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Activity of Eravacycline against *Enterobacteriaceae* and *Acinetobacter baumannii*, Including Multidrug-Resistant Isolates, from New York City

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Eravacycline demonstrated *in vitro* activity against a contemporary collection of more than 4,000 Gram-negative pathogens from New York City hospitals, with MIC_{50}/MIC_{90} values, respectively, for *Escherichia coli* of 0.12/0.5 μg/ml, *Klebsiella pneumoniae* of 0.25/1 μg/ml, *Enterobacter aerogenes* of 0.25/1 μg/ml, *Enterobacter cloacae* 0.5/1 μg/ml, and *Acinetobacter baumannii* of 0.5/1 μg/ml. Activity was retained against multidrug-resistant isolates, including those expressing KPC and OXA carbapenemases. For *A. baumannii*, eravacycline MICs correlated with increased expression of the *adeB* gene.

Nosocomial infections with multidrug-resistant Gram-negative bacilli have considerable clinical and economic burdens. Carbapenem-resistant members of the family *Enterobacteriaceae* and *Acinetobacter baumannii* have become commonplace in many medical centers around the world. In the United States, and particularly New York City, NY, spread of *Enterobacteriaceae* possessing the carbapenemase KPC and *A. baumannii* possessing OXA-type carbapenemases has been especially problematic (1–3). Since these isolates are often also resistant to aminoglycosides and fluoroquinolones, therapeutic options are very limited. Often the therapy of last resort are the polymyxins; however, there are lingering issues regarding dosing, susceptibility testing, and resistance with these agents (4, 5). As a result, the need for novel antibacterial agents with activity against multidrug-resistant isolates has been voiced (6).

Eravacycline is a novel fluorocycline of the tetracycline class; it was developed for both intravenous and oral use and has broad-spectrum activity against Gram-negative and Gram-positive aerobic and anaerobic pathogens (7). Like tigecycline, eravacycline is not affected by many of the tetracycline-specific resistance mechanisms found in Gram-negative bacteria, including acquired efflux systems and ribosomal protection proteins (7, 8). In this report, we determined the activity of eravacycline against *Enterobacteriaceae* and *A. baumannii* endemic to medical centers in New York City, NY.

For a 3-month period spanning November 2013 to January 2014, all single patient isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp., and *A. baumannii* were collected from 11 hospitals in Brooklyn and Queens, New York. Susceptibility tests were performed in a central research laboratory using the broth microdilution (for tigecycline and eravacycline) or agar dilution (all other agents) methods, according to CLSI standards (9). Ceftriaxone-resistant isolates were tested, by PCR, for the presence of bla_{KPC} and $bla_{OXA\text{-}23\text{-}type}$ and $bla_{OXA\text{-}24\text{-}type}$ using previously described primers (1, 10). Isolates of *K. pneumoniae* with bla_{KPC} and *A. baumannii* with $bla_{OXA23\text{-}type}$ underwent genetic fingerprinting by the repetitive sequence-based PCR (rep-PCR) method with enterobacterial repetitive intergenic consensus 2 (ERIC-2) primer, as previously described (2). Isolates were considered related if there was a 0- or 1-band difference. Finally, select isolates of *K. pneumoniae* were also examined for mutations in genes encoding RamR and ribosomal S10 proteins, using previously described primers and PCR conditions (11).

To examine the mechanisms contributing to eravacycline resistance in *A. baumannii*, susceptibility testing was performed using a collection of previously characterized isolates (12). Expression of *adeB*, *abeM*, and *ompA* was determined by real-time reverse transcription-PCR (RT-PCR) and normalized to that of *A. baumannii* ATCC 19606, as previously described (12). Multiple regression analysis was used to determine any correlation between expression of efflux genes *adeB*, *abeM*, and porin gene *ompA* with eravacycline MIC values. In addition, gene silencing of *adeB* was performed to further determine the role of this efflux system in eravacycline resistance. Two previously characterized isolates of *A. baumannii* were selected based on their disparate expression of *adeB* and susceptibility to kanamycin. Insertional inactivation of the *adeB* gene was performed by amplifying a 979-bp fragment of the gene (AccuPrime Pfx DNA polymerase; Invitrogen Corp., Carlsbad, CA). The amplicon was inserted into the pCR-Blunt II-TOPO vector using the Zero Blunt TOPO PCR cloning kit (Invitrogen Corp.). The plasmid was introduced into the clinical isolates by electroporation. Transformants were selected from plates containing 250 μg/ml of kanamycin. The presence of the insert in *adeB* was confirmed by PCR amplification and DNA sequencing using an M13 forward primer and a primer (5′-TTGGGCTGAT ATTACAGGGG-3′) located upstream of the insert.

