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# Eravacycline is Potent against Category A and B Pathogens

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## Abstract

**Background:** Eravacycline (ERA) is a novel broad-spectrum tetracycline being developed for the treatment of serious Gram-negative and Gram-positive aerobic and anaerobic bacterial infections. ERA is impervious to known tetracycline-specific-resistant mechanisms for efflux, ribosomal protection, and inactivation.

**Methods:** ERA and comparators were tested against 167 strains of *Bacillus anthracis, Yersinia pestis, Francisella tularensis, Burkholderia mallei*, and *Burkholderia pseudomallei* using standard CLSI microtiter-based methods to determine MIC values. Quality control of experimental conditions was established using *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213.

**Results:** $MIC_{90}$ results for ERA and comparators against biothreat pathogens are shown in the Table 1. To determine the impact of well characterized resistance-nodulation-cell-division (RND) efflux pumps on susceptibility, ERA and comparators were tested in isogenic strains of *B. pseudomallei* 1026b expressing 0 to 3 up-regulated or disabled RND pumps. Similar to doxycycline (DOX), ERA was effluxed by BpeEF-OprC and to a lesser extent by the AmrAB-OprA and BpeAB-OprB efflux pumps. However, the degree of efflux of ERA by a strain overexpressing BpeEF-OprC was significantly lower than that observed with DOX and the observed MIC (2 µg/mL) may well be within the therapeutic range.

**Table 1. Susceptibility ($MIC_{90}$ in µg/mL) of Biothreat Pathogens to Eravacycline and Comparators**

| Compound | Organism (n) | | | | |
|---|---|---|---|---|---|
| | *B. anthracis* (35) | *B. mallei* (30) | *B. pseudomallei* (35) | *F. tularensis* (33) | *Y. pestis* (34) |
| Eravacycline | 0.016 | 0.25 | 2 | 0.5 | 0.125 |
| Tetracycline | 0.031 | 0.125 | 8[a] | 0.5 | 2 |
| Penicillin | 0.125 | ND[b] | ND | 0.5 | 1 |
| Ciprofloxacin | 0.06 | 2 | ND | 0.125 | 0.031 |
| Gentamicin | ND | ND | ND | 0.5 | 1 |
| Ceftazidime | ND | 4 | 4 | ND | ND |
| Imipenem | ND | ND | 2 | ND | ND |

[a]Doxycycline; [b]ND = not done

**Conclusion:** ERA, a broad spectrum antibiotic with activity against tetracycline-resistant biothreat and public health pathogens, has the *in vitro* potency to be used as empiric therapy for the treatment of respiratory infections.

## Introduction

Eravacycline is a novel synthetic tetracycline that has broad-spectrum antimicrobial activity against aerobic and anaerobic Gram-negative and Gram-positive bacteria, with the exception of *Pseudomonas aeruginos*a (Sutcliffe, J., et al., poster F1-2158, 50th ICAAC, Sept. 2010). It has $MIC_{90}$ values against *Enterobacteriaceae* and non-fermenters, including those resistant to ≥3 antibiotic classes, of ≤2 µg/mL and $MIC_{90}$ values against all Gram-positive aerobes of ≤0.5 µg/mL. The use of a totally synthetic process provided an avenue to overcome tetracycline-specific resistance mechanisms and to avoid cross-resistance to known resistance mechanisms specific to all other antibiotic class (Clark, RB, et al., poster F1-2155, 50th ICAAC, Sept. 2010).

With funding from the Biomedical Advanced Research and Development Authority (BARDA; see Archives 2012 at www.tphase.com), eravacycline is currently being evaluated as a treatment for disease caused by biothreat pathogens (*Francisella tularensis, Yersinia pestis*, and *Bacillus anthracis*). As a first step, we wanted to ensure *in vitro* potency against clinical and environmental isolates of category A and B pathogens.

