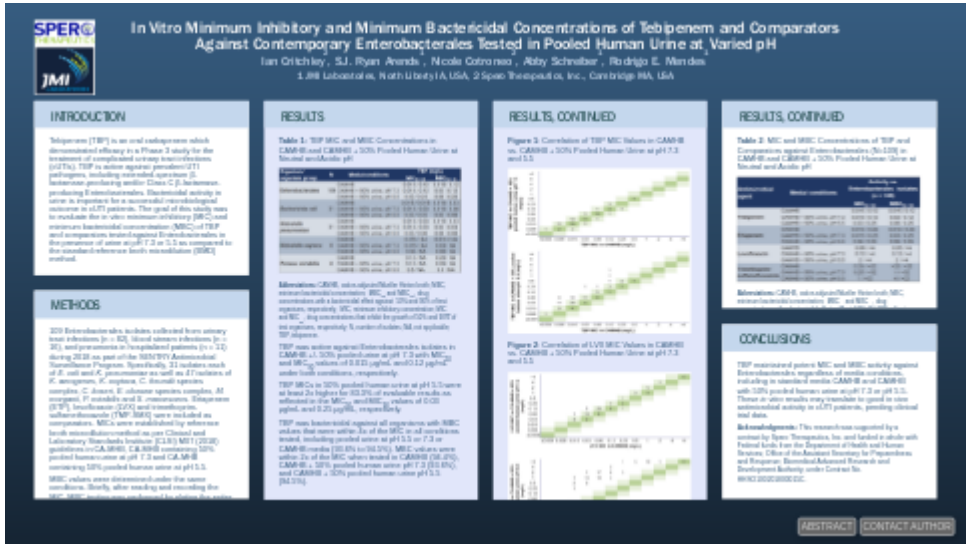
In Vitro Minimum Inhibitory and Minimum Bactericidal Concentrations of Tebipenem and Comparators Against Contemporary Enterobacterales Tested in Pooled Human Urine at Varied pH



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INTRODUCTION

Tebipenem (TBP) is an oral carbapenem which demonstrated efficacy in a Phase 3 study for the treatment of complicated urinary tract infections (cUTIs). TBP is active against prevalent UTI pathogens, including extended-spectrum β-lactamase-producing and/or Class C β-lactamase-producing Enterobacterales. Bactericidal activity in urine is important for a successful microbiological outcome in cUTI patients. The goal of this study was to evaluate the in vitro minimum inhibitory (MIC) and minimum bactericidal concentration (MBC) of TBP and comparators tested against Enterobacterales in the presence of urine at pH 7.3 or 5.5 as compared to the standard reference broth microdilution (BMD) method.

METHODS

109 Enterobacterales isolates collected from urinary tract infections (n = 82), blood stream infections (n = 16), and pneumonia in hospitalized patients (n = 11) during 2018 as part of the SENTRY Antimicrobial Surveillance Program. Specifically, 31 isolates each of *E. coli* and *K. pneumoniae* as well as 47 isolates of *K. aerogenes*, *K. oxytoca*, *C. freundii* species complex, *C. koseri*, *E. cloacae* species complex, *M. morganii*, *P. mirabilis* and *S. marcescens*. Ertapenem (ETP), levofloxacin (LVX) and trimethoprim-sulfamethoxazole (TMP-SMX) were included as comparators. MICs were established by reference broth microdilution method as per Clinical and Laboratory Standards Institute (CLSI) M07 (2018) guidelines in CA-MHB, CA-MHB containing 50% pooled human urine at pH 7.3 and CA-MHB containing 50% pooled human urine at pH 5.5.

MBC values were determined under the same conditions. Briefly, after reading and recording the MIC, MBC testing was performed by plating the entire well contents (0.1 mL) beginning at the MIC and including, as available, up to 2x, 4x, 8x, and 16x the MIC onto the surface of an agar plate and incubating at 35°C (± 2°C). The number of colony forming units (CFU)/mL was measured in duplicate after 48 hours. The average CFU/mL value was used to determine the cutoff CFU value required to achieve the MBC. The average inoculum density was 3.0×10^5 CFU/mL. The MBC was recorded as the lowest concentration of antimicrobial that led to a ≥99.9% reduction (i.e., a ≥3 $\log_{10}$ decrease) in the number of input bacterial cells.

