

# P 1149 The Novel Broad-Spectrum Fluorocycline TP-434 is Active Against MDR Gram-Negative Pathogens

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## Revised Abstract

**Background:** TP-434 is a novel broad-spectrum IV/oral fluorocycline antibiotic being developed by Tetraphase Pharmaceuticals. TP-434 has potent activity against multidrug-resistant (MDR) gram-negative pathogens associated with complicated nosocomial and community infections, including *Enterobacteriaceae* such as *Escherichia coli* and *Klebsiella* spp. expressing extended-spectrum beta-lactamases (ESBL) and/or carbapenemases, MDR *Acinetobacter baumannii*, and anaerobic bacteria such as *Bacteroides fragilis*.

**Methods:** Using standard CLSI methodology, TP-434 and clinical comparators were tested against recent isolates of *E. coli* (n=176, 97 were ESBL), *K. pneumoniae* (n=219, 90 were ESBL), *K. oxytoca* (n=41, 11 were ESBL), *A. baumannii* (n=89) and *B. fragilis* (n=30). The ESBL genotype of select isolates was characterized by PCR. *In vitro* bactericidal activity over 24 hours was determined using standard time-kill assays with vigorous aeration in rich media. *In vivo* activity was assessed in a murine septicemia model challenged intraperitoneally with ESBL (SHV)-producing *E. coli* strain EC133 and TP-434 administered intravenously, measuring survival at 48 hours.

**Results:** TP-434 showed MIC<sub>50</sub>/MIC<sub>90</sub> values (microgram/milliliter) of 0.25/0.5, 0.5/2, 0.25/1, 0.5/2 and 0.5/1 against multidrug-resistant panels of *E. coli*, *K. pneumoniae*, *K. oxytoca*, *A. baumannii*, and *B. fragilis*, respectively. The presence of TEM, SHV, CTX-M, OXA, DHA, FOX, CMY, ACT, KPC ESBL genes had no impact on TP-434; MIC<sub>50</sub>/MIC<sub>90</sub> values (micrograms/milliliter) for ESBL panels of *E. coli*, *K. pneumoniae* and *K. oxytoca* were 0.25/0.5, 0.5/2 and 0.5/1, respectively. Notably, TP-434 showed ≥2-fold improved potency over tigecycline, a tetracycline-class clinical comparator, for 28%, 42%, 44%, 76% and 77% of *E. coli*, *K. pneumoniae*, *K. oxytoca*, *A. baumannii*, and *B. fragilis* isolates, respectively. TP-434 was bactericidal *in vitro* against *E. coli*, *K. pneumoniae*, and *A. baumannii*, including ESBL-expressing isolates, showing ≥3-log reductions in colony forming units over 24 hours. The IV PD<sub>50</sub> of TP-434 in a mouse septicemia model was 1.2 mg/kg versus a 3.5 mg/kg for tigecycline.

**Conclusion:** TP-434 shows superior activity *in vitro* versus clinical comparators against problematic gram-negative bacteria, supporting its advancement into a Phase 2 clinical trial for complicated intra-abdominal infections.

## Introduction

TP-434, a novel fully synthetic fluorocycline-class antibiotic, was designed to have a broad antibacterial spectrum with potent activity against problematic multidrug-resistant gram-negative bacteria. It also provides coverage of all gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus*, streptococci, and vancomycin-resistant enterococci (not shown). TP-434 is unaffected by resistance mechanisms to other antibiotics and is also active against tetracycline-resistant strains. TP-434 shows efficacy at low doses in animal models of infection, including those challenged with gram-negatives intraperitoneally. The potency, spectrum, and promising IV and oral pharmacokinetics of TP-434 in humans (see poster 1509) offers the potential to treat serious hospital infections empirically, including intra-abdominal, skin and respiratory infections, by both IV and oral step-down therapy. TP-434 IV is currently in a Phase 2 clinical trial for complicated community-acquired intra-abdominal infections.

## Methods

**MIC assays.** TP-434 was tested against panels of recent clinical aerobic isolates, including quality control strains according to methods published by Clinical and Laboratory Standards Institute (CLSI) (1, 2). Recent clinical isolate collections include strains from Micromyx LLC, (Kalamazoo, MI), Eurofins Medinet (Chantilly, VA) and IHMA (Schaumburg, IL). PCR-characterization of extended spectrum β-lactamases was done at IHMA or by standard PCR methodology at Tetraphase Pharmaceuticals using published primers (3).