Eravacycline showed excellent efficacy in a Phase 2 trial for IV treatment of complicated intra-abdominal infections (cIAI) (93% and 100% efficacy in the microbiologically evaluable population at the test-of-cure visit for 1.5 mg/kg q24h and 1.0 mg/kg q12h respectively) (Solomkin, J, et al., Poster L1-1647a, 52nd ICAAC, Sept., 2012). An oral formulation is being developed for use in serious infections treated in the hospital that could highly benefit from an IV/oral step-down therapy. The successful treatment of serious infections caused by public health pathogens with eravacycline is expected to translate to success during use in an aerosolized biothreat attack.

## Methods

MIC values for eravacycline and control antibiotics (tetracycline, ciprofloxacin, penicillin, gentamicin, ceftazidime, doxycycline, and imipenem) were determined by broth microtiter dilution in 96-well plates according to the CLSI guidelines. Cation-adjusted Mueller-Hinton broth (CAMHB) or CAMHB plus 2% defined growth supplement (*F. tularensis* only) and quality control (QC) strains *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and/or *Pseudomonas aeruginosa* ATCC 27853, were used at all laboratories to validate test conditions and MIC values for control/comparator antibiotics. The MIC was defined as the lowest concentration of antibiotic/antimicrobial agent that completely inhibited growth of the organism in the microdilution wells. For calculation of $MIC_{50}$ and $MIC_{90}$ values, if there were two replicate values for an isolate and they were different, the higher value was reported. When there were more than two replicate values for an isolate, the mode is reported.

To determine the impact of specific tetracycline-resistance genes in isogenic *E. coli* strains, sequences encoding *tet*(A), *tet*(B), *tet*(K), *tet*(M), *tet*(X) and *E. coli* β-galactosidase (*lacZ*) as a negative control were amplified by PCR from clinical isolates confirmed by prior sequencing to have these tetracycline-resistance determinants. Genes were cloned into an L-arabinose inducible expression system without any affinity tags (pBAD-Myc-His, Invitrogen, Carlsbad, CA). Plasmids were transformed into *E. coli* DH10B cells (Invitrogen, Carlsbad, CA). Cloned inserts were verified by sequencing and comparing with reported sequences in GenBank (accession numbers: *tet*(A), AJ419171; *tet*(B), AP010961; *tet*(K), AJ888003; *tet*(M), X90939; *tet* (X), AB097942). In addition, the cloning of carbapenenase NDM-1 was constructed in a similar fashion and verified using GenBank HQ162469. Cells were grown in Mueller Hinton Broth containing ampicillin, 50 µg/ml, pre-induced for 30 minutes with 1% arabinose (*tet*(A), *tet*(B), *tet*(M), *tet*(X), $bla_{NDM-1}$) or 0.3% arabinose (tet(K)) at 30°C prior to use as inocula in MIC assays containing ampicillin, 50 µg/ml. Assays were incubated at 35°C as per CLSI guidelines.

The sources of public health pathogens were geographically diverse and primarily 2009-2012 isolates from US, Europe, and Asia. The sources of biothreat pathogens were also geographically diverse, with some isolates dating back to the 1930s.

