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The Effects of Varying Susceptibility Test Parameters on TP-434 *in vitro*

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Abstract

Background: TP-434 is a novel broad spectrum fluorocycline entering Phase 2 clinical trials for intra-abdominal infections. To support clinical development, a study was run to evaluate the effects of various susceptibility test conditions on TP-434 minimal inhibitory concentrations (MICs) for aerobic bacteria. Tigecycline (TIG), a tetracycline with known sensitivities to media age and divalent ion content, was used as the comparator (Peterson, et al. 2005. AAC 49:3910; Bradford, et al. 2005. AAC 49: 3903; Fernadez-Mazarrasa et al. 2009. JCM 47:827).

Methods: Broth microdilution and agar dilution were performed according to standard CLSI methodology. Varied assay parameters evaluated included: fresh/frozen media, aged (2 weeks at room temperature) media, added serum or blood, altered pH, added calcium, added polysorbate, *Haemophilus* test medium (HTM), carbon dioxide and altered inoculum size. A ≥4-fold difference in MICs was considered potentially significant.

Results: TP-434 MICs for *Staphylococcus aureus* (*Sa*) ATCC29213, *Enterococcus faecalis* (*Ef*) ATCC29212, *Streptococcus pneumoniae* (*Sp*) ATCC49619, *Escherichia coli* (*Ec*) ATCC25922 and *Haemophilus influenzae* (*Hi*) ATCC49247 were largely unaffected by most conditions. A 2- to 4-fold decrease in MIC was observed for *Sp* at pH 6.0 and *Ef* at pH 8.0. A 4-fold increase in MIC was observed only for *Ef* in 0.002% polysorbate and 5% and 10% human serum. No significant differences in MICs were observed for inocula ranging from 5 x 10^4 to 5 x 10^6 CFU/mL. A 4-fold increase in TIG MIC was noted for *Ef* in polysorbate. TP-434 MICs in aged media were unchanged or within one dilution of MICs in fresh media for 92.3% of all tested clinical isolates (n=30 isolates + *Ec, Sa, Ef* QC strains in triplicate); 7.6 % of the MICs were elevated ≥4-fold in aged media. This was in contrast to TIG where MIC values in aged media were unchanged (at or within one dilution of MICs in fresh media) for 69.2% of all tested isolates, while 30.7% showed ≥4-fold increases in MICs. Agar dilution and microdilution MICs for both TP-434 and TIG were similar, suggesting no significant interaction with agar.

Conclusions: In contrast to TIG, *in vitro* antibacterial activity of TP-434 appears to be largely unaffected by media age and media additives.

Introduction

The *in vitro* activity of tigecycline (TIG), a tetracycline-class antibiotic, is known to be affected by age and divalent cation content of media (1, 2, 3). As a result, CLSI guidelines specify the use of freshly prepared media, or media that has been frozen within less than 12 hours after preparation, for susceptibility testing with TIG. This study investigated whether the age of the cation-adjusted Mueller-Hinton broth (CA-MHB) and other testing parameters affected the activity of TP-434, similar to that seen with TIG, and thus whether any special accommodations need to be made for routine susceptibilty testing with TP-434. The *in vitro* activity profile under standard CLSI conditions and the impact of non-standard conditions (addition of serum or blood, *Haemophilus* Test Medium (HTM), altered pH, high/low inoculum, varied cation concentration, polysorbate addition, incubation in 5% CO_2) on the TP-434 activity profile was evaluated.

Methods

Isolates were tested for susceptibility by broth microdilution according to CLSI M100-S20, CLSI M7-A8, and Eurofins Medinet SOPs. Isolates were tested by agar dilution according to CLSI M100-S20, CLSI M7-A8, and Eurofins Medinet SOPs.

