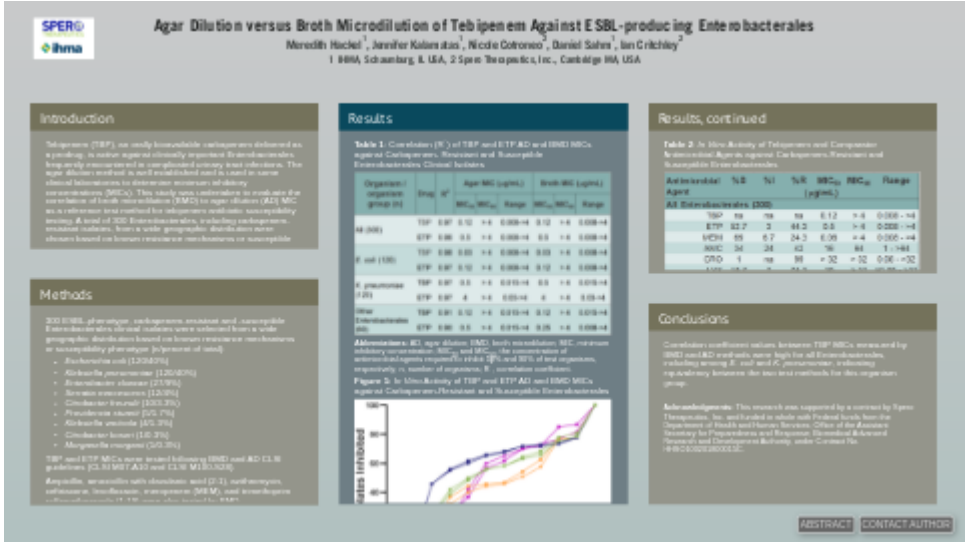
Agar Dilution versus Broth Microdilution of Tebipenem Against ESBL-producing Enterobacterales



Meredith Hackel[1], Jennifer Kalamatas[1], Nicole Cotroneo[2], Daniel Sahm[1], Ian Critchley[2]

1 IHMA, Schaumburg, IL USA, 2 Spero Therapeutics, Inc., Cambridge MA, USA

PRESENTED AT:

INTRODUCTION

Tebipenem (TBP), an orally bioavailable carbapenem delivered as a prodrug, is active against clinically important Enterobacterales frequently encountered in complicated urinary tract infections. The agar dilution method is well established and is used in some clinical laboratories to determine minimum inhibitory concentrations (MICs). This study was undertaken to evaluate the correlation of broth microdilution (BMD) to agar dilution (AD) MIC as a reference test method for tebipenem antibiotic susceptibility testing. A total of 300 Enterobacterales, including carbapenem-resistant isolates, from a wide geographic distribution were chosen based on known resistance mechanisms or susceptible phenotype. Isolates were tested by broth microdilution and agar dilution using the same inocula following CLSI guidelines. Ertapenem (ETP) was tested as a control, and results were evaluated using the coefficient of correlation (R^2 value).

METHODS

300 ESBL-phenotype, carbapenem-resistant and -susceptible Enterobacterales clinical isolates were selected from a wide geographic distribution based on known resistance mechanisms or susceptibility phenotype (n/percent of total):

- *Escherichia coli* (120/40%)
- *Klebsiella pneumoniae* (120/40%)
- *Enterobacter cloacae* (27/9%)
- *Serratia marcescens* (12/4%)
- *Citrobacter freundii* (10/3.3%)
- *Providencia stuartii* (5/1.7%)
- *Klebsiella variicola* (4/1.3%)
- *Citrobacter koseri* (1/0.3%)
- *Morganella morganii* (1/0.3%)

TBP and ETP MICs were tested following BMD and AD CLSI guidelines (CLSI M07-A10 and CLSI M100-S28).

Ampicillin, amoxicillin with clavulanic acid (2:1), azithromycin, ceftriaxone, levofloxacin, meropenem (MEM), and trimethoprim sulfamethoxazole (1:19) were also tested by BMD.

