Bacteria can persist as biofilms in chronic and device-related infections. TP-6076 is a novel tetracycline-class antibiotic with potent antibacterial activity against Gram-negative pathogens, including carbapenem-resistant Enterobacteriaceae (CRE). TP-6076 was evaluated in vitro against biofilms formed in panel of ten uropathogenic Escherichia coli (E. coli) isolates, a standard model for clinical urinary tract infections.

Methods: The minimal inhibitory concentration (MIC) of compounds was determined according to CLSI guidelines except that tryptic soy broth/yeast extract (TSB/YE) medium was used. Biofilm assays were performed in triplicate. Cultures were grown in TSB/YE at 35°C for 24 hours, allowing for attainment of 1.5 OD at 620 nm. Following incubation, cultures were diluted to ~10^6 colony forming units (CFU) in TSB/YE, and 500 µL of culture was added to 5 mL round bottom polystyrene tubes (BD Falcon #352054; BD, Franklin Lakes, NJ) and incubated for an additional 24 hrs at 35°C. For staining biofilms, planktonic cells were aspirated, tubes were rinsed with water and stained with 0.1% crystal violet (CV). For biofilm quantification, planktonic cells were aspirated, tubes were washed with water and stained with LIVE/DEAD Biofilm Viability Kit (LIVE = SYTO® 9, DEAD = propidium iodide (PI), Molecular Probes, Eugene, OR), and quantified by flow cytometry.

Results: Results from MIC and biofilm assays were consistent. TP-6076 at 2 µg/mL effectively cleared biofilms produced by all isolates, including those that were fluoroquinolone-resistant. This activity of TP-6076 was not an artifact of an initially fragile biofilm. For the levofloxacin-resistant isolates, 200 µg of levofloxacin produced less biofilm for one isolate. The TP-6076 and levofloxacin susceptibilities of 24 and 48 hours biofilms were similar for both conditions, confirming that the activity of TP-6076 was not an artifact of an initially fragile biofilm. This in vitro activity of TP-6076, if continued in vivo, would support its potential use in the clinical treatment of chronic biofilm infections.

Conclusions: TP-6076 at 2 µg/mL effectively cleared biofilms produced by all isolates, including those that were fluoroquinolone-resistant, reducing biofilm CFUs by >98% of the initial biofilm inoculum. The activity of TP-6076 against E. coli was similar between 24 and 48 hours established biofilms. This in vitro anti-biofilm activity of TP-6076, if confirmed in vivo, would support its potential use in the clinical treatment of chronic biofilm infections.