Panels containing CA-MHB reconstituted from powder that day (fresh CA-MHB) and CA-MHB which had aged on the benchtop at room temperature for two weeks were inoculated with all ATCC QC organisms (in triplicate) and clinical isolates. ATCC isolates of *E. coli*, *S. aureus*, and *E. faecalis* alongside 10 clinical isolates of each species from the Eurofins Medinet repository were used to evaluate the impact of aged media, frozen storage, and comparison of agar dilution MICs to broth microdilution MICs. An allotment of panels made from fresh CA-MHB powder were frozen at -70°C for 2 weeks for subsequent evaluation of stability of the drug in panels made with fresh CA-MHB after freezing. MICs were interpreted based on inhibition of growth and loss of button at the bottom of the well. Hazy growth was noted for all tetracyclines tested against *E. faecalis* but not taken into account when determining the MIC unless otherwise indicated.

For the evaluation of other test parameters, TP-434 was evaluated alongside TIG as a control. Assays run under CLSI standardized conditions were inoculated with 5x10^5 CFU/ml, contained 20-25 mg/L Ca^{2+}, had a neutral pH of 7.2-7.4, and were incubated in ambient air. In parallel, standard conditions were modified as follows:

- Human serum was added to test medium supplemented with either 5% serum or 10% serum.
- Inoculum size was varied by adding an initial concentration of 5x10^4 CFU/ml and 5x10^6 CFU/ml.
- Non-standard medium included CA-MHB supplemented with lysed horse blood (LHB) and HTM.
- CA-MHB, normally containing 25 mg/L Ca^{2+}, was supplemented with an additional 25 mg/L Ca^{2+} to yield a final concentration of 50 mg/L Ca^{2+}.
- Media pH was adjusted to 6.0 and 8.0.
- Media was supplemented with polysorbate at 0.002%.
- Plates were incubated in the presence of CO_2.

Any change in MIC beyond one doubling dilution of that observed under standard conditions was noted.

References

1. Peterson, et al. 2005. Effect of medium age and supplementation with biocatalytic oxygen-reducing reagent oxyrase on in vitro activitiies of tigecycline against recent clinical isolates. Antimicrob Agents Chemother 49:3910-3918.
2. Bradford, et al. 2005. Tigecycline MIC testing by broth dilution requires use of fresh medium or addition of the biocatalytic oxygen-reducing reagent oxyrase to standardize the test method. Antimicrob Agents Chemother 49:3903-3909.
3. Fernandez-Mazarrasa, et al. 2009. High concentrations of manganese in Mueller Hinton agar increase MICs of tigecycline as determined by Etest. J. Clini Microbiol 47:827-829.

Results

Table 1. Comparison of MICs when using fresh media versus aged media

Organism	Drug	N[a]	Log2 difference - aged vs fresh							% Log2 difference - aged vs fresh							Essential agreement[b]
			≥ -3	-2	-1	0	+1	+2	≥ +3	≥ -3	-2	-1	0	+1	+2	≥ +3	
overall	TP-434	39			3	22	11	1	2			7.7	56.4	28.2	2.6	5.1	92.3
	tetracycline	29			5	23	1					17.2	79.3	3.4			100.0
	tigecycline	39				2	25	10	2				5.1	64.1	25.6	5.1	69.2
E. coli	TP-434	13			1	8	4					7.7	61.5	30.8			100.0
	tetracycline	7			1	5	1					14.3	71.4	14.3			100.0
	tigecycline	13				1	9	3					7.7	69.2	23.1		76.9
S. aureus	TP-434	13			1	9	2	1				7.7	69.2	15.4	7.7		92.3
	tetracycline	13			3	10						23.1	76.9				100.0
	tigecycline	13				1	12						7.7	92.3			100.0
E. faecalis	TP-434	13			1	5	5		2			7.7	38.5	38.5		15.4	84.6
	tetracycline	9			1	8						11.1	88.9				100.0
	tigecycline	13					4	7	2					30.8	53.8	15.4	30.8