A total of 2,866 isolates of *E. coli* were gathered during the

Received 14 November 2014 **Returned for modification** 4 December 2014
Accepted 19 December 2014

Accepted manuscript posted online 22 December 2014

Citation Abdallah M, Olafisoye O, Cortes C, Urban C, Landman D, Quale J. 2015. Activity of eravacycline against *Enterobacteriaceae* and *Acinetobacter baumannii*, including multidrug-resistant isolates, from New York City. Antimicrob Agents Chemother 59:1802–1805. doi:10.1128/AAC.04809-14.

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doi:10.1128/AAC.04809-14

TABLE 1 Susceptibility results for *Enterobacteriaceae* and *A. baumannii* collected during the surveillance study

Bacterial species (no. of isolates)	MIC (μg/ml)			% susceptible
	50%	90%	Range	
E. coli (n = 2,866)				
Ceftriaxone	≤0.25	32	≤0.25–>64	87
Meropenem	≤0.125	≤0.125	≤0.125–>8	99.8
Gentamicin	1	>8	≤0.25–>8	85
Ciprofloxacin	≤0.125	>4	≤0.125–>4	69
Trimethoprim-sulfamethoxazole	≤0.5	>4	≤0.5–>4	64
Tetracycline	4	>16	≤0.25–>16	59
Eravacycline	0.12	0.5	≤0.015–4	
K. pneumoniae (n = 944)				
Ceftriaxone	≤0.25	>64	≤0.25–>64	67
Meropenem	≤0.125	4	≤0.125–>8	87
Gentamicin	0.5	>8	≤0.25–>8	78
Ciprofloxacin	≤0.125	>4	≤0.125–>4	67
Trimethoprim-sulfamethoxazole	≤0.5	>4	≤0.5–>4	65
Tetracycline	4	>16	≤0.25–>16	68
Tigecycline	0.5	2	≤0.015–16	95
Eravacycline	0.25	1	0.06–4	
E. aerogenes (n = 90)				
Ceftriaxone	≤0.25	32	≤0.25–>64	78
Meropenem	≤0.125	≤0.125	≤0.125–>8	94
Gentamicin	0.5	1	≤0.25–>8	94
Ciprofloxacin	≤0.125	0.5	≤0.125–>4	93
Trimethoprim-sulfamethoxazole	≤0.5	>4	≤0.5–>4	91
Tetracycline	2	>16	≤0.25–>16	82
Tigecycline	0.5	2	≤0.25–>16	97
Eravacycline	0.25	1	0.12–2	
E. cloacae (n = 124)				
Ceftriaxone	≤0.25	2	≤0.25–>64	77
Meropenem	≤0.125	≤0.125	≤0.125–>8	96
Gentamicin	0.5	2	≤0.25–>8	94
Ciprofloxacin	≤0.125	2	≤0.125–>4	89
Trimethoprim-sulfamethoxazole	≤0.5	>4	≤0.5–>4	82
Tetracycline	2	8	≤0.25–>16	79
Tigecycline	0.5	2	≤0.25–>16	96
Eravacycline	0.5	1	0.25–2	
A. baumannii (n = 158)				
Ceftazidime	16	>16	≤0.25–>16	33
Meropenem	>8	>8	≤0.125–>8	31
Gentamicin	8	>8	≤0.25–>8	45
Ciprofloxacin	>4	>4	≤0.125–>4	31
Trimethoprim-sulfamethoxazole	>4	>4	≤0.5–>4	34
Tetracycline	>16	>16	2–>16	10
Tigecycline	2	4	0.06–16	66
Eravacycline	0.5	1	≤0.015–8	

3-month surveillance study. Susceptibilities are given in Table 1; 13% were nonsusceptible to ceftriaxone, and five isolates carried bla_{KPC}. The eravacycline MIC_{50} and MIC_{90} values for all of the isolates were 0.12 and 0.5 μg/ml, respectively. Of the isolates resistant to ceftriaxone, eravacycline MIC_{50} and MIC_{90} values were 0.25 and 0.5 μg/ml, respectively.

