

# P 1142 In Vitro Activity of TP-2758 against Panels of Recent Bacterial Clinical Isolates

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## Revised Abstract

**Background:** TP-2758 is a novel, fully-synthetic IV/oral antibiotic based on the proven tetracycline core. TP-2758 was selected from more than 2000 analogs on the basis of its potent antibacterial activity, especially against multidrug-resistant (MDR) gram-negative bacteria.

**Methods:** Using standard CLSI methodology, TP-2758 and clinical comparators were tested against recent clinical isolates. *In vitro* bactericidal activity over 24 hours was determined using standard time-kill assays with vigorous aeration.

**Results:** MIC<sub>90</sub> results are in Tables 1-3. TP-2758 was bactericidal against some, but not all gram-negative isolates, including *Enterobacteriaceae* producing extended-spectrum beta-lactamases (ESBL), *Acinetobacter baumannii*, and *Proteus mirabilis*. TP-2758 also had good antimicrobial potency against MDR gram-positive pathogens, with representative MIC<sub>90</sub> values of 0.5, 0.13, 1, 2, and 0.13 µg/mL for MRSA (n=136), MSSA (n=50), *Enterococcus faecalis* (n=157), *Enterococcus faecium* (n=115), and *Streptococcus pneumoniae* (n=111) respectively.

**Conclusion:** TP-2758 is 2- to 4-fold more potent than tigecycline against gram-negative pathogens and less active against gram-positive pathogens. This novel tetracycline strongly targets MDR gram-negative bacteria and its potential as a unique IV/oral therapy for treatment of ESBL-producing and carbapenem-resistant

## Introduction

Complicated urinary tract infections (cUTIs) are predominantly caused by gram-negative bacteria - *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* - but nosocomial cUTIs have entered and staphylococci as part of the epidemiology. A recent population-based study noted an overall rate of pyelonephritis, the most severe manifestation of UTIs, of 15-17 cases per 10,000 women and 3-4 cases per 10,000 men (1). The in-hospital mortality rate for pyelonephritis varies from 7.3 cases per 1000 hospitalized women to 16.5 cases per 1000 hospitalized men (2). There has been an increase in trimethoprim-sulfamethoxazole (TMP-SMX) resistance, especially in *E. coli*, the most common cause of both nosocomial and community-acquired pyelonephritis (3). Thus, the IDSA guidelines for treatment of uncomplicated pyelonephritis recommend a fluoroquinolone (FQ) as first-line therapy (4). Given the 30% resistance to TMP-SMX, the use of FQs has significantly increased (doubled), and will undoubtedly result in FQ resistance being even more prevalent in UTI pathogens. The majority of these patients are seen in the emergency room where empiric therapy is utilized. The increasing rate of extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* and FQ resistance in the predominant pathogens will severely limit, or eliminate, oral options for treatment of ambulatory patients with even uncomplicated UTIs/pyelonephritis. Although fosfomycin and nitrofurantoin are possible oral options for cystitis, they are not recommended for pyelonephritis (4).

TP-2758, a novel fully synthetic tetracycline-class antibiotic modified at C7 and C8, was designed to have a broad antibacterial spectrum with potent activity against problematic multidrug-resistant gram-negative bacteria such as those found in urinary tract infections. It has the potential to be used as monotherapy for nosocomial cUTIs because it also has activity against all the major gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*. TP-2758 shows oral efficacy in animal models of infection UTI/pyelonephritis, including those challenged with ESBL-producing *E. coli* and *K. pneumoniae* (see abstract O 96). The oral and IV pharmacokinetics of TP-2758 in rat and monkey are promising for use of this compound in treating serious hospital infections empirically including cUTIs.

## Methods

**MIC assays.** TP-2758 was tested against panels of recent clinical aerobic isolates, including quality control strains according to methods published by Clinical and Laboratory Standards Institute (CLSI) (5, 6). Recent clinical isolate collections include strains from Eurofins Medinet (Chantilly, VA) and IHMA (Schaumburg, IL). PCR-characterization of extended spectrum beta-lactamases was done at IHMA or by standard PCR methodology at Tetraphase Pharmaceuticals using published primers (7).

**Time-kill assays.** The minimal inhibitory concentration (MIC) values were determined for antibiotic stocks as per CLSI standardized methodology prior to running time-kill assays. Time-kill assays were performed essentially as described by CLSI guidelines (8), with the following modifications: five milliliter cultures inoculated to a final starting density of ~1 x 10<sup>5</sup> - 1 x 10<sup>6</sup> colony forming units (CFU/ml) were shaken vigorously (300 rpm) at 35°C in 50 ml polypropylene conical tubes. Cultures were sampled at various time points, serially diluted in sterile saline, and plated on tryptic soy agar. The lower limit of detection per culture was 100 CFU/ml.

