Abstract #710

In vitro activity and performance of available susceptibility testing methods for eravacycline against carbapenem-resistant Enterobacteriaceae

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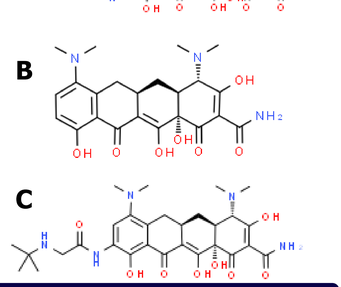
INTRODUCTION

- Carbapenem-resistant *Enterobacteriaceae* (CRE) infections are widespread in US hospitals and associated with high rates of morbidity and mortality
- The primary mechanism of carbapenem resistance is production of *Klebsiella pneumoniae* carbapenemase (KPC) enzymes
- Avibactam is a new DBO β-lactamase inhibitor that inhibits KPCs; in combination with ceftazidime, the combination demonstrates activity against genetically-diverse, KPC-producing *K. pneumoniae* isolates with ESBL and *ompK36* porin mutations
- In our clinical experience, treatment with ceftazidime-avibactam (CZA) results in clinical success rates of 55% and is well tolerated; however, microbiologic failures occur in 32% of patients and ceftazidime-avibactam resistance emerged in 10%
  - 14% of patients infected by KPC-producing *K. pneumoniae* developed resistance
  - Resistance was mediated by mutations in the Ω-loop of KPC-3
- Eravacycline (ERV) is a recently-approved, fully synthetic fluorocycline agent that demonstrates broad in vitro activity against multidrug-resistant pathogens
- Resistance to eravacycline is not mediated by β-lactamases or changes in outer membrane porins and is therefore predicted to maintain activity against CZA resistant isolates
- Our objectives were to:
  - 1) Compare the minimum inhibitory concentrations (MICs) of eravacycline and other available tetracycline agents against genetically-diverse CRE isolates
  - 2) Determine if combinations of ERV with ceftazidime-avibactam or meropenem-vaborbactam were synergistic

Figure 1: Chemical structures of eravacycline (A), minocycline (B), and tigecycline (C)



METHODS

Isolates:

- CRE isolates from UPMC were identified by non-susceptible carbapenem MICs
- Of the 148 isolates tested, 92 were *K. pneumoniae*, 20 *E. cloacae*, 11 *C. freundii*, 10 *E. coli*, 7 *K. aerogenes*, 4 *K. oxytoca*, and 4 *S. marcescens*
- 72% isolates were KPC positive and 19% of isolates were CZA resistant

Susceptibility Testing:

- Eravacycline, minocycline, and tigecycline MICs were measured by broth microdilution (BMD) using CLSI methods
- Eravacycline susceptibility was also measured by MIC test strip (MTS; Liofilchem)

Detection of and characterization of β-lactamases:

- β-lactamase genes were detected by PCR; variants identified by DNA sequencing

Time kill analysis:

- Flasks containing a high inoculum of $1x10^8$ cfu/mL were grown in the presence of 1x-MIC and 4x-MIC eravacycline alone, 1x-MIC ceftazidime-avibactam, and 1x-MIC meropenem-vaborbactam
- Synergy was investigated using combinations of eravacycline at 1x-MIC with 1x-MIC of ceftazidime-avibactam or 1x-MIC of meropenem-vaborbactam
- Bacterial burdens were determined over 24 hours of incubation at 37°C

RESULTS

Eravacycline, Minocycline and Tigecycline MICs

- By BMD, the median ERV MIC was 0.5 µg/mL; range 0.06-8 µg/mL
  - 55% of CRE isolates were susceptible to ERV (MIC ≤0.5 µg/mL)
- Eravacycline MIC_{50} and MIC_{90} were 0.5 and 2 µg/mL, respectively
- By MTS, the median ERV MIC was 0.5 µg/mL; range 0.19-0.5 µg/mL
  - 51% of CRE isolates were susceptible to ERV (MIC ≤0.5 µg/mL)

Figure 1: Correlation of Eravacycline MICs determined by BMD and MTS

Eravacycline BMD MICs \ Eravacycline MTS MICs	0	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16
16										1	
8								2	1		
4						3	4	10	5		
2						14	17	10			
1					5	27	12	2			
0.5					5	16	5	3		1	
0.25					2	1					
0.12					2						
0.06											
≤0.03											
0											

Note. Eravacycline susceptibility breakpoints are marked by the dotted horizontal and vertical lines; isolates with discrepant categorical interpretations are shaded in grey.