**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 (4), 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.

**Mouse systemic infection model.** Bacteria prepared from an overnight plate culture were diluted in hog gastric mucin for injection. Mice (six per group) were infected with a bacterial inoculum of 6.25 x 10<sup>6</sup> CFU mouse. Bacteria were delivered via intraperitoneal injection (0.5 ml). Animals were treated with a single dose of TP-434, tigecycline or imipenem at 1 hour post-infection by tail vein injection. Survival was assessed up to 48 hours post-infection and percent survival was calculated and the dose (mg/kg) effecting 50% survival, the protective dose 50% (PD<sub>50</sub>) was determined along with 95% confidence intervals as calculated by Probit analysis using GraphPad Prism version 4.03 (GraphPad Software). In this model, the PD<sub>50</sub> for imipenem was <0.3 mg/kg.

**Antibacterial activity against E. coli DH10B recombinantly expressing tetracycline-resistance genes.** Genes encoding *tet(A)*, *tet(B)*, *tet(K)*, *tet(M)*, *tet(X)* and *E. coli* β-galactosidase (*lacZ*) as a control were amplified by PCR from clinical isolates confirmed by prior sequencing to have these tetracycline-resistance determinants and cloned into an L-arabinose inducible expression system without any affinity tags (pBAD-Myc-His, Invitrogen, Carlsbad, CA). The class B carbapenemase NDM-1 was also cloned into the same inducible expression system. Plasmids were transformed and expressed in *E. coli* DH10B cells (Invitrogen, Carlsbad, CA). Cloned inserts were sequenced to verify the resistance gene sequence and compared to reported sequences in GenBank (accession numbers: *tet(A)*, AJ419171; *tet(B)*, AP0961; *tet(K)*, AJ888003; *tet(M)*, X90939.1; *tet(X)*, M37699; and NDM-1, HQ162469). Cells were grown in Mueller Hinton Broth containing ampicillin, 50 μg/ml, pre-induced for 30 minutes with 1% arabinose (*tet(A)*, *tet(B)*, *tet(M)*, *tet(X)*, NDM-1) or 0.1% arabinose (*tet(K)*) at 30°C prior to use as inocula in MIC assays containing ampicillin, 50 μg/ml. Assays were incubated at 35°C as per CLSI guidelines.

- Clinical and Laboratory Standards Institute (CLSI). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*; Approved Standard—Eighth Edition. CLSI document M07-A8 [ISBN 1-56238-689-1]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2009.
- Clinical and Laboratory Standards Institute (CLSI). *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*; Approved Standard—Seventh Edition. CLSI document M11-A7 [ISBN 1-56238-626-3]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007.
- Dallenne, C., A. Da Costa, D. Decore, C. Favier, G. Arlet. 2010. *Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae*. J. Antimicrob. Chemother. 65:490-495.
- CLSI. *Methods for determining bactericidal activity of antimicrobial agents*; approved guideline. CLSI document M26-A., vol. 19, CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania, USA, 1999.

## Results

Figure 1. TP-434 is More Potent than Tigecycline Against Significant Numbers of Isolates

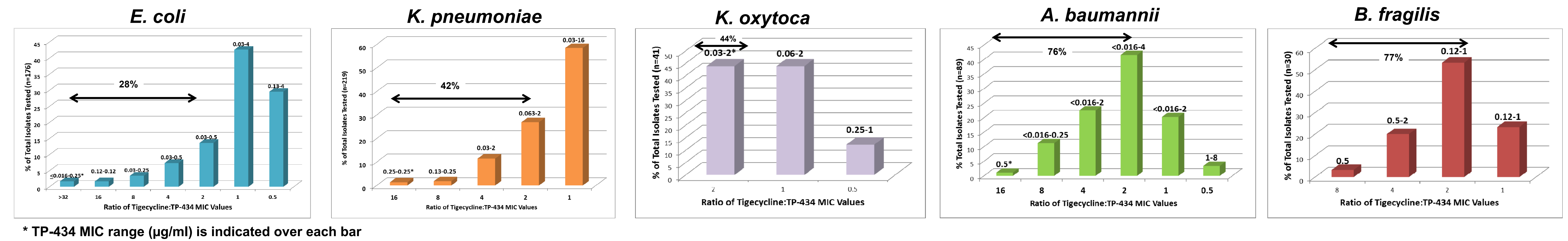


Table 1. TP-434 MIC<sub>50</sub>/MIC<sub>90</sub> Values for Panels of Gram-negative Isolates