## Results

**Table 2. Susceptibility of Biothreat Pathogens to Eravacycline and Comparators**

| Species | Compound | | | |
|---|---|---|---|---|
| ***B. anthracis* (35 isolates)** | **Eravacycline** | **Tetracycline** | **Ciprofloxacin** | **Penicillin** |
| **MIC Range (µg/mL)** | 0.004 – 0.016 | 0.016 – 0.06 | 0.016 – 0.06 | 0.016 – 0.25 |
| **$MIC_{50}$** | ≤0.016 | 0.03 | 0.03 | 0.03 |
| **$MIC_{90}$** | 0.016 | 0.03 | 0.06 | 0.12 |
| ***Y. pestis* (34 isolates)** | **Eravacycline** | **Tetracycline** | **Ciprofloxacin** | **Gentamicin** |
| **MIC Range (µg/mL)** | 0.016 - 0.25 | 0.12 - 4 | 0.008 - 0.06 | 0.12 - 2 |
| **$MIC_{50}$** | 0.06 | 1 | 0.016 | 0.5 |
| **$MIC_{90}$** | 0.12 | 2 | 0.03 | 1 |
| ***F. tularensis* (33 isolates)** | **Eravacycline** | **Tetracycline** | **Ciprofloxacin** | **Gentamicin** |
| **MIC Range (µg/mL)** | 0.016 - 1 | 0.06 - 2 | 0.016 - 0.5 | 0.016 - 2 |
| **$MIC_{50}$** | 0.12 | 0.25 | 0.03 | 0.25 |
| **$MIC_{90}$** | 0.5 | 0.5 | 0.12 | 0.5 |
| ***B. mallei* (30 isolates)** | **Eravacycline** | **Tetracycline** | **Ceftazidime[a]** | |
| **MIC Range (µg/mL)** | 0.008 - 1 | <0.03 - 1 | 0.5 - 8 | |
| **$MIC_{50}$** | 0.06 | 0.06 | 2 | |
| **$MIC_{90}$** | 0.25 | 0.12 | 4 | |
| ***B. pseudomallei*[b] (41 isolates)** | **Eravacycline** | **Doxycycline** | **Ceftazidime** | **Imipenem** |
| **MIC Range (µg/mL)** | ≤0.03 – 16 | 0.03 – 16 | 1 – 4 | 0.12 – 2 |
| **$MIC_{50}$** | 1 | 4 | 2 | 0.5 |
| **$MIC_{90}$** | 2 | 8 | 4 | 2 |

[a] 31 isolates; [b] includes clinical, environmental, and efflux pump mutants
All MIC values for eravacycline and control antibiotics against the quality control strains, *E. coli* ATCC 25922, *S. aureus* ATCC 29213, and *P. aeruginosa* ATCC 27853 were in the accepted ranges (data not shown).

**Figure 1. Eravacycline has In Vitro Potency against Isogenic *E. coli* strains Producing Tetracycline-Resistant Efflux, Ribosomal Protection Mechanisms, or Carbapenemase NDM-1**

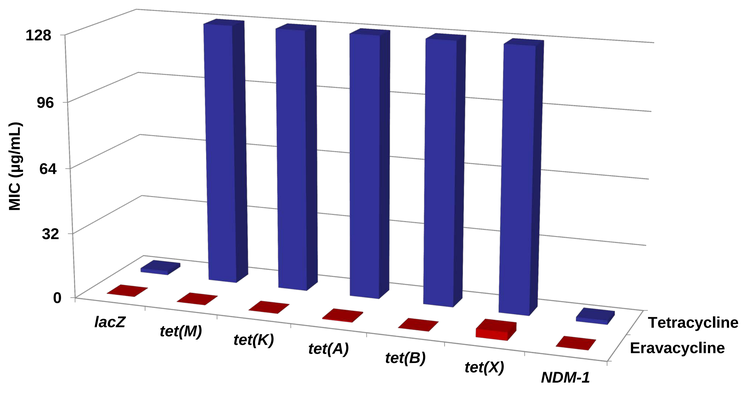


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**Table 3. MIC for Eravacycline and Comparators in *B. pseudomallei* Isolates Expressing Different Efflux Pumps[a]**

| Strain | Genotype | Efflux Pump(s) Expressed | Minimal Inhibitory Concentration (µg/mL) | | | |
|---|---|---|---|---|---|---|
| | | | Eravacycline | Ceftazidime | Doxycycline | Imipenem |
| **1026b** | Parental Strain | AmrAB-OprA, BpeAB-OprB | 0.25 | 2 | 0.5 - 1 | 0.25 |
| **Bp340** | 1026b Δ(*amrAB-oprA*) | BpeAB-OprB | ≤ 0.06 | 2 | 0.5 - 1 | 0.25 - 0.5 |
| **Bp227** | 1026b Δ(*bpeAB-oprB*) | AmrAB-OprA | 0.25 | 1 - 2 | 0.25 | 0.25 |
| **Bp207** | 1026b Δ(*amrAB-oprA*) Δ(*bpeAB-oprB*) | None Known | ≤ 0.06 | 2 | ≤ 0.03 | 0.5 |
| **Bp58** | 1026b Δ*bpeR* Δ(*amrAB-oprA*) | BpeAB-OprB | 0.25 | 4 | 4 | 0.25 - 0.5 |
| **Bp282** | Bp207 *bpeT* | BpeEF-OprC | 2 | 1 - 2 | 8 | 0.12 |
| **Bp320** | Bp282 Δ(*bpeEF-oprC*) | None Known | ≤ 0.06 | 1 | ≤ 0.06 | 0.5 |