Figure 2: MIC Distribution for Eravacycline, Minocycline, and Tigecycline

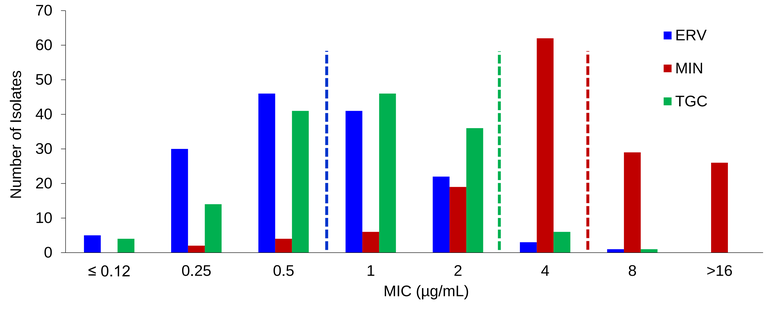
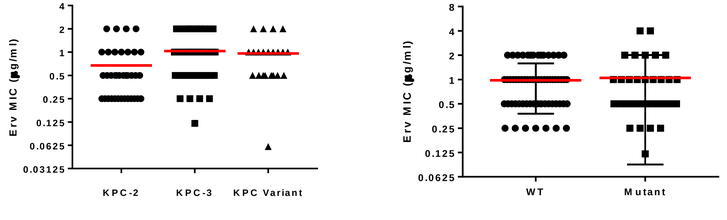
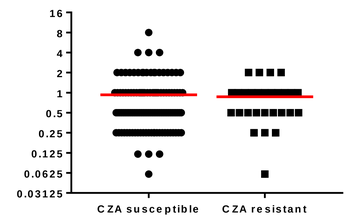


Figure 3: Eravacycline MICs by KPC and *ompK36* genotype



- Median eravacycline MICs were lower for KPC-2-producing isolates as compared to isolates harboring KPC-3 or KPC-variants (P=0.0018, P=0.0125, respectively)
- There was no difference in eravacycline MICs between wild type and mutant *ompK36*

Figure 4: Eravacycline MICs by ceftazidime-avibactam susceptibility

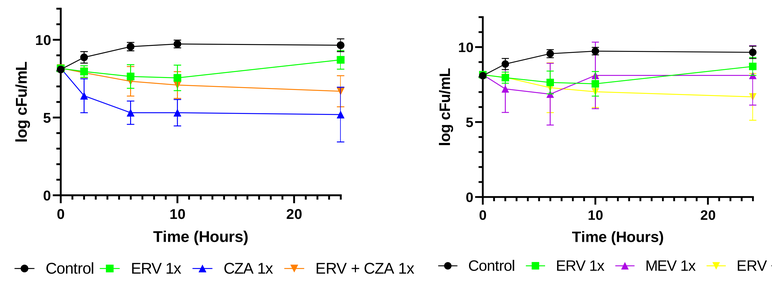


- Eravacycline MICs did not vary by ceftazidime-avibactam susceptibility

Eravacycline Synergy Testing

Figure 5: Eravacycline shows synergy with meropenem-vaborbactam

- Eight KPC-producing *K. pneumoniae* were tested by time-kill analysis
- Eravacycline was bactericidal against 50% of isolates at 4x-MIC (> 3-log_{10} cFu/mL kill)
  - Median log-kills at 1x and 4x MIC were -0.77 and -3.01cFu/mL, respectively
- In combination with ceftazidime-avibactam or meropenem-vaborbactam, eravacycline was synergistic against 25% and 75% of isolates, respectively (>1-log kill in combo)



CONCLUSIONS

- **Median eravacycline MICs were one 2-fold dilution lower than tigecycline; however, rates of susceptibility were dissimilar due to differences in the FDA-approved clinical breakpoints**
- **Eravacycline MICs by MTS showed high essential agreement, but lower rates of categorical agreement compared to BMD MICs**
- **Eravacycline MICs were not significantly influenced by KPC subtype or the presence of *ompK36* porin mutations in *K. pneumoniae* isolates**
- **Eravacycline shows synergy against most KPC-producing *K. pneumoniae* isolates in combination with meropenem-vaborbactam, but not with ceftazidime-avibactam; the combination of eravacycline plus meropenem-vaborbactam merits further investigation**

ACKNOWLEDGEMENTS

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