Organism	Isolates	N	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>E. coli</i>	total	176	0.25	0.5
	ESBL <sup>#</sup>	97	0.25	0.5
	MDR*	55	0.25	0.5
<i>K. pneumoniae</i>	total	219	0.5	1
	ESBL	90	0.5	2
	MDR	110	0.5	2
<i>K. oxytoca</i>	total	41	0.25	1
	ESBL	11	0.5	1
<i>A. baumannii</i>	total	89	0.5	2
	MDR	41	1	2
<i>B. fragilis</i>	total	30	0.5	1

\* Subset of total isolates confirmed to be non-susceptible/resistant to members of at least 3 major antibiotic classes using CLSI S/I/R guidelines for tetracycline, carbapenems, fluoroquinolones, 3rd generation cephalosporins, and gentamicin

<sup>#</sup> Majority of ESBL strains had more than one β-lactamase or carbapenemase gene.  
Number of *E. coli* ESBL isolates: SHV (n=10), TEM (n=2), OXA (n=3), CTXM (30), DHA-1 (n=1), ACT (n=1), CMY (n=2);  
Number of *K. pneumoniae* ESBL isolates: SHV (n=45), KPC (n=20), CTXM (n=19), DHA-1 (n=1), FOX (n=1), OXA (n=1), TEM (n=1);  
Number of *K. oxytoca* ESBL isolates: SHV (n=4), CTXM (n=8), OXA (n=4), DHA (n=1)

Figure 2. Activity of TP-434 (IV) in a Murine Septicemia Model with *E. coli* EC133 (SHV, *tet(B)*)

