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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 antibacterials among Enterobacteriaceae (EB) has increased. Tebipenem (SPR859) is the microbiologically active form of tebipenem-pivoxil (SPR994), an oral carbapenem, and is under development for treatment of complicated urinary tract infections (cUTI). It is important to understand the impact of a variety of conditions on the perceived in vitro activity of new agents. In this study, standard in vitro test parameters were altered to evaluate their impact on the potency of SPR859 and meropenem (MEM) against UTI pathogens.

Methods: Broth microdilution (BMD) susceptibility testing of SPR859 and MEM was conducted under standard conditions (CLSI M7-A10). One susceptible and one extended-spectrum beta-lactamase (ESBL) producing isolate each of Escherichia coli (EC), Klebsiella pneumoniae (KP), and Proteus mirabilis (PM) was tested. MICs were determined under different conditions such as media pH of 5 - 8, incubation in CO2, altered divalent cation concentration, inoculum size of ~104 – 107 CFU/mL, and prolonged incubation. The impact of testing in pooled normal human urine and heat-inactiveated serum (human and mouse) was also evaluated.

Results: Under standard conditions, SPR859 and MEM both had potent MICs against non-ESBL and ESBL EC (0.015 µg/mL), KP (0.03 – 0.12 µg/mL), and PM (0.06 – 0.12 µg/mL non-ESBL; 0.25 – 1 µg/mL ESBL). SPR859 and MEM potency decreased when testing with increased inoculum size (>5 x 106 CFU/mL) and for SPR859 when testing in 10% and 50% mouse serum (MICs increased > 4-fold). SPR859 and MEM MICs also increased > 4-fold in medium with low pH for some isolates (3/6 isolates). The remaining conditions (prolonged incubation, altered divalent cation concentration, low inoculum size, pH 8, incubation in CO2, and testing with human serum) had little to no impact on SPR859 and MEM activity (MICs were identical or within 2-fold of those under standard conditions). There was also no impact of testing in urine on the potency of SPR859 and MEM against EC and KP, but for PM MICs of SPR859 and MEM were 4-fold higher in urine than standard media.

Conclusions: The potency of SPR859 and MEM against susceptible and ESBL-positive EB by BMD is similarly stable to variation in test conditions excluding increased inoculum size and, in some instances, low pH where decreased potency was observed by MIC. The clinical significance, if any, of the decreased potency in mouse serum and against PM in urine remains to be determined through further clinical development.

INTRODUCTION

- Resistance among Enterobacteriaceae, including resistance mediated 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 *in vitro* activity.
- Standard susceptibility test parameters that can affect MIC test results when varied include:
  - Medium pH, cation 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 *in vitro* activity of new agents.
- The purpose of the current study was to determine the effect of testing under non-standard conditions on the *in vitro* activity of tebipenem and meropenem against UTI pathogens, including both non-ESBL and ESBL isolates.

METHODS

- Test organisms included a non-ESBL and ESBL isolate of *E. coli*, *K. pneumoniae*, and *P. mirabilis*.
- Test agents included tebipenem and meropenem.
- MICs were determined by broth microdilution in accordance with CLSI M7-A10 under standard and non-standard conditions in parallel.
- Testing conditions were modified as follows:
  - Medium pH adjusted to 5, 6, and 8 (standard = 7.2-7.4).
  - Media with altered divalent cation concentration (standard = 10-12.5 mg/L Mg^{2+}, 20-25 mg/L Ca^{2+}).
  - Low (~5 x 10^4 CFU/mL) and high (~5 x 10^{6-7} CFU/mL) inoculum size (standard = (~5 x 10^5 CFU/mL).
  - Prolonged incubation and altered atmosphere (6.5% CO_2).
  - Media with pooled heat-inactivated human serum (10% and 50%; v/v), heat-inactivated C57BL6 mouse serum (10% and 50%; v/v), and testing in 100% pooled human urine (pH adjusted to 7.2 – 7.4 to match CAMHB).