RESULTS

Table 1: TBP MIC and MBC Concentrations in CAMHB and CAMHB + 50% Pooled Human Urine at Neutral and Acidic pH

Organism/ organism group	N	Media/conditions	TBP (mg/L)	
			$MIC_{50 / 90}$	$MBC_{50 / 90}$
Enterobacterales	109	CAMHB	0.015 / 0.12	0.015 / 0.12
		CAMHB + 50% urine, pH 7.3	0.015 / 0.12	0.03 / 0.12
		CAMHB + 50% urine, pH 5.5	0.03 / 0.25	0.06 / 0.25
Escherichia coli	31	CAMHB	0.015 / 0.015	0.015 / 0.03
		CAMHB + 50% urine, pH 7.3	0.015 / 0.03	0.015 / 0.06
		CAMHB + 50% urine, pH 5.5	0.03 / 0.03	0.03 / 0.06
Klebsiella pneumoniae	31	CAMHB	0.015 / 0.03	0.015 / 0.03
		CAMHB + 50% urine, pH 7.3	0.015 / 0.03	0.03 / 0.03
		CAMHB + 50% urine, pH 5.5	0.03 / 0.06	0.06 / 0.06
Klebsiella oxytoca	6	CAMHB	0.015 / NA	0.015 / NA
		CAMHB + 50% urine, pH 7.3	0.015 / NA	0.03 / NA
		CAMHB + 50% urine, pH 5.5	0.06 / NA	0.06 / NA
Proteus mirabilis	6	CAMHB	0.12 / NA	0.25 / NA
		CAMHB + 50% urine, pH 7.3	0.12 / NA	0.25 / NA
		CAMHB + 50% urine, pH 5.5	0.5 / NA	0.5 / NA

Abbreviations: CAMHB, cation-adjusted Mueller Hinton broth; MBC, minimum bactericidal concentration; MBC_{50} and MBC_{90}, drug concentrations with a bactericidal effect against 50% and 90% of test organisms, respectively; MIC, minimum inhibitory concentration; MIC_{50} and MIC_{90}, drug concentrations that inhibit the growth of 50% and 90% of test organisms, respectively; N, number of isolates; NA, not applicable; TBP, tebipenem.

TBP was active against Enterobacterales isolates in CAMHB +/- 50% pooled urine at pH 7.3 with MIC_{50} and MIC_{90} values of 0.015 µg/mL and 0.12 µg/mL under both conditions, respectively.

TBP MICs in 50% pooled human urine at pH 5.5 were at least 2x higher for 83.3% of evaluable results as reflected in the MIC_{50} and MIC_{90} values of 0.03 µg/mL and 0.25 µg/mL, respectively.

TBP was bactericidal against all organisms with MBC values that were within 4x of the MIC in all conditions tested, including pooled urine at pH 5.5 or 7.3 or CAMHB media (93.6% to 94.5%). MBC values were within 2x of the MIC when tested in CAMHB (94.4%), CAMHB + 50% pooled human urine pH 7.3 (93.6%), and CAMHB + 50% pooled human urine pH 5.5 (94.5%).

RESULTS, CONTINUED

Figure 1: Correlation of TBP MIC Values in CAMHB vs. CAMHB + 50% Pooled Human Urine at pH 7.3 and 5.5

