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LETTER TO THE EDITOR

Eravacycline Is Active against Bacterial Isolates Expressing the Polymyxin Resistance Gene *mcr-1*

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Polymyxin antibiotics are considered a last line of defense in the treatment of severe infections caused by multidrug-resistant and extensively drug-resistant Gram-negative pathogens. However, the recent discovery of the plasmid-mediated polymyxin antibiotic resistance gene *mcr-1* and its apparent diffusion into environmental, food animal, and retail food sources and clinical settings threaten to stem the clinical utility of polymyxin antibiotics (1–5). The *mcr-1* gene encodes a phosphoethanolamine transferase which modifies lipid A in the outer membranes of *Enterobacteriaceae*, leading to decreased binding affinities of polymyxin antibiotics (1, 6).

Since there exists the possibility that modification of the outer membrane of Gram-negative bacteria has a more general effect on antibiotic susceptibility, the *in vitro* activity of the novel fluorocycline antibiotic eravacycline was evaluated against a diverse set of *mcr-1*-positive *Escherichia coli* clinical ($n = 16$) and animal ($n = 1$) isolates, *Salmonella enterica* animal isolates ($n = 4$), and a set of 4 carbapenem-resistant clinical isolates comprised of *E. coli*, *Enterobacter cloacae*, and *Klebsiella pneumoniae* engineered to overexpress the *mcr-1* gene.

Susceptibility to eravacycline and comparators was evaluated by broth microdilution MIC assays performed according to Clinical and Laboratory Standards Institute (CLSI) methodology (7). Antibiotics were obtained from Sigma-Aldrich (St. Louis, MO). The set of 21 nonclonal strains tested, carrying the *mcr-1* gene either chromosomally or on one of four different plasmids, was collected from sources in Switzerland ($n = 5$), South Africa ($n = 7$), France ($n = 3$), Portugal ($n = 4$), and the Centers for Disease Control (CDC0346, CDC349 [http://www.cdc.gov/drugresistance/resistance-bank/currently-available.html]; $n = 2$) and included representatives from at least 14 sequence types. The presence of the *mcr-1* gene was confirmed using published PCR primers and methods (1). The eravacycline MIC_{50} and MIC_{90} values against this panel were 0.25 and 0.5 μg/ml, respectively, similar to values for the in-class comparator tigecycline (Table 1). Nine isolates were resistant to 3rd- and/or 4th-generation cephalosporins, and 19 were resistant to tetracycline. The MIC_{50} and MIC_{90} values for colistin were 8 and 16 μg/ml, respectively, and those for polymyxin B were 4 and 8 μg/ml, respectively; 20/21 isolates showed MIC values of ≥4 μg/ml for colistin and/or polymyxin B.

To evaluate the specific effects of *mcr-1* expression in the absence of other resistance determinants that may be present in naturally occurring *mcr-1*-positive clinical isolates (8), the *mcr-1* gene was PCR amplified from *mcr-1*-positive isolate Af23 (9) using the forward and reverse primers GCGCGGTCATGATGCAGCATAC TTCTGTGTG and GCGGCCTCGAGTCAGCGGATGAATGCG GTG, with additions of 5′ BspHI and 3′ XhoI cloning sites, respectively. The *mcr-1* sequence was confirmed to be identical to that of GenBank accession number KX032519. The *mcr-1* gene was cloned into the NcoI and XhoI cloning sites of the $pBAD_B$-gentamicin vector under the control of an arabinose-inducible promoter, similar to what has previously been reported (6). The $pBAD_B$-gentamicin vector was constructed by cloning the *acc(3)-I* aminoglycoside 3-*N*-acetyltransferase gene from plasmid pUCGM (GenBank accession number U04610) into the $pBAD_B$ vector (Thermo Scientific, Waltham, MA) using KpnI and XbaI sites downstream of the multiple cloning site. A plasmid bearing the *E. coli lacZ* gene was cloned into the same expression vector and used as a negative control. Plasmids were transformed into the polymyxin-sensitive *E. coli* laboratory strain DH10B (Thermo Fisher Scientific, Waltham, MA) and into clinical isolates of *E. coli* (CDC0150 bla_{NDM-5}), *K. pneumoniae* [CDC0146 bla_{NDM} *tet*(A) and CDC0160 bla_{OXA-48}], and *E. cloacae* [CDC0050 bla_{KPC} *tet*(A)] from the carbapenemase detection and diversity panels available from the CDC (http://www.cdc.gov/drugresistance/resistance-bank/currently-available.html). Plasmid-containing strains were maintained in 5 μg/ml gentamicin and induced for 30 min at room temperature with 1% D-arabinose prior to the assay. MIC assays were conducted according to CLSI