RESULTS

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

Organism	Compound	Activity Under Standard Conditions[a]	Impact of Media pH: 5.0	Impact of Media pH: 6.0	Impact of Media pH: 8.0	Impact of Final Inoculum (CFU/mL): ~5.0 x 10^4	Impact of Final Inoculum (CFU/mL): ~5.0 x 10^6	Impact of Final Inoculum (CFU/mL): ~5.0 x 10^7	Impact of Divalent Cations (mg/L): 25 Ca^{++} 12.5 Mg^{++}	Impact of Divalent Cations (mg/L): 5 Ca^{++} 5 Mg^{++}	Impact of Divalent Cations (mg/L): 25 Ca^{++} 5 Mg^{++}	Impact of Divalent Cations (mg/L): 5 Ca^{++} 12.5 Mg^{++}	Impact of Divalent Cations (mg/L): 50 Ca^{++} 25 Mg^{++}
E. coli **ATCC 25922 (non-ESBL)**	**Tebipenem**	0.015	0.06 (4)	0.03 (2)	0.03 (2)	0.015 (0)	0.06 (4)	>32 (>2048)	0.015 (0)	0.015 (0)	0.015 (0)	0.015 (0)	0.015 (0)
	Meropenem	0.015 – 0.03	0.12 (8)	0.06 (4)	0.06 (4)	0.03 (2)	0.06 (4)	>32 (>2048)	0.03 (0)	0.03 (0)	0.03 (0)	0.03 (0)	0.03 (0)
E. coli **ATCC 35218 (ESBL)**	**Tebipenem**	0.015	0.03 (2)	0.015 (0)	0.015 (0)	0.015 (0)	0.03 (2)	>32 (>2048)	0.015 (0)	0.015 (0)	0.015 (0)	0.015 (0)	0.015 (0)
	Meropenem	0.015 – 0.03	0.06 (4)	0.03 (2)	0.06 (4)	0.015 (0)	0.03 (2)	>32 (>2048)	0.03 (0)	0.03 (0)	0.03 (0)	0.03 (0)	0.03 (0)
K. pneumoniae **ATCC 43816 (non-ESBL)**	**Tebipenem**	0.03	0.03 (0)	0.03 (0)	0.03 (0)	0.03 (0)	0.12 (4)	>32 (>1024)	0.03 (0)	0.03 (0)	0.03 (0)	0.03 (0)	0.03 (0)
	Meropenem	0.03 – 0.06	0.06 (2)	0.06 (2)	0.12 (4)	0.03 (0)	0.06 (2)	>32 (>1024)	0.03 (-2)	0.03 (-2)	0.03 (-2)	0.03 (-2)	0.06 (0)
K. pneumoniae **ATCC 700603 (ESBL)**	**Tebipenem**	0.06 – 0.12	0.06 (-2)	0.06 (-2)	0.12 (0)	0.06 (-2)	0.25 (2)	>32 (>256)	0.06 (-2)	0.06 (-2)	0.06 (-2)	0.06 (-2)	0.03 (-4)
	Meropenem	0.03 – 0.06	0.06 (0)	0.06 (0)	0.06 (0)	0.03 (-2)	0.25 (4)	>32 (>512)	0.03 (0)	0.03 (0)	0.03 (0)	0.03 (0)	0.03 (0)
P. mirabilis **ATCC 43071 (non-ESBL)**	**Tebipenem**	0.12	1 (8)	1 (8)	0.12 (0)	0.03 (-4)	8 (64)	>32 (>256)	0.12 (0)	0.12 (0)	0.12 (0)	0.12 (0)	0.12 (0)
	Meropenem	0.06	0.5 (8)	0.5 (8)	0.06 (0)	0.03 (-2)	2 (32)	>32 (>512)	0.06 (0)	0.06 (0)	0.06 (0)	0.06 (0)	0.12 (2)
P. mirabilis **MMX 6343 (ESBL)**	**Tebipenem**	0.5 – 1	1 (0)	1 (0)	0.5 (-2)	0.12 (-8)	8 (8)	>32 (>32)	0.5 (0)	0.5 (0)	0.5 (0)	0.5 (0)	0.5 (0)
	Meropenem	0.12 – 0.25	1 (4)	1 (4)	0.12 (-2)	0.03 (-4)	1 (4)	>32 (>128)	0.12 (0)	0.12 (0)	0.12 (0)	0.12 (0)	0.12 (0)