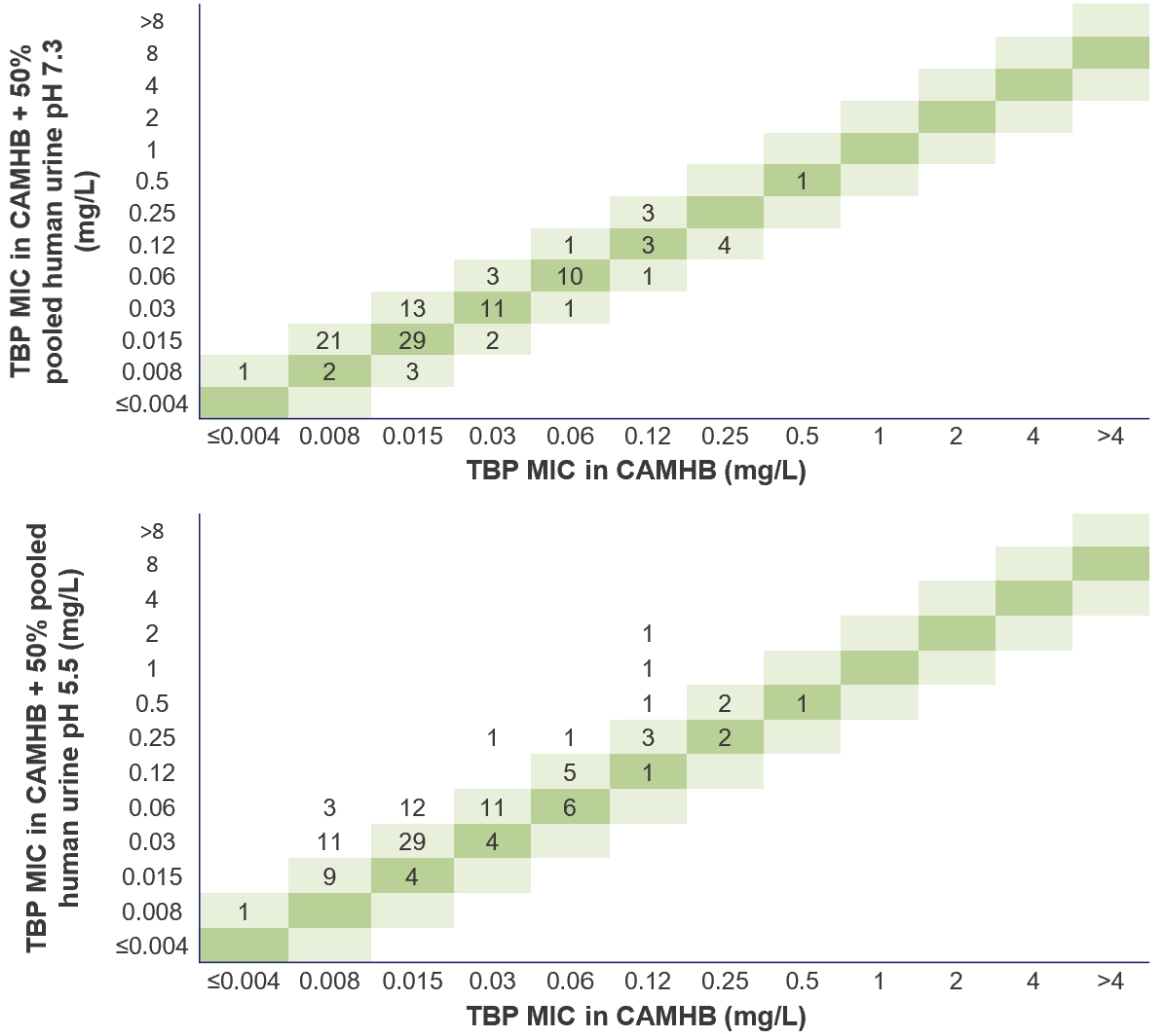
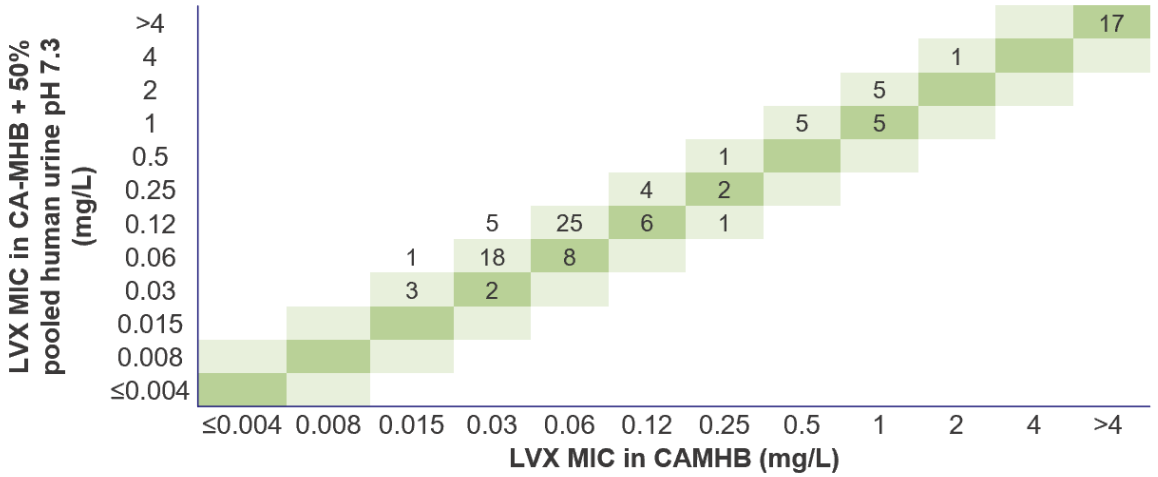
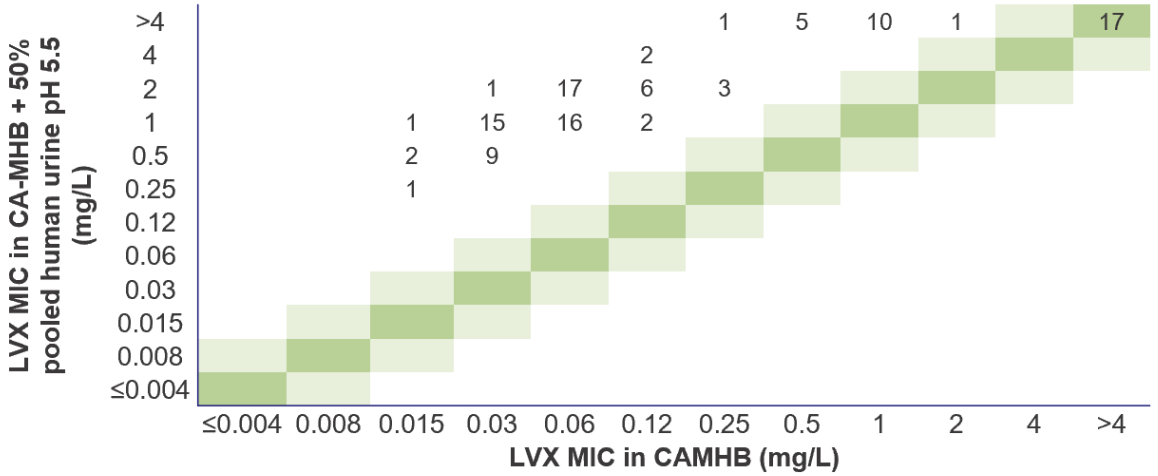