**Post-antibiotic effect (PAE) assays.** PAE assays were performed essentially as described by Craig et al. (9). Five milliliters of broth were inoculated with fresh colonies grown overnight on agar medium to a final starting density of ~1 x 10<sup>5</sup> - 1 x 10<sup>6</sup> colony forming units (CFU)/ml. Starter cultures were grown for two hours with vigorous aeration (300 rpm) at 35°C in 50 ml polypropylene conical tubes prior to drug exposure. At the start of antibiotic exposure, the starter culture (0.5 ml) was transferred to 4.5 ml pre-warmed medium containing final concentrations of 0X, 0.008X, 2X, 4X and 8X MIC TP-2758 in 50 ml conical tubes. Following one hour of drug exposure with vigorous aeration at 35°C, cultures were diluted either 1:100 or 1:1000 into pre-warmed TP-2758-free medium for monitoring post-antibiotic effect. The "0X" control was handled exactly as antibiotic-treated cultures except that no antibiotic was present during the antibiotic exposure period. The "0.008X" control contained a constant 0.008X MIC TP-2758 during antibiotic exposure and following the shift to TP-2758-free medium; this control confirmed that compound carry-over at the highest test concentration (8X MIC) had no significant effect on growth. Cultures were sampled at various timepoints (E0, start of exposure; E1, end of one hour exposure; S0, immediately post-dilution, and every hour following dilution into TP-2758-free medium). Samples were serially diluted in sterile saline, plated onto tryptic soy agar, and grown overnight at 35°C for viability counts. The lower limit of detection per culture was 100 CFU/ml.

## Summary

- TP-2758 demonstrated potent, broad-spectrum gram-negative antibacterial activity with MIC<sub>90</sub> values generally lower than or equivalent to comparators

- MIC<sub>90</sub> values against aerobic and anaerobic gram-positives were 0.06-2 µg/ml

- TP-2758 was active against *Enterobacteriaceae* isolates expressing one or more ESBLs, (TEM, SHV, CTX-M, OXA), AmpC beta-lactamases (DHA, FOX, ACT, CMY), and carbapenemases (OXA, KPC, and NDM-1)

- TP-2758 is bactericidal against some multidrug-resistant gram-negative isolates

- TP-2758 produced PAEs that may contribute to the pharmacodynamics of TP-2758

- TP-2758 is currently being investigated in a Phase 1 trial for safety, tolerability, and pharmacokinetics following oral administration