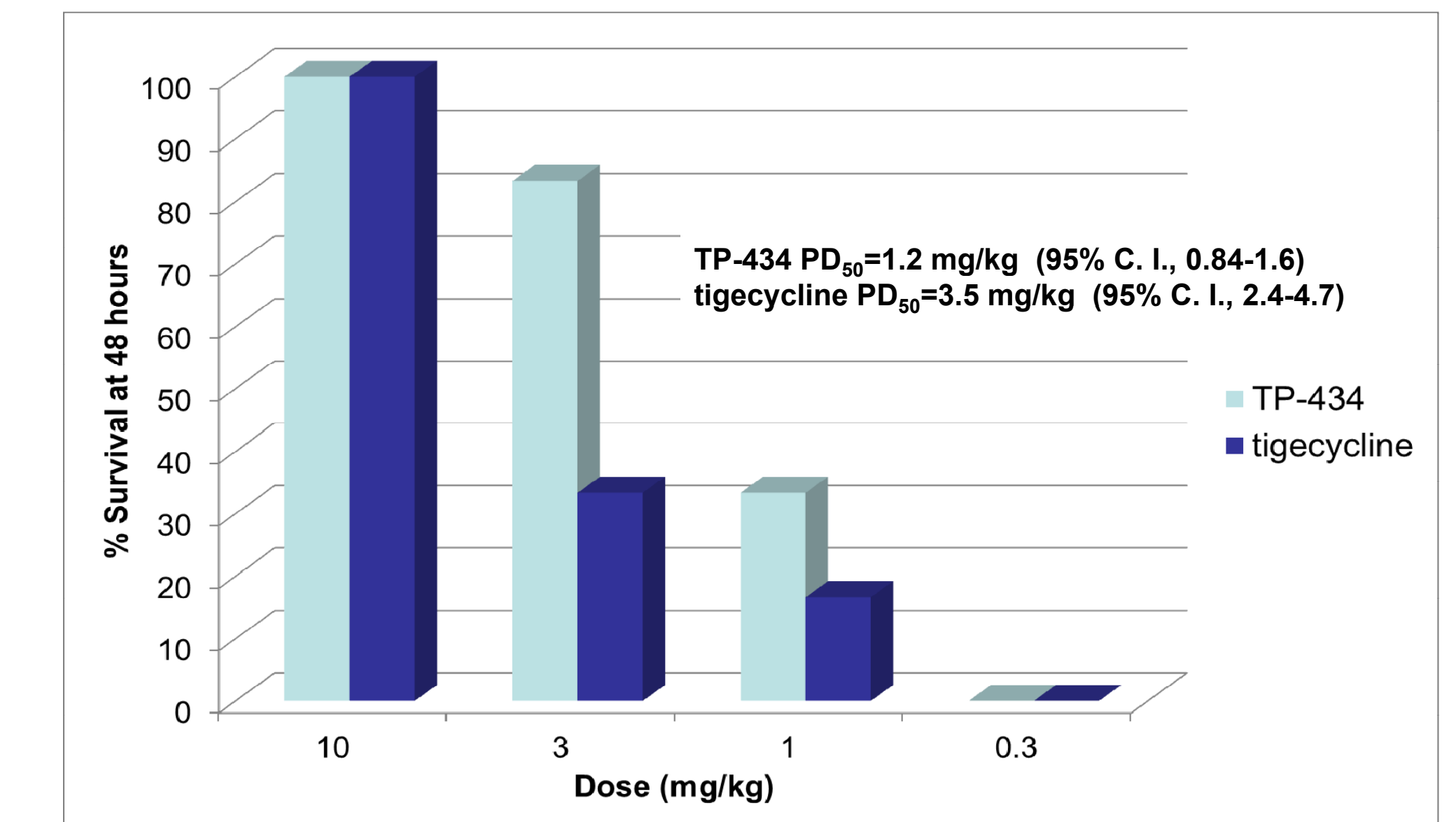


Figure 3. TP-434 Time Kill Assays

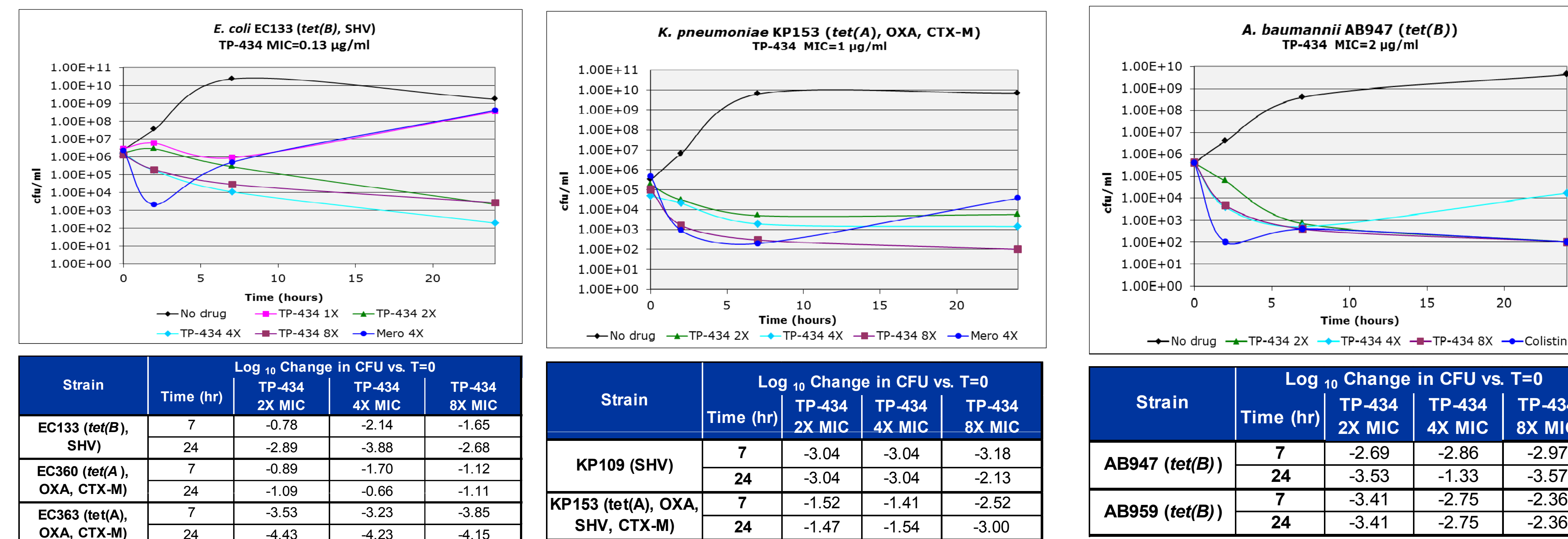
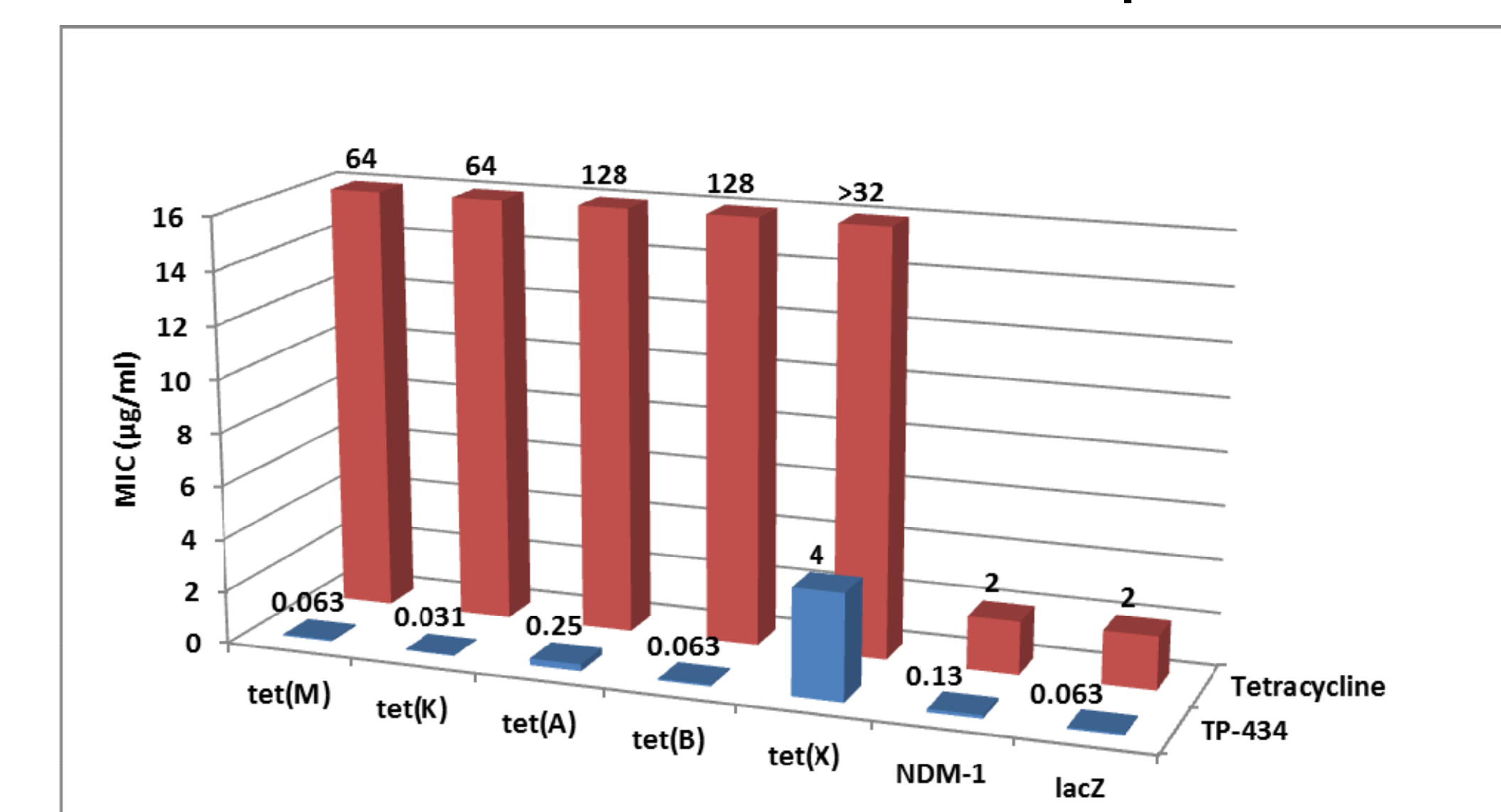


Figure 4. Activity Against Major Tetracycline-Resistance Mechanisms and NDM-1 Metallo-β-lactamase



Tetracycline and NDM-1 resistance genes are recombinantly over-expressed in *E. coli* DH10B; *lacZ* strain is negative control

## Conclusions

- TP-434 demonstrated potent, broad-spectrum gram-negative antibacterial activity, with ≥2-fold better potency over tigecycline against significant numbers of isolates.
- TP-434 showed superior activity over tetracycline, against major tetracycline-resistance mechanisms over-expressed recombinantly in *E. coli*.
- TP-434 was active against Enterobacteriaceae isolates expressing one or more ESBLs, including strains confirmed by PCR to contain genes encoding TEM, SHV, CTX-M, OXA, DHA, FOX, CMY, ACT β-lactamases, KPC and NDM-1 carbapenemases.
- TP-434 was bactericidal against some multidrug-resistant gram-negative isolates and exhibited >2-fold better potency vs. tigecycline in a mouse septicemia model against *E. coli* EC133, a tetracycline-resistant ESBL-expressing isolate.
- TP-434 is a clinical-stage antibiotic with excellent spectrum and potency necessary for the treatment of infections with a high incidence of gram-negative pathogens.