Among 944 isolates of *K. pneumoniae*, 33% were resistant to ceftriaxone, and 124 isolates carried bla_{KPC}. For all of the isolates, the MIC_{50} and MIC_{90} values for eravacycline were 0.25 and 1 μg/ml, respectively, which were 1 dilution lower than the corresponding values for tigecycline. For the tigecycline-resistant isolates, eravacycline MIC_{50} and MIC_{90} values were 2 and 8 μg/ml, respectively. For both cephalosporin-resistant isolates and those expressing bla_{KPC}, MIC_{50} and MIC_{90} values of eravacycline were 0.5 and 1 μg/ml, respectively. Twenty-three isolates with bla_{KPC}, from 11 different hospitals, underwent fingerprinting. Eight rep-PCR types were identified, including 12 isolates that belonged to one clone (data not shown).

Eleven isolates of *K. pneumoniae* with eravacycline MICs of ≥4 μg/ml underwent PCR analysis of *ramR* and *rpsJ* (encoding the

S10 ribosomal protein). For *ramR*, two isolates had a nonamplifiable gene, five isolates had mutations leading to premature stop codons, and two isolates had deletions leading to frameshift mutations. One isolate had a mutation leading to Thr50→Ile50, and one isolate had no mutation. For *rpsJ*, four isolates had a nonamplifiable gene, and only one had the previously described mutation leading to Val57→Leu57 (11).

There were 216 *Enterobacter* isolates, including 90 *Enterobacter aerogenes* isolates and 124 *Enterobacter cloacae* isolates. Three isolates of *E. aerogenes* and four isolates of *E. cloacae* harbored bla_{KPC}. For all 216 *Enterobacter* isolates, 96% were susceptible to tigecycline (MIC_{50} and MIC_{90} values of 0.5 and 2 μg/ml, respectively). The corresponding MIC_{50} and MIC_{90} values for eravacycline were 0.5 and 1 μg/ml, respectively. For both *E. aerogenes* and *E. cloacae*, eravacycline MIC_{90} values were 1 dilution lower than that of tigecycline (Table 1). For the tigecycline-resistant isolates, the eravacycline MICs ranged from 0.5 to 2 μg/ml. For the three isolates of *E. aerogenes* with bla_{KPC}, two had eravacycline MIC values of 0.25 and one had 0.5 μg/ml. For the four isolates of *E. cloacae* that had bla_{KPC}, two each had eravacycline MIC values of 0.5 and 1 μg/ml.

Only 31% of the 158 isolates of *A. baumannii* were susceptible to meropenem. Fifty-eight isolates were found to have $bla_{OXA\text{-}23\text{-}like}$, two carried $bla_{OXA\text{-}24\text{-}like}$, and one harbored bla_{KPC}. For all 158 isolates, 66% were susceptible to tigecycline (MIC ≤ 2 μg/ml). The MIC_{50} and MIC_{90} values of eravacycline were 0.5 and 1 μg/ml, which were 4-fold lower than the corresponding values for tigecycline. Among the tigecycline-resistant isolates, the MIC_{50} and MIC_{90} values of eravacycline were 1 and 2 μg/ml, respectively. For the 58 isolates that had $bla_{OXA\text{-}23\text{-}like}$, the MIC_{90} value for eravacycline was 1 μg/ml. Eighteen isolates, from eight hospitals, with $bla_{OXA\text{-}23\text{-}like}$ underwent fingerprinting. A total of 10 rep-PCR types were identified, including six belonging to a single clone (data not shown).