## MIC Results

Table 1. Activity of TP-2758 and comparators against gram-negative pathogens

Organism	N	MIC <sub>50</sub> /MIC <sub>90</sub> (µg/ml)						
		TP-2758	Tigecycline	Carbapenem	Fluoroquinolone	3 <sup>rd</sup> Generation Cephalosporin	Gentamicin	Piperacillin/Tazobactam
<i>Klebsiella pneumoniae</i>	164	0.25/0.5 0.063-8	0.5/2 0.13-16	0.063/16 0.03->32	8/>32 0.03->64	4/>32 0.03->64	8/>32 0.03->64	16/>128 0.5->128
<i>Klebsiella pneumoniae</i> ESBL	79	0.25/1 0.063-8	1/4 0.25-16	0.063/32 0.03->32	8/>32 0.03->64	>32/>64 2->64	>32/>64 0.5->128	16/>128 0.5->128
<i>Klebsiella pneumoniae</i> KPC	20	0.5/1 0.25-8	1/2 0.5-16	32/>32 4->32	>32/>32 4->32	>32/>32 2->32	>128/>128 >128->128	
<i>Klebsiella oxytoca</i>	41	0.25/0.5 ≤0.016-1	0.5/2 0.063-4	≤1-≤1 0.03-1	≤0.25/>4 0.03->32	≤0.5/>32 ≤0.5->32	0.5/>32 ≤0.5->32	2/32 ≤0.5->64
<i>Klebsiella oxytoca</i> (ESBL)	11	0.125/0.25 ≤0.016-0.5	0.25/0.5 0.063-1	0.063/0.25 0.03-1	0.5/>4 4->32	>32/>32 ≤0.5->32	8/32 0.5->32	8/32 0.5->32
<i>Escherichia coli</i>	121	0.12/0.25 ≤0.016-0.5	0.25/1 0.031->8	0.031/0.5 ≤0.016->32	0.5/>32 ≤0.016->32	32/>64 0.063->64	2/>32 ≤0.25->32	4/>64 ≤0.5->128
<i>Escherichia coli</i> (ESBL)	82	0.12/0.25 ≤0.016-0.5	0.25/2 0.063->8	0.031/0.13 ≤0.016->32	>4/32 0.03->32	>32/>64 0.5->64	8/>32 ≤0.5->32	8/>64 ≤0.5->128
<i>Proteus mirabilis</i>	98	2/2 0.25-8	4/8 2-16	4/16 0.063->32	0.063/8 ≤0.03->64	0.063/0.5 ≤0.03->64	2/32 0.5->64	0.25/1 ≤0.063-2
<i>Proteus vulgaris</i>	54	0.5/1 0.13-2	2/4 0.5-8	≤1/4 ≤0.5-4	≤0.25/0.25 ≤0.25-1	≤0.5/32 ≤0.03->64	1/4 ≤0.25->8	≤0.5/1 ≤0.5-4
<i>Enterobacter cloacae</i>	98	0.25/2 0.063-4	1/4 0.25-8	≤1/4 0.031->32	0.25/16 ≤0.016->32	>32/>64 0.13->64	1/16 ≤0.25->32	>64/>64 1->128
<i>Enterobacter aerogenes</i>	30	0.12/0.25 0.06-1	0.5/0.5 0.25-4	≤1/1 0.25-4	≤0.25/0.25 ≤0.25-4	≤0.5/16 ≤0.5->64	≤0.25/0.5 ≤0.5->64	2/16 ≤0.5->64
<i>Salmonella spp.</i>	30	0.12/0.12 0.06-0.25	0.25/0.5 0.12-1	≤1/1 ≤1-8	≤0.25/0.25 ≤0.25->4	≤0.5/0.5 ≤0.5->8	0.5/1 1-64	2/4 1-64
<i>Serratia marcescens</i>	35	0.25/0.5 0.12-0.5	1/2 0.5-2	≤1/4 ≤1-32	≤0.25/4 ≤0.25-16	≤0.5/>32 ≤0.5->64	0.5/16 ≤0.25->32	2/16 1-32
<i>Shigella spp.</i>	30	0.12/0.25 0.03-0.25	0.25/0.5 0.12-1	≤1/1 ≤1-51	≤0.25/0.5 ≤0.25-1	≤0.5/0.5 ≤0.008->2	1/1 ≤0.25->8	2/2 ≤0.5-4
<i>Citrobacter freundii</i>	50	0.25/0.5 0.06-0.5	0.5/1 0.12-2	≤0.5/1 ≤0.5-2	≤0.25/>4 ≤0.25->4	1/64 ≤0.5->64	0.5/8 ≤0.25->8	4/>64 ≤0.5->64
<i>Morganella morganii</i>	30	0.5/1 0.25-2	2/4 0.25-8	≤0.5/4 ≤0.5-16	≤0.25/4 ≤0.25-16	≤0.5/4 0.5->8	1/8 ≤0.5/1	≤0.5/1 ≤0.5/1
<i>Providencia stuartii</i>	48	1/2 0.12-8	2/8 0.25-16	1/2 ≤0.5-2	4/4 ≤0.25->4	≤0.03-4 ≤0.03-32	2/8 ≤0.25->8	2/8 1-32
<i>Acinetobacter baumannii</i>	59	0.13/1 ≤0.016-4	0.5/4 ≤0.016-8	4/32 0.13->32	4/32 0.063->32	>16/16 0.12->16	4/32 0.5->32	1/128 128->128
<i>Acinetobacter lwoffii</i>	29	0.06/0.12 ≤0.016-0.12	0.12/0.5 0.06-0.5	≤1/4 ≤1-8	≤0.25/0.25 ≤0.25-2	1/16 ≤0.5->64	≤0.25/1 ≤0.25->8	≤0.5/8 ≤0.5-16
<i>Stenotrophomonas maltophilia</i>	79	0.25/1 ≤0.016-8	0.5/2 0.031-8	>32/>32 0.031-8	1/8 0.13-32	>32/>32 1->64	16/>32 8->32	128/>128 8->32
<i>Burkholderia cenocepacia</i>	10	8/16 0.13->32	8/32 0.25->32	32/>32 4->32	4/8 0.5->32	16/32 2->32	>32/>32 >32->32	16/>128 0.5->128
<i>Pseudomonas aeruginosa</i>	36	16/32 0.5->32	16/32 1->32	1/32 0.5-32	2/32 0.25->32	4/32 1->32	ND ND	ND ND
<i>Moraxella catarrhalis</i>	64	0.03/0.03 ≤0.015-0.03	0.06/0.06 ≤0.016-0.12	≤0.25/≤0.25 <sup>a</sup> ≤0.25-≤0.25	0.06/0.06 ≤0.03-0.06	≤0.5/0.5 <sup>b</sup> ≤0.5-2	0.12/0.12 <sup>b</sup> ≤0.06-0.25	ND ND
<i>Haemophilus influenzae</i>	64	0.08/0.12 ≤0.016-0.12	0.12/0.25 ≤0.016-0.5	1/2 <sup>a</sup> 0.06-4	0.03/0.03 ≤0.016-0.13	≤0.016/≤0.03 ≤0.016-0.063	ND ND	ND ND
<i>Nisseria gonorrhoeae</i> <sup>a</sup>	40	0.015/0.06 0.008-0.12	0.06/0.25 ≤0.004-0.5	ND ND	0.008/2 ≤0.0005->2	0.004/0.03 ≤0.002-1	ND ≤0.25/2 <sup>a</sup>	ND ≤0.03->4
<i>Legionella pneumophila</i>	70	0.12/0.5 ≤0.004-1	ND ND	ND ND	ND ND	ND ND	ND ND	ND ND