[a] Strains from laboratory of Herbert Schweizer

**Table 4. The Susceptibility of Key Gram-negative Aerobic Bacteria to Eravacycline and Comparators**

| Organism | MIC Parameter | ERA | TET | TGC | CARB[a] | AG[b] | 3rd GC[c] | FQ[d] |
|---|---|---|---|---|---|---|---|---|
| *Acinetobacter baumannii* | $MIC_{50/90}$ | 0.25/1 | 8/>32 | 0.5/4 | 2/32 | 8/>32 | >16/>32 | >2/>2 |
| | Range | (0.016-8) | (≤0.25->32) | (≤0.016-8) | (0.13->32) | (≤0.25->32) | (0.13->64) | (0.016->32) |
| | n | 188 | 159 | 188 | 188 | 188 | 128 | 188 |
| *Citrobacter freundii* | $MIC_{50/90}$ | 0.25/1 | 1/>8 | 0.5/2 | 0.5/2 | 0.5/>8 | 1/32 | <0.25/>2 |
| | Range | (0.06-2) | (0.5->8) | (0.12-8) | (0.004->32) | (≤0.25->32) | (0.06->64) | (0.008->4) |
| | n | 115 | 65 | 115 | 103 | 115 | 115 | 115 |
| *Citrobacter freundii* 3rd Gen Ceph I/R[e] | $MIC_{50/90}$ | 0.5/1 | 2/8 | 1/2 | 1/16 | 0.5/>32 | >16/>64 | 1/>4 |
| | Range | (0.13-2) | (1->8) | (0.25-8) | (0.25->32) | (≤0.25->32) | (4->64) | (0.016->4) |
| | n | 42 | 16 | 42 | 39 | 42 | 42 | 42 |
| *Enterobacter cloacae* | $MIC_{50/90}$ | 0.5/2 | 2/>8 | 0.5/2 | 0.5/2 | 0.5/8 | 2/>64 | ≤0.25/>4 |
| | Range | (0.03-4) | (0.5->32) | (0.06-8) | (0.03->32) | (≤0.25->32) | (0.03->64) | (0.008->32) |
| | n | 270 | 218 | 270 | 270 | 270 | 246 | 270 |
| *Enterobacter cloacae* 3rd Gen Ceph I/R | $MIC_{50/90}$ | 0.5/2 | 4/>8 | 1/4 | 0.5/4 | 0.5/16 | >32/>64 | 0.25/>4 |
| | Range | (0.03-4) | (1->32) | (0.06-8) | (0.03->32) | (≤0.25->32) | (2->64) | (0.008->32) |
| | n | 122 | 93 | 122 | 122 | 122 | 122 | 122 |
| *Escherichia coli* | $MIC_{50/90}$ | 0.25/0.5 | 4/>32 | 0.25/0.5 | 0.25/0.5 | 1/>8 | ≤0.5/>32 | ≤0.25/>4 |
| | Range | (≤0.016-4) | (0.25->64) | (0.06->8) | (≤0.002->32) | (≤0.25->32) | (≤0.016->64) | (≤0.25->32) |
| | n | 445 | 390 | 445 | 445 | 445 | 445 | 445 |
| *Escherichia coli* 3rd Gen Ceph I/R | $MIC_{50/90}$ | 0.25/0.5 | >8/>32 | 0.25/1 | 0.06/0.5 | 2/>32 | >32/>64 | >4/32 |
| | Range | (≤0.016-1) | (0.5->32) | (0.03->8) | (≤1->32) | (≤0.25->32) | (2->64) | (≤0.25->32) |
| | n | 127 | 113 | 127 | 127 | 127 | 127 | 127 |
| *Klebsiella pneumoniae* | $MIC_{50/90}$ | 0.5/2 | 4/>32 | 0.5/2 | 0.25/>8 | 0.5/>8 | 8/>32 | 0.5/>32 |
| | Range | (0.03-16) | (≤0.25->64) | (0.13-16) | (≤0.002->32) | (≤0.25->32) | (≤0.016->64) | (≤0.25->64) |
| | n | 394 | 339 | 394 | 394 | 223 | 394 | 394 |
| *Klebsiella pneumoniae* 3rd Gen Ceph I/R | $MIC_{50/90}$ | 0.5/2 | 8/>32 | 1/4 | 1/16 | 4/16 | >32/64 | >4/>32 |
| | Range | (0.03-16) | (1->64) | (0.13-16) | (≤1->32) | (0.25->32) | (4->64) | (≤0.25->64) |
| | n | 210 | 187 | 210 | 210 | 82 | 210 | 210 |
| *Klebsiella pneumoniae* Carbapenem I/R[f] | $MIC_{50/90}$ | 0.5/2 | 8/>32 | 1/2 | >8/>32 | 4/>8 | >32/>32 | >4/>32 |
| | Range | (0.13-16) | (1->32) | (0.25-16) | (2->32) | (0.25->32) | (1->64) | (0.06->64) |
| | n | 90 | 81 | 90 | 90 | 50 | 90 | 90 |
| *Proteus mirabilis* | $MIC_{50/90}$ | 1/2 | >8/32 | 4/8 | 2/4 | 1/>8 | ≤0.5/1 | ≤0.25/>4 |
| | Range | (0.25-16) | (2->64) | (0.5-16) | (0.008->32) | (≤0.25->64) | (≤0.016->64) | (0.016->64) |
| | n | 166 | 111 | 166 | 166 | 166 | 166 | 166 |
| *Providencia stuartii* | $MIC_{50/90}$ | 1/2 | >8/>8 | 2/4 | 2/4 | 4/32 | <0.5/16 | >2/>4 |
| | Range | (0.13-8) | (<0.25->8) | (0.06-16) | (0.25-16) | (<0.25->32) | (≤0.016->64) | (0.016->4) |
| | n | 101 | 51 | 101 | 101 | 101 | 101 | 101 |
| *Pseudomonas aeruginosa* | $MIC_{50/90}$ | 8/32 | >8/64 | 16/32 | 2/>8 | 2/>8 | >16/>32 | 1/>4 |
| | Range | (1->32) | (8-64) | (1->32) | (0.12->32) | (0.12->32) | (1->64) | (0.06->32) |
| | n | 145 | 93 | 145 | 145 | 145 | 145 | 145 |

