P0054

25th ECCMID
25 April – 28 April 2015
Copenhagen, Denmark

TP-6076 is Active In Vitro Against Biofilms Formed by a Panel Containing Fluoroquinolone-Resistant and Susceptible Uropathogenic *Escherichia coli* Clinical Isolates

Kathy Kerstein, Joyce A. Sutcliffe, Trudy H. Grossman
Tetraphase Pharmaceuticals, Inc., Watertown, MA

Contact:
Teresa Dahlman
Tetraphase Pharmaceuticals, Inc.
TDahlman@tphase.com

Revised Abstract

Objective: 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. TP-6076 was evaluated *in vitro* against biofilms formed by a panel of ten uropathogenic *Escherichia coli* clinical isolates versus levofloxacin, a standard of care for complicated 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 done in at least triplicate. Cultures were grown in TSB/YE for 2 hours at 35°C, diluted to ~10^6 colony forming units (CFU) in TSB/YE, and 500 µL of culture was added to 5 mL polystyrene tubes and allowed to form biofilms at 35°C for 24 hrs, without shaking. At 24 hrs, planktonic cells were aspirated and biofilms were fed with either 600 µL of fresh TSB/YE, or TSB/YE containing TP-6076 (2 or 20 µg/mL) or levofloxacin (20 or 200 µg/mL), 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 saline, cells were released from biofilms by sonication in saline and plated for CFUs. The % CFU reduction, versus the initial biofilm inoculum, was calculated. For three levofloxacin-resistant isolates whose 24 hr biofilms appeared less robust by CV staining, 24 hr biofilms were re-fed with fresh media and grown for an additional 24 hours prior to testing with drug. **Results:** All ten isolates were highly susceptible to TP-6076, with MIC values ranging from 0.031 to 0.13 µg/mL. Seven of the ten strains were highly levofloxacin-resistant, with levofloxacin MIC values ranging from 32 to 128 µg/mL; the three levofloxacin-susceptible isolates had levofloxacin MIC values ranging from 0.031 to 0.13 µg/mL. TP-6076 at 2 µg/mL effectively cleared biofilms from all isolates, reducing biofilm CFUs by >98% of the initial biofilm inoculum. Levofloxacin at 20 µg/mL effectively cleared biofilms formed by the three susceptible isolates (>99% CFU reduction), but failed to clear the biofilms of the seven levofloxacin-resistant isolates. For the levofloxacin-resistant isolates, 200 µg/mL of levofloxacin produced >99% CFU reduction for four levofloxacin-resistant isolates, >95% reduction for two isolates and failed to reduce biofilm CFUs for one isolate. The TP-6076 and levofloxacin susceptibilities of 24 hr and 48 hr biofilms were similar for the three isolates tested under both conditions, confirming that the activity of TP-6076 was not an artifact of an initially fragile biofilm. **Conclusion:** This *in vitro* activity of TP-6076, if confirmed *in vivo*, would support its potential use in the clinical treatment of chronic biofilm infections.

Background

Infections caused by multidrug-resistant, and, in some cases, pan-resistant Gram-negative microorganisms, are increasingly common problems in hospital or nursing home settings. In a 2013 report [1], the CDC estimated over 9000 healthcare-associated infections, and over 600 deaths were caused by *Klebsiella* spp. and *Escherichia coli*. The majority of these cases (85%) were due to *Klebsiella* spp.. Carbapenem-resistant Enterobacteriaceae (CRE) are of such concern that the CDC has designated CRE as an *urgent threat.*

Many bacterial pathogens associated with chronic infections can persist as inherently antibiotic- and host defense-tolerant biofilms, embedded in complex extracellular matrices attached to inert surfaces, dead or living tissue, and on medical devices during mild or serious infections. Restricted antibiotic diffusion across the extracellular matrix, up-regulation of intrinsic efflux pumps, generally lower metabolic activity, and the presence of persister cells are all thought to be significant factors contributing to increased antimicrobial tolerance of bacteria growing in biofilms [2-6].

TP-6076 is a novel, fully synthetic, tetracycline-class antibiotic with a unique heterocyclic substituent at C8, along with novel modifications at C4 and C7 [7]. TP-6076 has excellent activity *in vitro* against CRE [7, and see poster P0243] and was efficacious in a mouse pneumonia model with a KPC-expressing *Klebsiella pneumoniae* isolate [8]. In the present study we show that TP-6076 possesses potent anti-biofilm activity against 10 uropathogenic *E. coli* isolates, including strains resistant to fluoroquinolone antibiotics.