Table 3. Activity of TP-2758 and comparators against anaerobes

Organism	N	MIC <sub>50</sub> /MIC <sub>90</sub> (µg/ml)					
		TP-2758	Tigecycline	Imipenem	Clindamycin	Metronidazole	Piperacillin/Tazobactam
<i>Bacteroides fragilis</i>	10	1/2 0.13-8	1/8 0.5-8	0.25/1 0.13-2	1/16 ≤0.03->16	1/1 1-1	0.5/2 0.13-4
<i>Bacteroides spp. non-fragilis</i>	10	1/8 0.06-8	2/16 0.25-16	0.5/8 0.12-8	4/16 1-16	1/1 0.25->64	8/>128 1->128
<i>Clostridium difficile</i>	10	0.03/0.06 0.015-0.12	0.06/0.12 0.06-0.12	4/8 1-8	8/16 1-16	0.25/0.5 0.25-1	4/8 2-8
<i>Clostridium perfringens</i>	10	0.06/2 0.06-4	0.25/1 0.12-2	0.12/0.25 0.03-2	2/8 0.06->16	1/2 0.25-2	≤0.06/1 ≤0.06-16
<i>Eubacterium lentum</i>	11	0.12/0.25 0.06-0.5	0.12/0.25 0.12-0.5	0.5/0.5 ≤0.016-1	0.25/64 0.12->64	2/2 1->64	16/32 ≤0.06-32
<i>Fusobacterium spp.</i>	12	0.015/0.06 0.008-0.12	0.03/0.12 ≤0.004-0.25	≤0.016/0.03 ≤0.016-1	≤0.03/0.5 ≤0.03-8	≤0.06/0.5 ≤0.06->64	≤0.06/≤0.06 ≤0.06-2
<i>Propionibacterium acnes</i>	10	0.06/0.06 0.03-0.06	0.12/0.12 0.06-0.12	≤0.016/≤0.016 ≤0.016-0.03	0.06/0.06 ≤0.03-0.06	>64/>64	≤0.06/≤0.06 ≤0.06-1
<i>Porphyromonas asaccharolytica</i>	9	0.016/NA 0.015-0.25	0.03/NA 0.03-12	0.03/NA ≤0.015-0.03	≤0.03/NA ≤0.03-16	0.25/NA ≤0.06-0.25	≤0.06/NA ≤0.06-12
<i>Peptostreptococcus spp.</i>	11	0.06/0.25 0.03-0.25	0.06/0.12 0.03-0.5	≤0.016/0.5 ≤0.016-0.5	0.12/16 ≤0.03-16	1/1 0.25->64	≤0.06-2 ≤0.06-8
<i>Prevotella spp.</i>	10	0.12/1 0.06-4	0.25/0.5 0.12-8	≤0.016/0.5 ≤0.015->16	≤0.03/16 ≤0.03->16	1/4 0.25-4	≤0.06/32 ≤0.06-64

