

# TP-6076 is active against bacterial isolates carrying emergent resistance types

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## Abstract

**Background.** Transferrable and mutation-based resistance amongst clinically relevant Gram-negative pathogens remains an ongoing impediment to the development of novel antimicrobial therapies. While there are a number of new agents in development for the treatment of multidrug-resistant and carbapenem-resistant Enterobacteriaceae (CRE) and *Acinetobacter baumannii* (CRAB), coverage gaps due to resistance persist. TP-6076 was screened against a number Enterobacteriaceae and *Acinetobacter* isolates from the CDC antimicrobial resistance bank and clinical sources and analyzed for activity against various resistance types. TP-6076 was assayed for activity against isolates carrying various RNA methylase genes, genes conferring fosfomycin resistance, tetracycline efflux pump genes, porin mutations, and those isolates resistant to ceftazidime-avibactam (Caz-Avi).

**Methods.** Susceptibility testing by broth microdilution minimal inhibitory concentration (MIC) assays was done using CLSI methodology. Isolates screened were from the CDC Antimicrobial Resistance Bank panels and the laboratory of Patrice Nordmann. Isolate resistance information is as provided by CDC whole genome sequencing data (RNA methylase, porin mutation, fosfomycin resistance) or PCR and sequencing performed for this study.

**Results.** The TP-6076 MIC<sub>50/90</sub> values for total Enterobacteriaceae, total *Acinetobacter*, and resistance subgroups are presented in the Table. Overall, TP-6076 MIC<sub>50/90</sub> values for resistant pathogens remained within one dilution of the MIC<sub>50/90</sub> value for all strains, 0.063/0.5 µg/ml for *Enterobacteriaceae* and 0.016/0.063 µg/ml for *A. baumannii*.

**Conclusions.** TP-6076 retained potency *in vitro* against a number of emergent resistance types and represents a promising candidate in development for the treatment of multidrug resistant Gram-negative pathogens.

Resistance Group (n)	TP-6076 MIC <sub>50/90</sub> (Range) µg/mL	Resistance Group (n)	TP-6076 MIC <sub>50/90</sub> (Range) µg/mL
Total Enterobacteriaceae (176)	0.063/0.5 (0.004 – 1)	Total <i>Acinetobacter</i> (55)	0.016/0.063 (0.004 – 0.25)
RNA methylase (26)	0.063/0.5 (0.004 – 1)	RNA methylase (21)	0.008/0.031 (0.004 – 0.063)
<i>fosA</i> (22)	0.063/0.25 (0.008 – 0.5)	Caz-Avi Resistant (54)	0.016/0.063 (0.004 – 0.25)
metallo-beta-lactamase positive (41)	0.063/0.5 (0.004 – 1)	Tetracycline Efflux Pump (28)	0.016/0.063 (0.004 – 0.25)
ΔompK35/36 (33)	0.063/0.5 (0.008 – 1)		
Caz-Avi Resistant (47)	0.031/0.5 (0.004 – 1)		
Tetracycline Efflux Pump (59)	0.031/0.5 (0.004 – 1)		

## Background

Transferrable and mutation-based resistance amongst clinically relevant Gram-negative pathogens remains an ongoing impediment to the development of novel antimicrobial therapies. While there are a number of new agents in development for the treatment of multidrug-resistant and carbapenem-resistant Enterobacteriaceae (CRE) and *Acinetobacter baumannii* (CRAB), coverage gaps due to resistance persist. Novel antimicrobial agents in the aminoglycoside class have been shown to be hindered in activity by transferable 16S ribosomal RNA methyltransferase genes, which are often colocalized with the *bla*<sub>NDM</sub> gene in Enterobacteriaceae<sup>1</sup>. Resistance to ceftazidime-avibactam has been reported in a number of species due to mutations in the *bla*<sub>KPC</sub> gene or due to porin mutations<sup>2,3</sup>. Porin loss has similarly been associated with reduced activity of the meropenem-vaborbactam combination due to reduced uptake and competition for transport<sup>4</sup>. Increasing resistance to current therapies has renewed interest in older antimicrobial agents such as fosfomycin, however plasmid-mediated fosfomycin resistance genes remain a concern for the increased use of this drug<sup>5</sup>.

To better understand how these transferrable resistance elements may impact TP-6076, a fully synthetic tetracycline derivative antibiotic currently in development for treatment of Gram-negative infections, it was screened against a number of multidrug resistant Enterobacteriaceae and *Acinetobacter* isolates and analyzed for activity against various resistance types. TP-6076 maintained potency against isolates carrying rRNA methyltransferase genes, transferable fosfomycin resistance, tetracycline efflux pump genes, metallo-beta-lactamase genes, and porin mutations, as well as isolates classified by the CDC as resistant to ceftazidime-avibactam through various known and as yet undefined mechanisms.