Organism	Compound	Activity Under Standard Conditions[a]	Impact of Incubation Time and Atmosphere: 24h incubation	Impact of Incubation Time and Atmosphere: 48h incubation	Impact of Incubation Time and Atmosphere: 6.5% CO_2	Impact of Human/Mouse Serum and Human Urine: 50% Human Serum	10% Human Serum	50% Mouse Serum	10% Mouse Serum	100% Human Urine[b]
E. coli **ATCC 25922 (non-ESBL)**	**Tebipenem**	0.015	0.015 (0)	0.06 (4)	0.015 (0)	0.03 (2)	0.03 (2)	0.5 (32)	0.06 (4)	0.015 (0)
	Meropenem	0.015 – 0.03	0.015 (0)	0.015 (0)	0.015 (0)	0.03 (0)	0.03 (0)	0.03 (0)	0.03 (2)	0.03 (2)
E. coli **ATCC 35218 (ESBL)**	**Tebipenem**	0.015	0.015 (0)	0.015 (0)	0.015 (0)	0.03 (2)	0.03 (2)	0.5 (32)	0.03 (2)	0.015 (0)
	Meropenem	0.015 – 0.03	0.015 (0)	0.015 (0)	0.015 (0)	0.03 (0)	0.03 (0)	0.03 (2)	0.015 (0)	0.015 (0)
K. pneumoniae **ATCC 43816 (non-ESBL)**	**Tebipenem**	0.03	0.03 (0)	0.03 (0)	0.03 (0)	0.06 (2)	0.03 (0)	1 (32)	0.06 (2)	0.03 (0)
	Meropenem	0.03 – 0.06	0.03 (0)	0.06 (2)	0.06 (2)	0.03 (-2)	0.03 (-2)	0.03 (0)	0.03 (0)	0.03 (0)
K. pneumoniae **ATCC 700603 (ESBL)**	**Tebipenem**	0.06 – 0.12	0.06 (-2)	0.12 (0)	0.12 (0)	0.12 (0)	0.06 (-2)	2 (32)	0.12 (2)	0.06 (0)
	Meropenem	0.03 – 0.06	0.06 (0)	0.06 (0)	0.06 (0)	0.06 (2)	0.03 (0)	0.12 (4)	0.03 (0)	0.06 (2)
P. mirabilis **ATCC 43071 (non-ESBL)**	**Tebipenem**	0.12	0.12 (0)	0.12 (0)	0.25 (2)	0.25 (2)	0.25 (2)	2 (16)	0.25 (2)	0.5 (4)
	Meropenem	0.06	0.06 (0)	0.12 (2)	0.12 (2)	0.12 (2)	0.12 (2)	0.06 (0)	0.06 (0)	0.25 (4)
P. mirabilis **MMX 6343 (ESBL)**	**Tebipenem**	0.5 – 1	1 (0)	1 (0)	1 (0)	0.5 (0)	1 (2)	4 (8)	1 (2)	2 (4)
	Meropenem	0.12 – 0.25	0.25 (0)	0.25 (0)	0.5 (2)	0.12 (0)	0.12 (0)	0.25 (2)	0.5 (4)	0.5 (4)

[a]MIC range from four independent inocula [b]Urine with pH adjusted to 7.35

- Instances where the MIC increased ≥4-fold are shown in red font; instances where the MIC decreased ≥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 both tebipenem and meropenem decreased with increasing inoculum size and for select isolates at acidic pH.
- Tebipenem had 16- to 32-fold less activity when tested in 50% mouse serum. However, there was no notable change in activity when tested in 50% or 10% human serum, 10% mouse serum, or 100% human urine with the exception of *P. mirabilis* where the activity of both tebipenem and meropenem decreased 4-fold.
- There was little to no change in the activity of tebipenem and meropenem in the presence of varied divalent cations, with prolonged incubation time, or when incubated in CO_2.

CONCLUSIONS

- The activity of tebipenem and meropenem was largely stable by broth microdilution 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 testing 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%) excluding *P. mirabilis* where tebipenem and meropenem activity decreased 4-fold.
- The clinical significance, 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 medium pH and increased inoculum size highlights the need for adherence to CLSI guidelines during the broth microdilution testing of both tebipenem and meropenem.