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 DBD 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 D-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

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 1x10⁸ cfu/mL were grown in the presence of 1xMIC and 4xMIC eravacycline alone, 1xMIC ceftazidime-avibactam, and 1xMIC meropenem-vaborbactam
  - Synergy was investigated using combinations of eravacycline at 1xMIC with 1xMIC of ceftazidime-avibactam or 1xMIC 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 MIC₅₀ and MIC₉₀ were 0.5 and 4 µg/mL, respectively

Synergistic MICs by BMD and MTS

ERV MICs by ceftazidime-vaborbactam susceptibility

Eravacycline Synergy Testing

- Eight KPC-producing K. pneumoniae were tested by time-kill analysis
- Eravacycline was bactericidal against 50% of isolates at 4xMIC (3-log₉ cfu/mL kill)
- Median log-kills at 1x and 4x MIC were -0.77 and -3.01µF/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

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