## Materials / Methods

Susceptibility testing by broth microdilution minimal inhibitory concentration (MIC) assays was done using CLSI methodology<sup>6,7</sup>. Isolates screened were selected from the CDC Antimicrobial Resistance Bank panels and the laboratory of Dr. Patrice Nordmann (University of Fribourg). CDC panel isolates for this study were combined from the Enterobacteriaceae Carbapenem Breakpoint, Gram Negative Carbapenemase Detection, Enterobacteriaceae Carbapenemase Diversity, *Acinetobacter baumannii*, Novel Antibiotic Resistance, Ceftolozane/Tazobactam, Ceftazidime/Avibactam, Enteric Pathogen Diversity, and Imipenem/Relebactam panels. Due to the limited potency of tetracycline antibiotics against isolates of *P. aeruginosa*, those strains were excluded from analysis.

Isolate genotypic resistance information was analyzed as provided by CDC either as whole genome sequencing or targeted sequencing information, with additional tetracycline resistance typing done by PCR and sequencing performed at Tetraphase for this study as previously described<sup>8,9</sup>. Comparator antibiotics were purchased commercially through commercial sources (Sigma Aldrich, BioVision, or MedChem Express) and results checked against published QC ranges for ATCC control strains<sup>7</sup>.

## Results

**Table 1. TP-6076 activity against total and resistant subgroups of Enterobacteriaceae**

	Enterobacteriaceae								
	MIC <sub>50/90</sub> range	Total (221)	RNA Methylase (30)	<i>fosA</i> (23)	ΔompK35/36 (33)	Caz-Avi Resistant (47)	Metallo-beta-lactamase+ (47)	NDM+ (37)	Tetracycline Resistance (71)
<b>TP-6076</b>	<b>0.031/0.25</b>	<b>0.063/0.25</b>	<b>0.063/0.25</b>	<b>0.063/0.5</b>	<b>0.031/0.5</b>	<b>0.063/0.5</b>	<b>0.063/0.5</b>	<b>0.063/0.5</b>	<b>0.031/0.5</b>
	<b>0.004 - 1</b>	<b>0.004 - 1</b>	<b>0.008 - 0.5</b>	<b>0.008 - 1</b>	<b>0.004 - 1</b>	<b>0.004 - 1</b>	<b>0.004 - 1</b>	<b>0.004 - 1</b>	<b>0.004 - 1</b>
<b>Tigecycline</b>	0.25/2	0.5/4	0.5/1	0.5/2	0.25/4	0.5/4	0.5/4	0.5/4	0.25/2
	0.016 - 8	0.063 - 4	0.063 - 2	0.031 - 4	0.063 - 4	0.063 - 4	0.063 - 4	0.063 - 4	0.016 - 8
<b>Minocycline</b>	8/>32	8/>32	8/32	8/>32	8/>32	16/>32	16/>32	16/>32	8/>32
	0.25 - >32	1 - >32	1 - >32	1 - >32	0.5 - >32	0.5 - >32	0.5 - >32	0.5 - >32	0.5 - >32
<b>Meropenem</b>	4/>32	>32	32/>32	8/>32	>32	>32	>32	>32	1/>32
	≤0.016 - >32	0.031 - >32	0.031 - >32	0.063 - >32	≤0.016 - >32	2 - >32	4 - >32	4 - >32	≤0.016 - >32
<b>Meropenem/Vaborbactam</b>	0.063/>32	32/>32	0.5/>32	1/>32	32/>32	>32	>32	>32	0.063/>32
	≤0.016 - >32	0.031 - >32	0.031 - >32	≤0.016 - >32	≤0.016 - >32	1 - >32	2 - >32	2 - >32	≤0.016 - >32
<b>Ceftazidime/Avibactam</b>	1/>32	>32	2/>32	2/>32	>32	>32	>32	>32	1/>32
	0.063 - >32	1 - >32	0.125 - >32	0.5 - >32	16 - >32	>32	>32	>32	0.25 - >32
<b>Gentamicin</b>	4/>32	>32	16/>32	>32	>32	>32	>32	>32	32/>32
	0.125 - >32	4 - >32	0.25 - >32	0.25 - >32	0.5 - >32	0.5 - >32	0.5 - >32	0.5 - >32	0.5 - >32
<b>Levofloxacin</b>	16/>32	32/>32	8/>32	32/>32	32/>32	32/>32	32/>32	32/>32	16/>32
	0.031 - >32	0.063 - >32	0.031 - >32	0.125 - >32	0.5 - >32	0.031 - >32	0.063 - >32	0.031 - >32	0.031 - >32
<b>Colistin</b>	0.25/>32	0.25/16	0.25/8	0.25/>32	0.25/32	0.25/16	0.25/32	0.25/32	0.25/32
	0.063 - >32	0.063 - >32	0.063 - >32	0.063 - >32	0.125 - >32	0.125 - >32	0.125 - >32	0.125 - >32	0.063 - >32
<b>Sulopenem</b>	8/>32	>32	32/>32	32/>32	>32	>32	>32	>32	4/>32
	≤0.016 - >32	0.125 - >32	0.031 - >32	0.25 - >32	0.063 - >32	4 - >32	4 - >32	4 - >32	0.031 - >32