Figure 2: Correlation of LVX MIC Values in CAMHB vs. CAMHB + 50% Pooled Human Urine at pH 7.3 and 5.5





Abbreviations: CAMHB, cation-adjusted Mueller Hinton broth; LVX, levofloxacin; MIC, minimum inhibitory concentration; TBP, tebipenem.

RESULTS, CONTINUED

Table 2: MIC and MBC Concentrations of TBP and Comparators against Enterobacterales (N=109) in CAMHB and CAMHB + 50% Pooled Human Urine at Neutral and Acidic pH

Antimicrobial agent	Media/ conditions	Activity vs. Enterobacterales isolates (n = 109)	
		$MIC_{50 / 90}$	$MBC_{50 / 90}$
Tebipenem	CAMHB	0.015 / 0.12	0.015 / 0.12
	CAMHB + 50% urine, pH 7.3	0.015 / 0.12	0.03 / 0.12
	CAMHB + 50% urine, pH 5.5	0.03 / 0.25	0.06 / 0.25
Ertapenem	CAMHB	0.015 / 0.25	0.015 / 0.25
	CAMHB + 50% urine, pH 7.3	0.015 / 0.25	0.03 / 0.25
	CAMHB + 50% urine, pH 5.5	0.06 / 0.25	0.06 / 0.25
Levofloxacin	CAMHB	0.06 / >4	0.05 / >4
	CAMHB + 50% urine, pH 7.3	0.12 / >4	0.12 / >4
	CAMHB + 50% urine, pH 5.5	2 / >4	2 / >4
Trimethoprim-sulfamethoxazole	CAMHB	0.25 / >32	>32 / >32
	CAMHB + 50% urine, pH 7.3	0.25 / >32	1 / >32
	CAMHB + 50% urine, pH 5.5	1 / >32	4 / >32

Abbreviations: CAMHB, cation-adjusted Mueller Hinton broth; MBC, minimum bactericidal concentration; MBC_{50} and MBC_{90}, drug concentrations with a bactericidal effect against 50% and 90% of test organisms, respectively; MIC, minimum inhibitory concentration; MIC_{50} and MIC_{90}, drug concentrations that inhibit the growth of 50% and 90% of test organisms, respectively; N, number of isolates.