Results were evaluated using the coefficient of correlation (R2).

Quality control organisms included *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603.

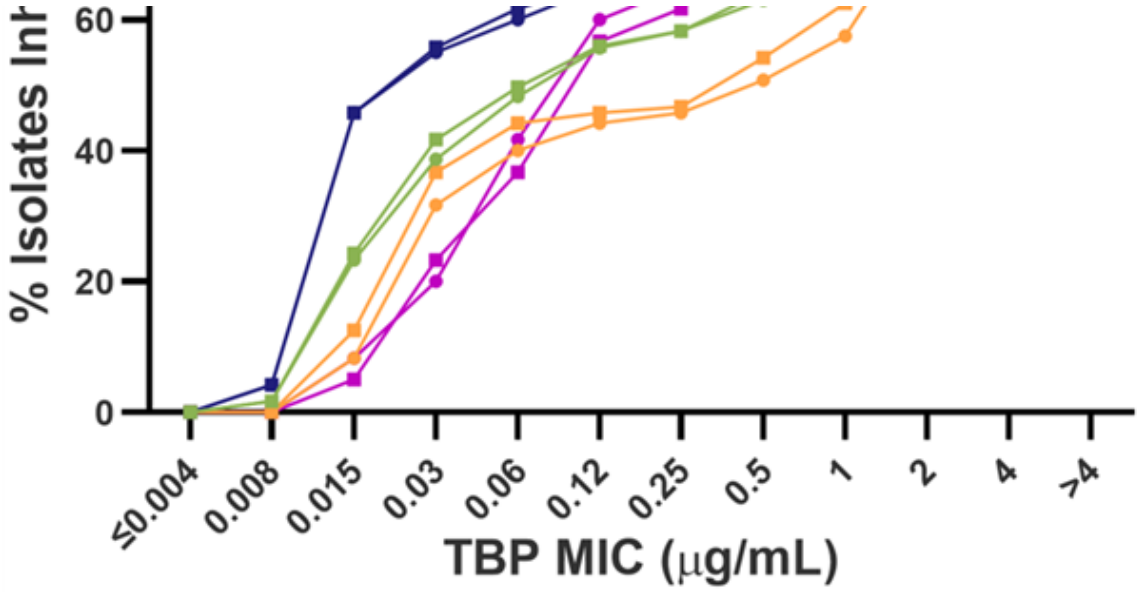
RESULTS

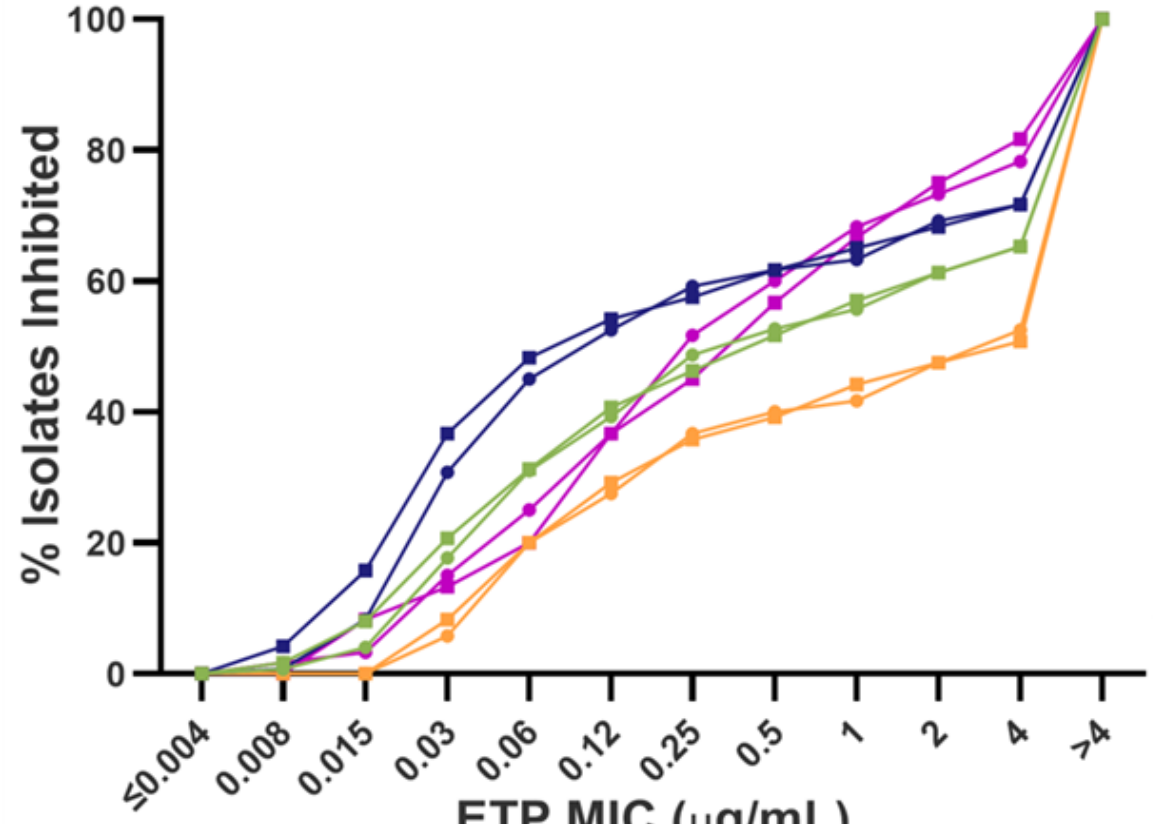
Table 1: Correlation (R^2) of TBP and ETP AD and BMD MICs against Carbapenem- Resistant and Susceptible Enterobacterales Clinical Isolates

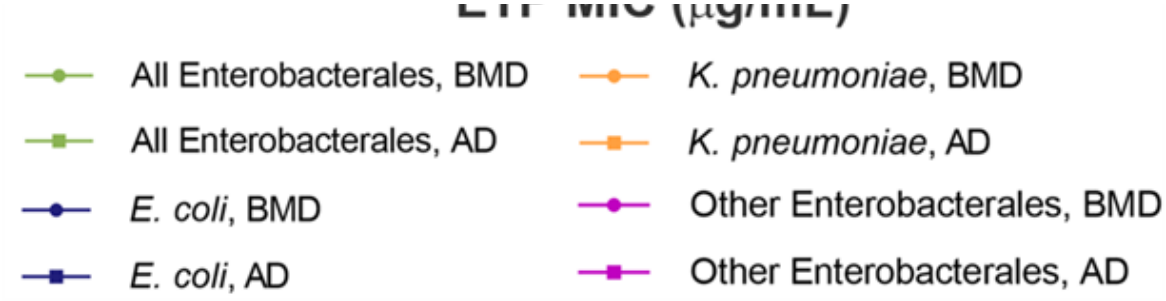
Organism / organism group (n)	Drug	R^2	Agar MIC (μg/mL)			Broth MIC (μg/mL)		
			MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
All (300)	TBP	0.97	0.12	> 4	0.008->4	0.12	> 4	0.008->4
	ETP	0.96	0.5	> 4	0.008->4	0.5	> 4	0.008->4
E. coli (120)	TBP	0.98	0.03	> 4	0.008->4	0.03	> 4	0.008->4
	ETP	0.97	0.12	> 4	0.008->4	0.12	> 4	0.008->4
K. pneumoniae (120)	TBP	0.97	0.5	> 4	0.015->4	0.5	> 4	0.015->4
	ETP	0.97	4	> 4	0.03->4	4	> 4	0.03->4
Other Enterobacterales (60)	TBP	0.91	0.12	> 4	0.015->4	0.12	> 4	0.015->4
	ETP	0.90	0.5	> 4	0.015->4	0.25	> 4	0.008->4

Abbreviations: AD, agar dilution; BMD, broth microdilution; MIC, minimum inhibitory concentration; MIC_{50} and MIC_{90}, the concentration of antimicrobial agents required to inhibit 50% and 90% of test organisms, respectively; n, number of organisms; R^2, correlation coefficient.

