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In vitro Activity of Tebipenem against Relevant Clinical Isolates in the Presence of Pulmonary Surfactant

S.J.R. Arends[1], A.L. Klauer[1], N. Cotroneo[2], I.A. Critchley[2], R.E. Mendes[1]

[1]JMI Laboratories, North Liberty, Iowa, USA, [2]Spero Therapeutics, Cambridge, MA, USA

Introduction

- Tebipenem is an orally administered broad-spectrum carbapenem antibiotic.
- Tebipenem has recently completed a Phase 3 clinical trial evaluating its safety and efficacy for the treatment of complicated urinary tract infection and acute pyelonephritis.
- The objective of this study was to evaluate the effect of bovine pulmonary surfactant (BPS) on the *in vitro* activity of tebipenem and ertapenem against a recent collection of clinical isolates to support the feasibility for tebipenem use in the treatment of bacterial pneumonia.

Materials and Methods

- In this study, 10 clinical isolates were recovered from patients with documented infections in 2018 as a part of the SENTRY Antimicrobial Surveillance Program.
- These isolates were sent to a central monitoring laboratory (JMI Laboratories, North Liberty, Iowa, USA) and included one isolate from each of the following species:
  - *Escherichia coli*
  - *Enterobacter cloacae*
  - *Klebsiella pneumoniae*
  - *Citrobacter freundii*
  - Methicillin-susceptible *Staphylococcus aureus*
  - *Moraxella catarrhalis*
  - *Streptococcus pneumoniae*
  - *Streptococcus pyogenes*
  - *Haemophilus influenzae*
  - *Haemophilus parainfluenzae*
- Bacterial species were identified by JMI Laboratories using standard microbiology methods and matrix-assisted laser desorption ionization-time of flight mass spectrometry (Bruker Daltonics, Bremen, Germany).
- Isolates were tested for antimicrobial susceptibility by the broth microdilution method appropriate for the organism or group of organisms being tested according to the Clinical and Laboratory Standards Institute (CLSI) M07 (2018) and M45 (2015) guidelines.
- JMI Laboratories produced frozen-form 96-well panels with cation-adjusted Mueller-Hinton broth (CAMHB) as the testing medium. Exceptions were CAMHB supplemented with 2.5–5% lysed horse blood for testing streptococci and *Haemophilus* Test Medium broth for *Haemophilus* spp.
- Panels were supplemented with BPS (Infasurf from ONY Biotech Lot # 149805190) to final concentrations of 1%, 5%, and 10% by volume.
- MIC values for daptomycin were obtained against *S. aureus* ATCC 29213 in the presence and absence of BPS as a positive control to monitor BPS effects in CAMHB, which contained 50 mg/L of calcium and 10–12.5 mg/L of magnesium per CLSI guidelines.
- As BPS can introduce considerable cloudiness to the MIC testing media, antimicrobial growth inhibition also was evaluated using the colorimetric metabolic indicator resazurin when necessary. After visual MIC value determinations, 10 µL of a resazurin solution (BioRad; catalog #BUF012B; 6.75–7.0 mg/mL in H.O) was added to the test wells in each panel. The panels incubated for an additional 3 hours at 35°C in ambient atmosphere, followed by a second MIC determination.
- MIC values in the presence of BPS with results >2-fold higher than those results obtained without BPS were considered different.
- All categorical interpretations used CLSI M100 (2021), where published.

