 8 of which were NDM types. All 15 organisms were resistant to classical tetracyclines. Two were susceptible at tigecycline's EUCAST breakpoint of ≤1 μg/ml, four intermediate (MIC, 2 μg/ml), and nine resistant, with MICs of >2 μg/ml. For eravacycline, 12/15 MICs were from 1 to 4 μg/ml (Table 3), with 10/15 values 2-fold below those for tigecycline.

MICs of eravacycline and tigecycline for the carbapenem-resistant *A. baumannii* series were unimodally distributed (Table 4), with eravacycline values mostly 2- to 4-fold below tigecycline, clustering at 0.5 to 1 μg/ml versus 1 to 4 μg/ml. MICs of minocycline were widely scattered, with most isolates highly resistant to tetracycline. As with *Enterobacteriaceae*, eravacycline and tigecycline MIC_{50}s and MIC_{90}s for the carbapenem-resistant groups exceeded those for the susceptible controls. Five *A. baumannii* isolates, all with OXA-23 carbapenemase, were included based on previously found tigecycline resistance: four "retained" tigecycline MICs of 8 to 16 μg/ml, and MICs of eravacycline for these were 4 to 8 μg/ml.

Two key findings emerge. First, eravacycline is 2- to 4-fold more active than tigecycline against carbapenem-resistant *Enterobacteriaceae* and *A. baumannii* isolates, but with qualitatively similar behaviors, leading to close correlation between MICs of both molecules (Fig. 1). Second, although (unsurprisingly) little relationship existed between eravacycline MICs and specific carbapenem resistance mechanisms, MIC_{90}s of eravacycline and tigecycline were 2- to 4-fold higher for carbapenem-resistant *Enterobacteriaceae* and *A. baumannii* isolates than for the carbapenem-susceptible controls, with a similar MIC_{50} shift for eravacycline. The likely explanation is that a subset of carbapenem-resistant isolates have upregulated endogenous efflux or reduced permeability, a view supported by a recent Chinese study reporting frequent upregulation of the AcrAB pump in *K. pneumoniae* isolates with KPC enzymes (14). Upregulation of such pumps is the principal mode of tigecycline resistance in *Enterobacteriaceae*

and *A. baumannii* (15) and accounts for the intrinsic resistance of *Proteeae* (16).

The small but consistent gains in activity against carbapenem-resistant *Enterobacteriaceae* and *A. baumannii* isolates compared with tigecycline, coupled with higher serum drug levels, better tolerability, and more straightforward pharmacokinetics, may translate to an advantage for eravacycline, and clinical investigation is warranted.

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