Figure 1: *In Vitro* Activity of TBP and ETP AD and BMD MICs against Carbapenem-Resistant and Susceptible Enterobacterales


% Isolates Inh
60
40
20
0
≤0.004
0.008
0.015
0.03
0.06
0.12
0.25
0.5
1
2
4
>4
TBP MIC (μg/mL)


% Isolates Inhibited
100
80
60
40
20
0
≤0.004
0.008
0.015
0.03
0.06
0.12
0.25
0.5
1
2
4
>4



- Correlation between the tebipenem MICs obtained via the two methods as represented by R^2 values was high at 0.97 for all isolates combined.
- Correlation between TBP MICs obtained via the two methods was similarly high among *E. coli* (R^2 was 0.98), *K. pneumoniae* (R^2 was 0.97), and for all other Enterobacterales (R^2 was 0.91).

RESULTS, CONTINUED

Table 2: *In Vitro* Activity of Tebipenem and Comparator Antimicrobial Agents against Carbapenem-Resistant and Susceptible Enterobacterales

Antimicrobial Agent	%S	%I	%R	MIC_{50} (μg/mL)	MIC_{90}	Range
All Enterobacterales (300)						
TBP	na	na	na	0.12	> 4	0.008 - >4
ETP	52.7	3	44.3	0.5	> 4	0.008 - >4
MEM	69	6.7	24.3	0.06	> 4	0.008 - >4
AMC	34	24	42	16	64	1 - >64
CRO	1	na	99	> 32	> 32	0.06 - >32
LVX	18.7	7	74.3	16	> 32	≤0.06 - >32
SXT	18.3	na	81.7	> 32	> 32	0.25 - >32
***E. coli* (120)**						
TBP	na	na	0	0.03	> 4	0.008 - >4
ETP	61.7	1.7	36.7	0.12	> 4	0.008 - >4
MEM	71.7	2.5	25.8	0.03	> 4	0.008 - >4
AMC	52.5	17.5	30	8	32	2 - >64
CRO	2.5	na	97.5	> 32	> 32	0.06 - >32
LVX	13.3	2.5	84.2	16	> 32	≤0.06 - >32
SXT	24.2	na	75.8	> 32	> 32	0.25 - >32
***K. pneumoniae* (120)**						
TBP	na	na	na	0.5	> 4	0.015 - >4
ETP	40	1.7	58.3	4	> 4	0.03 - >4
MEM	64.2	10.8	25	0.5	> 4	0.03 - >4
AMC	26.7	38.3	35	16	> 64	1 - >64
CRO	na	na	100	> 32	> 32	8 - >32
LVX	16.7	6.7	76.7	16	> 32	≤0.06 - >32

SXT	11.7	na	88.3	> 32	> 32	0.5 - >32
Other Enterobacterales (60)						
TBP	na	na	na	0.12	> 4	0.015 - >4
ETP	60	8.3	31.7	0.25	> 4	0.008 - >4
MEM	73.3	6.7	20	0.06	> 4	0.015 - >4
AMC	11.7	8.3	80	32	64	1 - >64
CRO	na	na	100	> 32	> 32	4 - >32
LVX	33.3	16.7	50	1	16	≤0.06 - >32
SXT	20	na	80	> 32	> 32	0.5 - >32

- Enterobacterales clinical isolates assessed in this study demonstrated a broad range of susceptibility to carbapenems, with 24.3% and 44.3% being resistant to MEM and ETP, respectively.
- TBP $MIC_{50/90}$ values for all Enterobacterales by broth microdilution and agar dilution were the same for both test methods (MIC_{50}, 0.12 µg/mL; MIC_{90}, >4 µg/mL).
- Similarly, ETP $MIC_{50/90}$ values for broth microdilution and agar dilution were the same for both test methods (MIC_{50}, 0.5 µg/mL; MIC_{90}, >4 µg/mL).

