The Impact of Varied Test Conditions on the In Vitro Activity of Tebipenem (SPR859) and Meropenem Against Urinary Pathogens, Including Those Expressing Extended-Spectrum Beta-lactamases (ESBL)

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ABSTRACT

Background: Resistance to antibiotics among Enterobacteriaceae (EB) has increased. Tebipenem (SPR859) is the only active extended-spectrum b-lactam antibiotic (ESBL) among EB, and is under development for treatment of complicated urinary tract infections (cUTIs). It is important to understand the impact of a variety of conditions on the potency of SPR859 and meropenem (MEMP) against EB pathogens, including those expressing ESBL.

Methods: MICs from broth microdilution were tested under standard conditions (CLSI M7-A10). One susceptible and one extended-spectrum beta-lactamase (ESBL) producing isolate each of ESBL (Klebsiella pneumoniae 857; AmpC ESBL (PM)) was tested. MICs were determined under different conditions such as media pH of 5-8, in CLO2, added divalent cation concentration (size of 10-100 µM), CLO2, and pH. The impact of pooled heat-inactivated human urine on MIC values was measured.

Results: MICs under standard conditions (CLSI M7-A10) were different for both isolates and were lower for PM than for MEMP. MICs were based on broth microdilution in accordance with CLSI M7-A10 under standard and non-standard conditions in parallel. Testing conditions were modified as follows: media pH adjusted to 5, 6, and 8 (standard = 7.2-7.4), and with added divalent cation concentration (standard = +10-12.5 mg/mL, 20-25 mg CaCl2/L, and 25 mg MgCl2/L), high and low concentrations (standard = +5x106 CFU/mL, inoculum size (standard = +5x105 CFU/mL), the impact of pooled heat-inactivated human urine on MIC values was measured.

Conclusions: The impact of pH and human urine on MIC values was not significant, but a higher percentage of SPR859 MICs were lower than for MEMP. Overall, there was no impact of the added cation concentration, and a non-significant decrease in MICs was noted.

INTRODUCTION

- Resistance among Enterobacteriaceae, including resistance meditated by ESBLs, has increased.
- Tebipenem (SPR859) is an orally available carbapenem approved in Japan for respiratory tract infections in children; tebipenem is currently under development in the US for complicated UTI.
- Prior to widespread susceptibility testing of tebipenem, it is important to understand the impact of variations to the standard CLSI testing methods on broth microdilution.

- Standard susceptibility test parameters that can affect MIC test results when varied include: medium pH concentration, supplementation, inoculum size, incubation duration, and atmosphere.

- In addition, it is important to understand the effect of bodily fluids (human urine and serum) on its activity in vitro.

- The purpose of the current study was to determine the impact of testing under non-standard conditions on the in vitro activity of tebipenem and meropenem against UTI pathogens, including both ESBL and non-ESBL Enterobacteriaceae.

METHODS

- Tests included organisms a non-ESBL and ESBL isolate of E. coli, K. pneumoniae, and P. mirabilis.
- All tests included broth microdilution in accordance with CLSI M7-A10 under standard and non-standard conditions in parallel.
- Testing conditions were modified as follows: media pH adjusted to 5, 6, and 8 (standard = 7.2-7.4), and with added divalent cation concentration (standard = +10-12.5 mg/mL, 20-25 mg CaCl2/L, and 25 mg MgCl2/L), high and low concentrations (standard = +5x106 CFU/mL, inoculum size (standard = +5x105 CFU/mL), the impact of pooled heat-inactivated human urine on MIC values was measured.

RESULTS

Organism  Compound  Activity Under Standard Conditionsa Impact of Incubation Time and Atmosphere Impact of Human/Mouse Serum and Human Urine

Medicant Inhibition Impact of 5% Inhibition Impact of 5% Inhibition 5% Human 5% Human Human

MICs (µg/mL) of tebipenem and meropenem under standard and non-standard conditions (fold-change in MIC relative to standard conditions shown in parenthesis)

<table>
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<tr>
<th>Organism</th>
<th>Compound</th>
<th>Impact of Media pH:</th>
<th>Impact of Final Inoculum (CFU/mL):</th>
<th>25 Ca⁺⁺ (µg/mL)</th>
<th>38 Ca⁺⁺ (µg/mL)</th>
<th>50 Ca⁺⁺ (µg/mL)</th>
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<td>Tebipenem</td>
<td>0.015 (4)</td>
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**Notes:**
- The MIC of tebipenem and meropenem is shown in red font; instances where the MIC decreased by ≥4-fold are shown in green font.
- Under standard conditions, both tebipenem and meropenem had similar activity, and the observed activity was not impacted by ESBL phenotype.
- The activity of tebipenem and meropenem decreased with increased inoculum size and for isolates at acidic pH; tebipenem had 16- to 32-fold less activity when tested in 50% mouse serum, whereas there was no notable decrease in activity when tested in 50% human serum, 10% mouse serum, of 100% human urine with the exception of P. mirabilis where the activity of both tebipenem and meropenem decreased 4 folds.
- There was no little to no change in the activity of tebipenem and meropenem in the presence of pooled heat inactivated cations, with prolonged incubation time, or when incubated in CO2.

**CONCLUSIONS**

- The activity of tebipenem and meropenem was largely stable by both microbiology when testing under non-standard conditions, with the exception of low pH and increased inoculum size which resulted in decreased activity.
- Tebipenem activity was reduced when tested with high concentrations of mouse serum (50%) but was not notably affected when tested at a lower concentration of mouse serum (10%); human serum (10% and 50%) and in human urine (100%) had no impact on the activity of tebipenem and meropenem. Untested concentration of P. mirabilis where the activity of both tebipenem and meropenem decreased 4 folds.
- The clinical significance of, if any, of decreased activity as observed with mouse serum and human urine (P. mirabilis only) has yet to be determined.
- The impact of low mald pH and increased inoculum size highlights the need for adherence to CLSI guidelines during the broth microdilution testing of both tebipenem and meropenem.