TABLE 1 Activities of eravacycline against *mcr-1*-positive *E. coli* and *S. enterica* isolates[a]

Antibiotic	MIC_{50}, MIC_{90} (μg/ml)	Range (μg/ml)	% susceptible
Eravacycline	0.25, 0.5	0.031 to 0.5	NA
Colistin	8, 16	1 to 16	NA
Polymyxin B	4, 8	0.5 to 8	NA
Tetracycline	>32, >32	0.5 to >32	9.5
Tigecycline	0.25, 0.5	0.063 to 1	100
Gentamicin	2, >32	0.5 to >32	71.4
Aztreonam	0.25, >32	0.063 to >32	57.1
Meropenem	0.031, 0.063	≤0.016 to 0.063	100
Cefepime	1, >32	0.13 to >32	57.1
Ceftazidime	0.5, >32	0.25 to >32	66.7
Cefotaxime	0.25, >32	0.063 to >32	57.1
Levofloxacin	8, >32	0.063 to >32	42.8

[a] MIC_{50} and MIC_{90} values were determined by broth microdilution MIC assays, performed in duplicate (at least) for each isolate, and representative data are shown. Data shown are for a total of 21 isolates. NA, not available. % susceptible, percentage of susceptible isolates according to CLSI (10) or FDA (for tigecycline) (11) guidelines.

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TABLE 2 Activities of eravacycline and comparators against carbapenem-resistant *E. coli*, *K. pneumoniae*, and *E. cloacae* clinical isolates recombinantly expressing *mcr-1*

	MIC (μg/ml) of the following strain with the indicated characteristic:													
	E. coli DH10B		*E. coli* CDC0150 bla_{NDM-5}			*K. pneumoniae* CDC0146 bla_{NDM} *tet*(A)			*K. pneumoniae* CDC0160 bla_{OXA-48}			*E. cloacae* CDC0050 bla_{KPC} *tet*(A)		
Antibiotic	*lacZ*	*mcr-1*	No plasmid	*lacZ*	*mcr-1*	No plasmid	*lacZ*	*mcr-1*	No plasmid	*lacZ*	*mcr-1*	No plasmid	*lacZ*	*mcr-1*
Eravacycline	0.13	0.13	0.13	0.063	0.13	0.5	1	0.5	0.25	0.25	0.5	0.5	0.25	0.25
Colistin	0.063	4	0.25	0.13	8	0.25	0.13	8	0.5	0.25	8	0.13	0.13	8
Polymyxin B	0.063	4	0.25	0.063	4	0.13	0.13	8	0.25	0.13	8	0.13	0.13	4
Tetracycline	2	2	>32	>32	>32	>32	>32	>32	8	4	4	4	2	2
Tigecycline	0.13	0.13	0.25	0.25	0.5	1	2	1	0.5	0.5	0.5	0.5	0.5	0.5
Aztreonam	0.25	0.13	>32	32	32	>32	>32	32	0.25	0.25	0.13	>32	>32	>32
Meropenem	0.031	0.031	>32	16	16	>32	8	4	32	8	8	1	0.25	0.25
Cefepime	1	0.5	>32	>32	>32	>32	>32	>32	8	4	2	>32	8	16
Ceftazidime	0.5	0.5	>32	>32	>32	>32	>32	>32	1	0.5	0.5	>32	>32	>32
Cefotaxime	0.063	0.063	>32	>32	>32	>32	>32	>32	8	2	1	>32	4	8
Levofloxacin	≤0.016	≤0.016	32	32	32	>32	>32	>32	0.063	0.063	0.063	16	8	8

MIC values were determined by broth microdilution MIC assays, performed in duplicate (at least) for each isolate according to CLSI standard procedures, with the exception that gentamicin at 5 μg/ml and 1% D-arabinose were present in assays with plasmid-containing strains. Representative results are shown.