- Similar to TBP, ETP was active against Enterobacterales isolates (98.2% susceptible, MIC_{50}, 0.015 mg/L and MIC_{90}, 0.25 mg/L) with MICs within 2x when grown in the presence of 50% pooled human urine at pH 7.3 (MIC_{50}, 0.015 mg/L and MIC_{90}, 0.25 mg/L) and at least 2x higher (82.4% of evaluable results) when grown in the presence of 50% pooled human urine at pH 5.5 (MIC_{50}, 0.06 mg/L and MIC_{90}, 0.25 mg/L. All ETP MBC values within one dilution of the MIC.
- LVX was less active in CAMHB ($MIC_{50/90}$ 0.06/>4 mg/L) and $MIC_{50/90}$ values were 2x higher in urine at pH 7.3 and 8x higher in urine at pH 5.5. LVX was bactericidal under all conditions with MBCs ≤ 2x the MIC.
- TMP-SMX was the least active agent ($MIC_{50/90}$ 0.25/>32 mg/L) with MICs generally within 2x in urine at pH 7.3, and 4x higher in urine at pH 5.5. TMP-SMX $MBC_{50/90}$ values were >32/>32 in CAMHB, 1/>32 in CAMHB + 50% pooled urine at pH 7.3, and 4/>32 in CAMHB + 50% pooled urine at pH 5.5.

CONCLUSIONS

TBP maintained potent MIC and MBC activity against Enterobacterales regardless of media conditions, including in standard media CAMHB and CAMHB with 50% pooled human urine at pH 7.3 or pH 5.5. These *in vitro* results may translate to good *in vivo* antimicrobial activity in cUTI patients, pending clinical trial data.

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ABSTRACT

Background: Tebipenem (TBP) is an oral carbapenem which demonstrated efficacy in a Phase 3 study for the treatment of complicated urinary tract infections (cUTIs). TBP is active against prevalent UTI pathogens, including extended-spectrum β-lactamase-producing and/or Class C β-lactamase-producing Enterobacterales that exhibit considerable co-resistance to oral agents such as the fluoroquinolones and trimethoprim-sulfamethoxazole (TMP-SMX), limiting their empiric use. Bactericidal activity in urine is important for a successful microbiological outcome in cUTI patients. The goal of this study was to evaluate the in vitro minimum inhibitory (MIC) and bactericidal (MBC) concentrations of TBP and comparators tested against Enterobacterales in the presence of urine at pH 7.3 or 5.5 as compared to the standard reference broth microdilution (BMD) method.

Methods: A total of 109 Enterobacterales isolates, including resistant phenotypes, from documented UTI, bloodstream and respiratory infections during 2018 (including 31 each of *Escherichia coli* and *Klebsiella pneumoniae*) were evaluated. MICs and MBCs were determined in accordance with CLSI methods using standard conditions in cation-adjusted Mueller Hinton Broth (CAMHB) and in CAMHB containing 50% pooled human urine adjusted to pH 7.3 or pH 5.5. Comparators were ertapenem (ETP), TMP-SMX and levofloxacin (LVX).

Results: TBP was active in CAMHB ($MIC_{50/90}$ 0.015/0.12 µg/mL). Compared to the reference method, TBP MICs were ≤2 x in CAMHB + 50% pooled urine at pH 7.3 ($MIC_{50/90}$ 0.015/0.12 µg/mL) and were often ≥ 2x higher in CAMHB + 50% pooled urine at pH 5.5 ($MIC_{50/90}$, 0.03/0.25 µg/mL). Regardless of media, TBP was bactericidal against all isolates with MBCs within 4x the MIC. ETP ($MIC_{50/90}$ 0.015/0.25 µg/mL) was similarly active under all conditions with MBC values ≤ 2x the MIC. LVX was less active in CAMHB ($MIC_{50/90}$ 0.06/>4 µg/mL) and $MIC_{50/90}$ values were 2x higher in urine at pH 7.3 and 8x higher in urine at pH 5.5. LVX was bactericidal under all conditions with MBCs ≤ 2x the MIC. TMP-SMX was the least active agent ($MIC_{50/90}$ 0.25/>32 µg/mL) with MICs within 2x in urine at pH 7.3, and 4x higher in urine at pH 5.5. TMP-SMX MBCs shifted at least 2x higher than MIC in the majority of instances.

Conclusions: TBP maintained potent MIC and MBC activity against Enterobacterales regardless of media conditions including standard media CAMHB and CAMHB with 50% pooled human urine at pH 7.3 or pH 5.5. These *in vitro* results may translate to good *in vivo* antimicrobial activity in cUTI patients, pending clinical trial data.