[a]N = isolates with defined MICs available for analysis
[b]essential agreement is defined as MICs at or within one doubling dilution of standard (fresh)

Table 2. Comparison of MICs when using fresh media versus frozen media

Organism	Drug	N[a]	Log2 difference - frozen vs fresh							% Log2 difference - frozen vs fresh							Essential agreement[b]
			≥ -3	-2	-1	0	+1	+2	≥ +3	≥ -3	-2	-1	0	+1	+2	≥ +3	
overall	TP-434	38				5	28	5					13.2	73.7	13.2		100.0
	tetracycline	28					19	8	1					67.9	28.6	3.6	96.4
	tigecycline	39			1	23	14	1				2.6	59.0	35.9	2.6		97.4
E. coli	TP-434	13				1	11	1					7.7	84.6	7.7		100.0
	tetracycline	7					6	1						85.7	14.3		100.0
	tigecycline	13			1	10	2					7.7	76.9	15.4			92.3
S. aureus	TP-434	13					10	3						76.9	23.1		100.0
	tetracycline	13					7	5	1					53.8	38.5	7.7	92.3
	tigecycline	13				2	11						15.4	84.6			100.0
E. faecalis	TP-434	12				4	7	1					33.3	58.3	8.3		100.0
	tetracycline	8					6	2						75.0	25.0		100.0
	tigecycline	13				11	1	1					84.6	7.7	7.7		100.0

[a]N = isolates with defined MICs for both conditions available for analysis
[b]essential agreement is defined as MICs at or within one doubling dilution of standard (fresh)

Table 3. Comparison of MICs when using fresh broth microdilution (BMD) versus agar dilution (AD) reading haze as growth for BMD with *E. faecalis*

Organism	Drug	N[a]	Log2 difference - AD vs BMD							% Log2 difference - AD vs BMD							Essential agreement[b]
			≥ -3	-2	-1	0	+1	+2	≥ +3	≥ -3	-2	-1	0	+1	+2	≥ +3	
overall	TP-434	36				16	20						44.4	55.6			100.0
	tetracycline	26			6	18	2					23.1	69.2	7.7			100.0
	tigecycline	36				23	13						63.9	36.1			100.0
E. coli	TP-434	12				6	6						50.0	50.0			100.0
	tetracycline	6				5	1						83.3	16.7			100.0
	tigecycline	12			1	9	2					8.3	75.0	16.7			100.0
S. aureus	TP-434	12				6	6						50.0	50.0			100.0
	tetracycline	12			2	10						16.7	83.3				100.0
	tigecycline	12			1	1	9	1				8.3	8.3	75.0	8.3		91.7
E. faecalis	TP-434	12				4	8						33.3	66.7			100.0
	tetracycline	8			4	3	1					50.0	37.5	12.5			100.0
	tigecycline	12			2	9	1					16.7	75.0	8.3			100.0

[a]N = isolates with defined MICs for both conditions available for analysis
[b]essential agreement is defined as MICs at or within one doubling dilution of standard (fresh broth microdilution)

Table 4. TP-434 and tigecycline MICs (µg/ml) and the effects of human serum

Organisms	Isolate ID	Replicate Agent	0% Serum[a] 1	0% Serum[a] 2	5% Serum 1	5% Serum 2	10% Serum 1	10% Serum 2
S. aureus	ATCC 29213	TP-434	0.06	0.03	0.06	0.06	0.03	0.03
		tigecycline	0.12	0.12	0.12	0.12	0.12	0.12
E. faecalis	ATCC 29212	TP-434	0.015	0.015	0.06	0.06	0.06	0.03
		tigecycline	0.03	0.03	0.06	0.06	0.06	0.06
S. pneumoniae	ATCC 49619	TP-434	0.015	0.015	0.008	0.008	0.008	0.008
		tigecycline	0.03	0.03	0.03	0.03	0.015	0.03
E. coli	ATCC 25922	TP-434	0.12	0.06	0.06	0.06	0.06	0.06
		tigecycline	0.12	0.12	0.12	0.12	0.06	0.06
H. influenzae	ATCC 49247	TP-434	0.25	0.25	0.25	0.25	0.25	0.25
		tigecycline	>2	1	2	2	2	2