methodology, with the exception that both gentamicin and arabinose were present. Results showed that while overexpression of *mcr-1* in each clinical isolate raised the MIC values for colistin and polymyxin B by 32- to 64-fold, there was no effect on susceptibility to eravacycline and other comparators (i.e., MIC values with and without *mcr-1* overexpression were within 2-fold of those of the *lacZ* control strain) (Table 2). It was noted that the MIC values of the cell wall-inhibiting antibiotics meropenem, cefepime, ceftazidime, and cefotaxime for plasmid-containing strains (expressing either *lacZ* or *mcr-1*) generally were lower (when endpoints were available) than those for the corresponding "no-plasmid" controls. Since similar MIC values were obtained for strains expressing either *lacZ* or *mcr-1*, the lower MIC values may be attributed to general effects on growth rate due to recombinant expression or antibiotic interactions due to the presence of gentamicin in assays with the plasmid-containing strains.

In conclusion, the present study showed that eravacycline was potent *in vitro* against naturally occurring *mcr-1*-positive bacterial isolates and unaffected by the overexpression of *mcr-1* in *Enterobacteriaceae*.

REFERENCES

1. **Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J.** 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis **16:**161–168. http://dx.doi.org/10.1016/S1473-3099(15)00424-7.
2. **Zurfuh K, Poirel L, Nordmann P, Nuesch-Inderbinen M, Hachler H, Stephan R.** 2016. Occurrence of the plasmid-borne mcr-1 colistin resistance gene in extended-spectrum-beta-lactamase-producing Enterobacteriaceae in river water and imported vegetable samples in Switzerland. Antimicrob Agents Chemother **60:**2594–2595. http://dx.doi.org/10.1128/AAC.00066-16.
3. **Castanheira M, Griffin MA, Deshpande LM, Mendes RE, Jones RN, Flamm RK.** 2016. Detection of *mcr-1* among *Escherichia coli* clinical isolates collected worldwide as part of the SENTRY Antimicrobial Surveillance Program during 2014-2015. Antimicrob Agents Chemother http://dx.doi.org/10.1128/AAC.01267-16.
4. **Figueiredo R, Card RM, Nunez J, Pomba C, Mendonca N, Anjum MF, Da Silva GJ.** 20 June 2016. Detection of an mcr-1-encoding plasmid mediating colistin resistance in *Salmonella enterica* from retail meat in Portugal. J Antimicrob Chemother http://dx.doi.org/10.1093/jac/dkw240.
5. **Paterson DL, Harris PN.** 2016. Colistin resistance: a major breach in our last line of defence. Lancet Infect Dis **16:**132–133. http://dx.doi.org/10.1016/S1473-3099(15)00463-6.
6. **Ye H, Li Y, Li Z, Gao R, Zhang H, Wen R, Gao GF, Hu Q, Feng Y.** 2016. Diversified mcr-1-harboring plasmid reservoirs confer resistance to colistin in human gut microbiota. mBio **7:**e00177-16. http://dx.doi.org/10.1128/mBio.00177-16.
7. **CLSI.** 2015. Methods for dilution of antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—tenth edition. CLSI document M07-A10. Clinical and Laboratory Standards Institute, Wayne, PA.
8. **Zhang H, Seward CH, Wu Z, Ye H, Feng Y.** 2016. Genomic insights into the ESBL and MCR-1-producing ST648 *Escherichia coli* with multi-drug resistance. Sci Bull **61:**875–878. http://dx.doi.org/10.1007/s11434-016-1086-y.
9. **Poirel L, Kieffer N, Brink A, Coetze J, Jayol A, Nordmann P.** 2016. Genetic features of MCR-1-producing colistin-resistant *Escherichia coli* isolates in South Africa. Antimicrob Agents Chemother **60:**4394–4397. http://dx.doi.org/10.1128/AAC.00444-16.
10. **CLSI.** 2016. Performance standards for antimicrobial susceptibility testing; twenty-six informational supplement. CLSI document. Clinical and Laboratory Standards Institute, Wayne, PA.
11. **Wyeth Pharmaceuticals.** 2016. Tygacil—tigecycline injection, powder, lyophilized, for solution. Prescribing information. Wyeth Pharmaceuticals, Philadelphia, PA.