Results

- BPS effect on activity of tebipenem
  - The 10 isolates displayed tebipenem MIC values ranging from ≤0.004 to 0.06 mg/L in media without BPS (Table 1).
  - No instances of a >2-fold shift toward lower potency in BPS were observed.
  - The addition of resazurin was required to determine the MIC endpoint value for the 2 *Haemophilus* spp. strains grown in HTM at 5% and 10% BPS. The MIC values determined with this method displayed no shift from those observed in HTM media or HTM media with 1% BPS.
- BPS effect on activity of ertapenem
  - Ertapenem was less active (≥4-fold higher MIC values) than tebipenem among 4 of the 10 isolates tested in media without BPS.
  - Ertapenem MIC values ranged from 0.015 to 0.25 mg/L in media without BPS (Table 2).
  - No instances of a >2-fold shift toward lower potency in BPS were observed.
  - Similar to tebipenem, resazurin was required to determine the MIC endpoint value for the 2 *Haemophilus* spp. strains grown in HTM at 5% and 10% BPS. MIC values found with this method displayed no shift from those values observed for these strains in HTM media or HTM media with 1% BPS.
- Daptomycin MIC values against the *S. aureus* ATCC 29213 strain in media with varying concentrations of BPS are shown in Table 3.
  - The daptomycin MIC value of 0.25 mg/L in CAMHB containing 50 mg/L of calcium and 10–12.5 mg/L of magnesium was within the CLSI published range.
  - As expected, the addition of BPS to a final concentration of 1%, 5%, or 10% caused a large shift in the observed MIC values to >8 mg/L for all 3 BPS concentrations.

Conclusions

- Tebipenem was active against all isolates tested with MIC values ≤0.06 mg/L.
- The addition of BPS to the testing medium did not affect the *in vitro* MIC values of tebipenem or ertapenem against these species.
- As expected, the observed daptomycin MIC values of the QC strain ATCC 29213 shifted from 0.25 mg/L to >8 mg/L for all conditions when BPS was present.
- These results further support the clinical development of tebipenem for potential use against respiratory tract infections.

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Table 1. Effect of bovine pulmonary surfactant (BPS) on tebipenem MIC values

Species	Tebipenem MIC (mg/L)			
	No BPS	1% BPS	5% BPS	10% BPS
Escherichia coli	0.015	0.015	0.03	0.015
Enterobacter cloacae species complex	0.03	0.03	0.03	0.015
Klebsiella pneumoniae	0.015	0.015	0.015	0.008
Citrobacter freundii species complex	0.015	0.015	0.015	0.015
Methicillin susceptible *Staphylococcus aureus*	0.015	0.015	0.015	0.008
Moraxella catarrhalis	0.015	0.015	0.008	0.008
Streptococcus pneumoniae	≤0.004	≤0.004	≤0.004	≤0.004
Streptococcus pyogenes	≤0.004	0.008	0.008	0.008
Haemophilus influenzae	0.06	0.06	0.06[a]	0.06[a]
Haemophilus parainfluenzae	0.03	0.03	0.03[a]	0.03[a]

[a] Required addition of resazurin for MIC determination.

Table 2. Effect of bovine pulmonary surfactant (BPS) on ertapenem MIC values

Species	Ertapenem MIC (mg/L)			
	No BPS	1% BPS	5% BPS	10% BPS
Escherichia coli	0.015	0.008	0.015	0.015
Enterobacter cloacae species complex	0.12	0.12	0.06	0.03
Klebsiella pneumoniae	0.015	0.008	0.008	0.015
Citrobacter freundii species complex	0.015	0.008	0.008	0.015
Methicillin susceptible *Staphylococcus aureus*	0.25	0.25	0.25	0.25
Moraxella catarrhalis	0.015	0.015	0.015	0.015
Streptococcus pneumoniae	0.03	0.015	0.015	0.015
Streptococcus pyogenes	0.03	0.008	0.015	0.015
Haemophilus influenzae	0.03	0.03	0.06[a]	0.06[a]
Haemophilus parainfluenzae	0.03	0.03	0.03[a]	0.03[a]

[a] Required addition of resazurin for MIC determination.

Table 3. Tebipenem and comparator MIC occurrences tested against *S. aureus* ATCC 29213

Antimicrobial agent	No. of occurrences at MIC (mg/L) of [a]:											
	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8
Tebipenem			1									
Daptomycin						1						
Daptomycin with 1% BPS												1
Daptomycin with 5% BPS												1
Daptomycin with 10% BPS												1

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Contact

R.E. Mendes
JMI Laboratories
345 Beaver Kreek Centre, Suite A
North Liberty, IA 52317
Phone: 319-665-3370
Fax: (319) 665-3371
Email: Rodrigo-mendes@jmilabs.com

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