A collection of 38 previously characterized isolates of *A. baumannii*, representing 10 different strains, were examined (12). Of the 38 isolates, 26 were resistant to meropenem. None harbored bla_{KPC}, $bla_{OXA23\text{-}type}$, or $bla_{OXA24\text{-}type}$ carbapenemases. The eravacycline MIC values ranged from 0.06 to 4 μg/ml, with MIC_{50} and MIC_{90} values of 0.5 and 2 μg/ml, respectively. By multiple regression analysis, there was a significant correlation between the eravacycline MICs and expression of *adeB* ($P = 0.002$); there was no correlation with expression of *abeM* and *ompA*. For five isolates with negligible *adeB* expression (less than 0.10 times that of the control), the average tigecycline and eravacycline MICs were 0.37 ± 0.35 and 0.24 ± 0.17 μg/ml, respectively. For 28 isolates with expression 0.64 to 6 times that of the control, the average tigecycline and eravacycline MICs were 1.1 ± 0.51 and 0.84 ± 0.68 μg/ml, respectively. The remaining isolates had *adeB* expression 7 to 42 times that of the control, and the average tigecycline and eravacycline MICs were 2.6 ± 1.3 and 1.8 ± 1.3 μg/ml, respectively.

The *adeB* gene was disrupted in two isolates. For the first isolate, there was hyperexpression of *adeB* (40 times higher than that of the control *A. baumannii* ATCC 19606). For this isolate, disruption of the *adeB* gene reduced the eravacycline MIC 8-fold, from 2 to 0.25 μg/ml. Expression of *adeB* was normal in the second isolate (1.4 times that of the control). The eravacycline MIC (at 0.25 μg/ml) was unchanged when *adeB* was disrupted in this isolate.

New antimicrobial agents are sorely needed to treat infections due to multidrug-resistant Gram-negative pathogens. Eravacycline is a novel fluorocycline, with a tetracycline core, that binds to the 70S ribosome of bacteria (8). However, unlike tetracycline, eravacycline is largely unaffected by commonly encountered efflux pumps (e.g., Tet A and Tet B) and ribosomal protection proteins (Tet M and Tet O) found in clinical isolates (8). In one report, MIC_{90} values for most Gram-negative bacilli ranged from 0.5 to 2 μg/ml; however, there was reduced activity against *Pseudomonas aeruginosa* and *Burkholderia cenocepacia* (7). Microbiological cure rates of complicated intra-abdominal infections following eravacycline therapy reached 93 to 100%, which were comparable to that of ertapenem therapy (13). Intrapulmonary concentrations of eravacycline are higher than free plasma eravacycline concentrations, suggesting this agent may also be useful against respiratory tract infections (14).

In this report, eravacycline demonstrated activity against clinical isolates of *E. coli*, *K. pneumoniae*, and *Enterobacter* spp. from hospitals in New York City, NY. It retained activity against multidrug-resistant isolates, including those with extended-spectrum β-lactamases and the KPC carbapenemases. Consistent with another study (7), activity was also preserved against tetracycline-resistant isolates, and MIC values were generally 1 dilution lower than that for tigecycline for *K. pneumoniae* and *Enterobacter* spp. As has been reported for tigecycline, reduced susceptibility to eravacycline in isolates of *K. pneumoniae* may be related to mutations involving *ramR* (affecting expression of the regulator RamA and the AcrAB efflux system) and *rpsJ* (affecting the S10 ribosomal protein).

In addition, multidrug-resistant *A. baumannii* is another well-established pathogen in many medical centers, particularly in New York City, NY. Options for therapy for this pathogen are also quite limited and often involve a polymyxin and/or tigecycline. Eravacycline also demonstrated activity against these isolates and was 4-fold more active than tigecycline by comparison of MIC_{90} values. In our collection of well-characterized isolates consisting of multiple clones, increased MIC values to eravacycline (reaching 2 to 4 μg/ml) were associated with increased expression of the native efflux pump AdeABC. Increased expression of this efflux system has also been associated with reduced activity of tigecycline (12). Because of limited therapeutic options, infection control efforts should be emphasized to limit the spread of *A. baumannii* with reduced susceptibility to tigecycline and eravacycline.

In summary, eravacycline is a novel antimicrobial agent with enhanced *in vitro* activity against multidrug-resistant Gram-negative pathogens endemic in New York City. Eravacycline activity is preserved against tetracycline-resistant isolates, and its activity is generally 1 or 2 dilutions lower than that of tigecycline.

ACKNOWLEDGMENT

This work was supported by a research grant from Tetraphase Pharmaceuticals, Inc.

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