[a]Standard concentration

Table 5. TP-434 and tigecycline MICs (µg/ml) and the effects of non-standard inocula

Organisms	Isolate ID	Replicate Agent	Inoculum 5X10^{5a} 1	Inoculum 5X10^{5a} 2	Inoculum 5X10^4 1	Inoculum 5X10^4 2	Inoculum 5x10^6 1	Inoculum 5x10^6 2
S. aureus	ATCC 29213	TP-434	0.06	0.03	0.03	0.03	0.06	0.06
		tigecycline	0.12	0.12	0.06	0.06	0.12	0.12
E. faecalis	ATCC 29212	TP-434	0.015	0.015	0.015	0.015	0.015	0.015
		tigecycline	0.03	0.03	0.03	0.03	0.03	0.03
S. pneumoniae	ATCC 49619	TP-434	0.015	0.015	0.008	0.015	0.015	0.015
		tigecycline	0.03	0.03	0.015	0.03	0.03	0.06
E. coli	ATCC 25922	TP-434	0.12	0.06	0.06	0.06	0.06	0.06
		tigecycline	0.12	0.12	0.12	0.12	0.12	0.12
H. influenzae	ATCC 49247	TP-434	0.25	0.25	0.25	0.25	0.25	0.25
		tigecycline	>2	1	1	1	2	2

[a]Standard inocula

Table 6. TP-434 and tigecycline MICs (µg/ml) and impact of non-standard testing media

Organisms	Isolate ID	Replicate Agent	MHB[a] 1	MHB[a] 2	MHB+LHB[b] 1	MHB+LHB[b] 2	HTM[c] 1	HTM[c] 2
S. aureus	ATCC 29213	TP-434	0.06	0.03	0.06	0.06	0.06	0.06
		tigecycline	0.12	0.12	0.12	0.12	0.5	1
E. faecalis	ATCC 29212	TP-434	0.015	0.015	0.03	0.03	0.03	0.03
		tigecycline	0.03	0.03	0.06	0.06	0.5	1
S. pneumoniae	ATCC 49619	TP-434	0.015	0.008	0.015	0.015	0.015	0.015
		tigecycline	0.12	0.12	0.03	0.03	1	1
E. coli	ATCC 25922	TP-434	0.12	0.06	0.06	0.06	0.12	0.12
		tigecycline	0.12	0.12	0.12	0.12	0.12	0.12
H. influenzae	ATCC 49247	TP-434	NG	NG	0.12	0.12	0.25	0.25
		tigecycline	NG	NG	0.12	0.12	>2	1

[a]Standard media for *S. aureus* , *E. faecalis* , and *E. coli*
[b]Standard media for *S. pneumoniae* only
[c]Standard media for *H. influenzae* only
NG, no growth

Table 7. TP-434 and tigecycline MICs (µg/ml) and the effects of supplemented calcium

Organisms	Isolate ID	Replicate Agent	Calcium 20-25 µg/ml[a] 1	Calcium 20-25 µg/ml[a] 2	Calcium 50 µg/ml 1	Calcium 50 µg/ml 2
S. aureus	ATCC 29213	TP-434	0.06	0.03	0.03	0.03
		tigecycline	0.12	0.12	0.12	0.12
E. faecalis	ATCC 29212	TP-434	0.015	0.015	0.015	0.015
		tigecycline	0.03	0.03	0.03	0.03
S. pneumoniae	ATCC 49619	TP-434	0.015	0.015	0.015	0.015
		tigecycline	0.03	0.03	0.03	0.03
E. coli	ATCC 25922	TP-434	0.12	0.06	0.12	0.06
		tigecycline	0.12	0.12	0.12	0.12
H. influenzae	ATCC 49247	TP-434	0.25	0.25	0.25	0.25
		tigecycline	>2	1	1	1