Methods

***E. coli* isolates.** All isolates used in this study were cultured from urine from urinary tract infections. Strain information is provided in Table 1.

MIC assays. The minimal inhibitory concentration (MIC) of compounds was determined according to CLSI guidelines [9] except that tryptic soy broth/yeast extract (TSB/YE) medium was used.

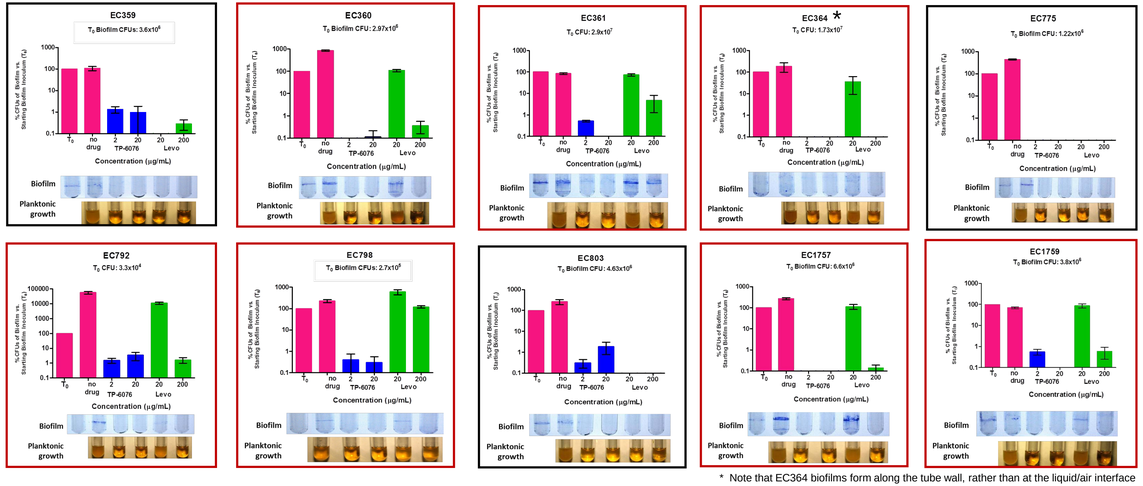
Biofilm assays were performed in at least triplicate. For biofilm assays, cells from a fresh Tryptic Soy Agar (TSA, BBL BD # 221283) plate grown overnight at 35°C were suspended in 0.9% saline to a 0.5 McFarland standard, diluted ten-fold into Tryptic Soy Broth/1% Yeast Extract medium (TSB, Bacto BD#211825 and YE, Bacto BD#210929) and grown at 35°C into log phase for 2 hours. 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 allowed to form biofilms at 35°C for 24 hrs, without shaking. At 24 hrs, planktonic cells were aspirated and biofilms were fed with either 600 µL of fresh TSB/YE, or TSB/YE containing TP-076 (2 or 20 µg/mL; synthesized at Tetraphase Pharmaceuticals) or levofloxacin (20 or 200 µg/mL; Sigma-Aldrich, St. Louis, MO), 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; #C3886-100G0, Sigma-Aldrich, St. Louis, MO). For quantification of biofilm CFUs after the second overnight incubation, with or without drug, planktonic cells were aseptically removed by aspiration and 1 mL of sterile 0.9% saline was added to each tube along with two sterile 6 mm borosilicate glass beads (Kimax # 89001-520; VWR, Arlington Heights, IL). Replicate tubes were sonicated in a Branson 5510 water bath at room temperature for one minute and then placed on ice. The sonicates. containing cells dispersed from the biofilm. were serially diluted and plated on TSA plates and incubated at 35°C for CFU quantification. The % CFU reduction, versus the initial biofilm inoculum, was calculated. For three levofloxacin-resistant isolates whose 24 hr biofilms appeared less robust by CV staining, 24 hr biofilms were re-fed with fresh media and grown for an additional 24 hours prior to testing with drug.