Abbreviations: AMC, amoxicillin-clavulanate (2:1); CRO, ceftriaxone; ETP, ertapenem; I, intermediate-susceptible; MEM, meropenem; MIC, minimum inhibitory concentration; MIC50 and MIC90, the concentration of antimicrobial agents required to inhibit 50% and 90% of test organisms, respectively; n, number of organisms; R, resistant; R2, correlation coefficient; S, susceptible; SXT, trimethoprim-sulfamethoxazole (1:19); TBP, tebipenem.

CONCLUSIONS

Correlation coefficient values between TBP MICs measured by BMD and AD methods were high for all Enterobacterales, including among *E. coli* and *K. pneumoniae*, indicating equivalency between the two test methods for this organism group.

Acknowledgments: This research was supported by a contract by Spero Therapeutics, Inc. and funded in whole with Federal funds from the Department of Health and Human Services; Office of the Assistant Secretary for Preparedness and Response; Biomedical Advanced Research and Development Authority, under Contract No. HHSO100201800015C.

ABSTRACT

Background: Effective oral agents are needed for complicated urinary tract infections (cUTIs) where *Escherichia coli* is the dominant causative agent. Increasing prevalence of extended-spectrum β-lactamase-(ESBL) producing organisms results in co-resistance to many currently available oral agents such as the β-lactams, fluoroquinolones and trimethoprim-sulfamethoxazole (SXT). The carbapenems represent the most potent and broad-spectrum β-lactams and are minimally affected by most β-lactamases. Tebipenem (TBP) is an orally bioavailable carbapenem with activity against clinically important Enterobacterales frequently encountered in cUTIs, including those resistant to currently available oral therapeutics.

The agar dilution method is well established and is used in some clinical laboratories to determine minimum inhibitory concentrations (MICs). This study evaluated the correlation of MIC as determined by broth microdilution (BMD) to agar dilution as a reference test method for TBP susceptibility testing.

Methods: A total of 300 Enterobacterales from a wide geographic distribution were tested, including (n) *E. coli* (120), *Klebsiella pneumoniae* (120), and other Enterobacterales: *Enterobacter cloacae* (27), *Serratia marcescens* (12), *Citrobacter freundii* (10), *Providencia stuartii* (5), *Klebsiella variicola* (4), *Citrobacter koseri* (1), and *Morganella morganii* (1). Isolates were chosen based on known resistance mechanisms or susceptibility phenotype and were positive for ≥1 ESBL. Isolates were tested by BMD and agar dilution following CLSI guidelines. Ertapenem (ETP) was tested as a control, and results were evaluated using the coefficient of correlation (R2 value).

Results: 44.3%, 74.3% and 81.7% of all isolates were resistant to ETP, LVX and SXT, respectively. TBP $MIC_{50/90}$ values for BMD and agar dilution were the same for both test methods (0.12/>4 μg/mL). Correlation between the two methods as represented by R^2 values for TBP was 0.97 for all isolates combined, 0.98 for *E. coli*, 0.97 for *K. pneumoniae* (0.97), and 0.91 for other Enterobacterales. Similarly, ETP $MIC_{50/90}$ values were 0.5 and >4 μg/mL, respectively, by both methods. R^2 values for ETP were 0.96 for all isolates combined, 0.97 for *E. coli*, 0.97 for *K. pneumoniae*, and 0.90 for other Enterobacterales.

Conclusions: R^2 values between TBP MIC values measured by BMD and agar dilution were high for all Enterobacterales, including the subgroups of *E. coli* and *K. pneumoniae*, indicating equivalency between the two test methods for this organism group.