Table 2. Activity of TP-2758 and comparators against gram-positive pathogens

Organism	N	MIC <sub>50</sub> /MIC <sub>90</sub> (µg/ml)				
		TP-2758	Tigecycline	Linezolid	Levofloxacin	Vancomycin
<i>Staphylococcus aureus</i> (MRSA)	105	0.13/0.5 0.03-4	0.13/0.25 0.06-1	2/4 1-64	4/16-64 0.13->64	1/1 0.5-2
<i>Staphylococcus aureus</i> (MRSA, PVL+)	30	0.06/0.06 0.03-0.06	0.12/0.13 0.06-0.13	1/2 1-2	0.25/2 0.25-2	1/1 1-1
<i>Staphylococcus aureus</i> (MSSA)	50	0.06/0.13 0.06-0.13	0.12/0.25 0.06-0.25	2/4 1-4	0.25/1 0.13->2	1/1 1-2
<i>Staphylococcus epidermidis meth-R</i>	26	0.06/0.25 0.03-0.25	0.06/0.13 0.03-0.25	<0.5/1 ≤0.5-2	>4/4 0.12->4	1/2 0.5-2
<i>Staphylococcus epidermidis meth-S</i>	26	0.06/0.13 0.03-0.25	0.06/0.25 0.03-0.25	1/1 ≤0.5-2	0.25/4 0.12->4	2/2 1-2
<i>Staphylococcus saprophyticus</i>	49	0.13/0.25 0.06-0.25	0.13/0.25 0.06-0.25	2/4 1-8	0.5/0.5 0.25-1	1/1 0.5-2
<i>Enterococcus faecalis</i>	157	0.5/1 0.031-16	0.06/0.13 ≤0.016-1	2/2 ≤0.5->32	>4/32 0.5->32	2/32 1->32
<i>Enterococcus faecalis</i> VRE	64	0.5/1 0.031-4	0.06/0.13 ≤0.016-0.5	2/2 ≤0.5-32	>4/32 1->32	>16/32 16->32
<i>Enterococcus faecalis</i> VSE	93	0.5/2 0.031-16	0.06/0.13 ≤0.016-1	2/4 1->32	1/32 0.5->32	1/2 1-4
<i>Enterococcus faecium</i>	115	0.06/0.5 ≤0.016->4	0.06/0.13 ≤0.016-1	2/4 ≤0.5->32	>4/32 0.5->32	>16/32 ≤0.25->32
<i>Enterococcus faecium</i> VRE	65	0.12/1 ≤0.016->4	0.06/0.13 ≤0.016-1	2/4 ≤0.5->32	>4/32 4->32	>16/32 8->32
<i>Enterococcus faecium</i> VSE	50	0.06/0.5 0.03-0.5	0.06/0.06 ≤0.016-0.13	2/2 ≤0.5-2	>4/4 0.5->32	0.5/1 ≤0.25-4
<i>Streptococcus pneumoniae</i>	111	0.03/0.13 ≤0.016-0.5	0.06/0.06 ≤0.016-0.12	1/1 0.5-1	0.5/1 0.5->4	0.25/0.5 0.13-0.5
<i>Streptococcus anginosus</i>	42	0.03/0.5 ≤0.008-4	0.016/0.06 0.008-0.25	1/2 ≤0.25-2	0.5/1 ≤0.25-2	0.5/1 ≤0.06-1
<i>Streptococcus intermedius</i>	30	0.03/0.12 ≤0.008-0.25	0.03/0.12 ≤0.008-0.25	1/1 ≤0.25-1	1/2 ≤0.25->4	0.5/0.5 ≤0.06-0.5
<i>Streptococcus mitis</i>	29	0.03/0.25 ≤0.008-1	0.03/0.12 ≤0.008-0.25	1/1 0.5-1	1/2 0.5-2	0.5/0.5 ≤0.06-0.5
<i>Streptococcus sanguis</i>	18	0.015/0.12 ≤0.008-0.25	0.03/0.06 ≤0.008-0.12	0.5/1 ≤0.25-1	0.5/2 ≤0.25-2	0.5/1 ≤0.06-1
<i>Streptococcus pyogenes</i>	64	0.03/0.25 ≤0.008-1	0.06/0.06 ≤0.016-0.12	1/2 0.5-4	0.5/1 ≤0.004-2	0.5/0.5 ≤0.06-0.5

Table 1. Carbapenem=ertapenem, imipenem or meropenem; FQ=levofloxacin or ciprofloxacin; 3<sup>rd</sup> GC=cefotaxime, ceftazidime or ceftioxime; ND = not done; \*H. influenzae, 50 isolates; \*M. catarrhalis, 50 isolates; \*P. mirabilis, 89 isolates; \*Data for penicillin: \*17.5% and 30% of isolates were tetracycline-resistant and intermediate resistant, respectively; ND=not done

## Time Kill Assays