References

1) **Centers for Disease Control and Prevention,** 2013. Antibiotic resistant threats in the United States, 2013.
2) **Fux, C. A., J. W. Costeron, P. S. Steward, P. Stoodley.** 2005. Survival strategies of infectious biofilms. TRENDS Microbiol 13:34-40.
3) **Costerton, J. W., R. Veeh, M. Shirtliff, M. Pasmore, C. Post, G. Ehrlich.** 2003. The application of biofilm science to the study and control of chronic bacterial infections. J. Clin Inves. 112:1466-1477.
4) **Hung, C., Y. Zhou, J. S. Pinkner, K. W. Dodson, J. R. Crowley, J. Heuser, M. R. Chapman, M. Hadjifrangiskou, J. P. Henderson, S. J. Hultgren.** 2013. *Escherichia coli* biofilms have an organized and complex extracellular matrix structure. mBio 4: e00645-13
5) **Costerton, J. W., P. S. Steward, E. P. Greenberg.** 1999. Bacterial Biofilms: A Common Cause of Persistent Infections. Science 284: 1318-1322.
6) **Donlan, R. M., J. W. Costerton.** 2002. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 15:167-193.
7) **Sun C, Hunt D, Clark R, Fyfe C, Kerstein K, Grossman T, Sutcliffe J, Xiao, X.** In vitro potency of novel totally synthetic tetracycline against MDR Gram-negative pathogens including carbapenem-resistant Enterobacteriaceae, Abstr. P0299, 24th ECCMID, 10-13 May, 2014, Barcelona, Spain
8) **Grossman T, Fyfe C, Kerstein K, Sun C, Hunt D, Clark R, Xiao X, Sutcliffe J**. In vivo efficacy of novel, fully synthetic tetracyclines in a murine lung infection model challenged with KPC-producing Klebsiella pneumoniae, Abstr. P0300, 24th ECCMID, 10-13 May, 2014, Barcelona, Spain
9) **CLSI.** 2014. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard - tenth edition. CLSI document M07-A10E. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA.

Results

Figure 1. TP-6076 is active against 24 hour-established *E. coli* biofilms after 24 hour treatment
(graphs with levofloxacin-resistant strains are outlined in red)



* Note that EC364 biofilms form along the tube wall, rather than at the liquid/air interface

Figure 2. TP-6076 is active against 48 hour-established (mature) biofilms after 24 hour treatment

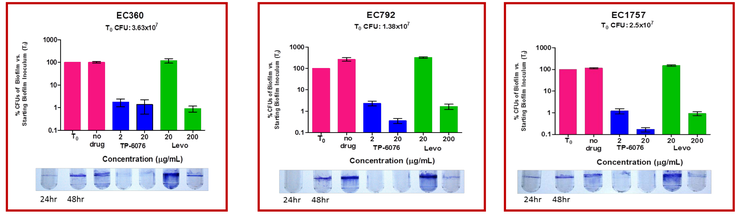


Table 1. Demographics and susceptibility of uropathogenic *E. coli* isolates used in this study

Tetraphase Strain No.	MIC (µg/mL) TP-6076	MIC (µg/mL) Levofloxacin	MIC (µg/mL) Tigecycline	Supplier	Patient Sex	Year of Isolation	Geographic Region of Isolation	Known Genotype/Phenotype
EC359	0.0312	0.125	0.25	Eurofins Medinet	Female	2008	South Atlantic, US	ESBL +
EC360	0.0625	32	0.5	Eurofins Medinet	Female	2008	South Atlantic, US	*tet*(A), $bla_{TEM-1/2/15}$, ST131
EC361	0.0625	64	0.25	Eurofins Medinet	Male	2008	South Atlantic, US	$bla_{CTXM-1/2/15}$, ST131
EC364	0.125	64	0.5	Eurofins Medinet	Female	2008	Mountain, US	*tet*(B), *tet*(D), $bla_{CTXM-9/14}$
EC775	0.0625	0.0625	0.5	Eurofins Medinet	Female	2009	Mid-Atlantic, US	*tet* (A)
EC792	0.0625	32	0.25	Eurofins Medinet	Female	2009	Pacific, US	
EC798	0.125	128	0.5	Eurofins Medinet	Female	2009	West South Central, US	*tet* (B)
EC803	0.0625	0.0312	0.5	Eurofins Medinet	Female	2009	Mountain, US	
EC1757	0.125	32	0.5	IHMA	Female	2011	Bulgaria	*tet* (A)
EC1759	0.0625	64	0.5	IHMA	Female	2011	Bulgaria	*tet* (B)

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* biofilms was similar against 24-hr and 48-hr 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.**