[a]carbapenem: imipenem, carbapenem, or ertapenem; [b]aminoglycoside: gentamicin or tobramycin; [c]3rd generation cephalosporin: ceftazidime, cefotaxime or ceftriaxone; [d]fluoroquinolone: levofloxacin or ciprofloxacin; [e]3rd Gen Ceph I/R isolates were defined as ceftazidime MIC ≥8 µg/mL, cefotaxime MIC ≥2 µg/mL, or ceftriaxone, MIC ≥2 µg/mL; [f]carbapenem I/R isolates were defined as imipenem MIC ≥2 µg/mL, meropenem MIC ≥2 µg/mL, or ertapenem, MIC ≥1 µg/mL

## Conclusions

- Eravacycline demonstrated *in vitro* potency against all five Category A and B pathogens with $MIC_{90}$ values of 0.016 – 2 µg/mL
- Eravacycline was effluxed by *B. pseudomallei* BpeEF-OprC and, to a lesser extent, AmrAB-OprA and BpeAB-OprB efflux pumps, but to a lesser degree than other tetracyclines
- These results support eravacycline as a broad-spectrum antibiotic with activity against biothreat pathogens and support future *in vivo* efficacy studies in relevant animal models of infection
- If efficacious in future studies under the Animal Rule, eravacycline could be used as an for empiric therapy during an aerosolized biothreat attack
- Broad-spectrum potency and efficacy demonstrated in a phase 2 trial for cIAI support the development of eravacycline as an important new antibiotic the treatment of serious infections treated in the hospital