[a]Standard concentration

Table 8. TP-434 and tigecycline MICs (µg/ml) and the effects of pH

Organisms	Isolate ID	Replicate Agent	pH 7.2-7.4[a] 1	pH 7.2-7.4[a] 2	pH 6.0 1	pH 6.0 2	pH 8.0 1	pH 8.0 2
S. aureus	ATCC 29213	TP-434	0.06	0.03	0.06	0.06	0.03	0.03
		tigecycline	0.12	0.12	0.25	0.25	0.12	0.12
E. faecalis	ATCC 29212	TP-434	0.015	0.015	0.03	0.015	0.004	haze
		tigecycline	0.03	0.03	0.06	0.06	haze	haze
S. pneumoniae	ATCC 49619	TP-434	0.015	0.015	0.004	0.008	0.015	0.015
		tigecycline	0.03	0.03	0.015	0.015	0.03	0.03
E. coli	ATCC 25922	TP-434	0.12	0.06	0.12	0.25	0.12	0.12
		tigecycline	0.12	0.12	0.25	0.25	0.12	0.12
H. influenzae	ATCC 49247	TP-434	0.25	0.25	0.12	0.25	NG	NG
		tigecycline	>2	1	0.5	0.5	NG	NG

[a]Standard pH
NG, no growth

Table 9. TP-434 and tigecycline MICs (µg/ml) and the effects of polysorbate at 0.002%

Organisms	Isolate ID	Replicate Agent	w/out polysorbate[a] 1	w/out polysorbate[a] 2	w/ polysorbate (0.002%) 1	w/ polysorbate (0.002%) 2
S. aureus	ATCC 29213	TP-434	0.06	0.03	0.12	0.12
		tigecycline	0.12	0.12	0.12	0.12
E. faecalis	ATCC 29212	TP-434	0.015	0.015	0.06	0.06
		tigecycline	0.03	0.03	0.12	0.12
S. pneumoniae	ATCC 49619	TP-434	0.015	0.015	0.015	0.015
		tigecycline	0.03	0.03	0.03	0.03
E. coli	ATCC 25922	TP-434	0.12	0.06	0.06	0.06
		tigecycline	0.12	0.12	0.12	0.12
H. influenzae	ATCC 49247	TP-434	0.25	0.25	0.25	0.25
		tigecycline	>2	1	1	1

[a]Standard concentration

Table 10. TP-434 and tigecycline MICs (µg/ml) and the effects of incubation in CO_2

Organisms	Isolate ID	Replicate Agent	ambient air[a] 1	ambient air[a] 2	with 5% CO_2 1	with 5% CO_2 2
S. aureus	ATCC 29213	TP-434	0.06	0.03	0.03	0.03
		tigecycline	0.12	0.12	0.12	0.12
E. faecalis	ATCC 29212	TP-434	0.015	0.015	0.03	0.015
		tigecycline	0.03	0.03	0.03	0.03
S. pneumoniae	ATCC 49619	TP-434	0.015	0.015	0.015	0.015
		tigecycline	0.03	0.03	0.03	0.03
E. coli	ATCC 25922	TP-434	0.12	0.06	0.06	0.06
		tigecycline	0.12	0.12	0.12	0.12
H. influenzae	ATCC 49247	TP-434	0.25	0.25	0.25	0.25
		tigecycline	>2	1	1	2

[a]Standard concentration

Conclusions

- The *in vitro* antibacterial activity of TP-434 appears to be largely unaffected by media age, media additives and non-standard growth conditions.
- For testing TP-434, TIG and TET against *E. faecalis*, the strongest correlation of broth microdilution MIC values versus agar dilution MIC values was obtained when “haze” was read as growth.