Enterobacteriaceae include *E. coli* (63), and isolates from various species of *Klebsiella* (73), *Enterobacter* (30), *Proteus* (7), *Citrobacter* (7), *Salmonella* (13), *Shigella* (7), *Serratia* (12), *Morganella* (6), *Providencia* (2), *Kluyvera* (1). Resistance classifications were adapted from whole genome sequencing and resistance data provided by the CDC Antimicrobial Resistance Bank where available with supplemental and confirmatory PCR and sequencing performed by Tetraphase Pharmaceuticals. Metallo-beta-lactamase producing strains included the *bla*<sub>NDM</sub> (36), *bla*<sub>KPC</sub> (4) and *bla*<sub>OXA-48</sub> (6) families, while rRNA methylase genes included *armA* (19), *rmtB* (2), *rmtC* (8), *rmtF* (2), and *rmtG* (1) genes with some isolates harboring more than one gene. Tetracycline resistance genes included tetracycline specific efflux pumps *tet(A)*, *tet(B)*, *tet(C)*, and *tet(J)* identified alone or in tandem by PCR. Multiple resistance types were frequently identified within single isolates; 21 of 30 strains reported positive for rRNA methylase genes were also positive for metallo-beta-lactamase enzymes, 19 of these 30 were resistance to ceftazidime-avibactam, 13 of 30 screened positive for a tetracycline specific efflux pump, and 9 of 30 had reported mutations associated with *ompK35* or *ompK36*. Likewise, 19 isolates screened positive for both a metallo-beta-lactamase and tetracycline efflux pump, 14 carried an *ompK35/36* mutation and a tetracycline efflux gene, and four carried both *fosA* and a tetracycline efflux pump.

- TP-6076 MIC<sub>50/90</sub> and range values for total *Enterobacteriaceae* were 0.031/0.25 µg/ml and 0.004 – 1 µg/ml respectively, and remained within a dilution of these values across all resistance types screened in this analysis.

**Table 2. TP-6076 activity against total and resistant subgroups of *A. baumannii***

	<i>Acinetobacter baumannii</i>			
	MIC <sub>50/90</sub> range	Total (55)	RNA Methylase (21)	Tetracycline Resistance (28)
<b>TP-6076</b>	<b>0.016/0.063</b>	<b>0.008/0.31</b>	<b>0.016/0.063</b>	<b>0.016/0.063</b>
	<b>0.004 - 0.25</b>	<b>0.002 - 0.063</b>	<b>0.004 - 0.25</b>	<b>0.004 - 0.25</b>
<b>Tigecycline</b>	2/4	2	2/4	2/4
	0.031 - 2	0.5 - 4	0.125 - 8	0.125 - 8
<b>Minocycline</b>	8/16	16	8/16	8/16
	0.016 - 32	1 - 16	0.016 - 32	1 - 32
<b>Meropenem</b>	>32	>32	>32	>32
	2 - >32	2 - >32	2 - >32	4 - >32
<b>Meropenem/Vaborbactam</b>	>32	>32	>32	>32
	2 - >32	2 - >32	2 - >32	4 - >32
<b>Ceftazidime/Avibactam</b>	>32	>32	>32	>32
	8 - >32	32 - >32	16 - >32	32 - >32
<b>Gentamicin</b>	>32	>32	>32	>32
	8 - >32	>32	8 - >32	8 - >32
<b>Levofloxacin</b>	16/>32	16/>32	16/>32	16/>32
	4 - >32	4 - >32	4 - >32	4 - >32
<b>Colistin</b>	0.5/4	0.5/4	0.5/4	0.5/2
	0.063 - >32	0.125 - >32	0.125 - >32	0.125 - >32
<b>Sulopenem</b>	>32	32/>32	>32	>32
	4 - >32	8 - >32	4 - >32	8 - >32

Resistance classifications were adapted as previously described. The rRNA methylase genes reported present in *A. baumannii* were strictly *armA*, while tetracycline specific efflux pumps were mainly represented by the *tet(B)* gene.

- TP-6076 MIC<sub>50/90</sub> and range values for total *A. baumannii* were 0.016/0.063 µg/ml and 0.004 – 0.25 µg/ml respectively, with the MIC<sub>50/90</sub> remaining within a two-fold dilution across each subset.

## Conclusions

TP-6076 retained a high degree of *in vitro* potency against isolates carrying a number of transferable resistance mechanisms, including those that have the potential to reduce efficacy of recently approved antimicrobial agents. TP-6076 is currently undergoing phase I trials and represents a promising new candidate for the treatment of infections caused by multidrug-resistant and carbapenem-resistant Gram-negative organisms.

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