Functionalized Polymers Enhance Permeability of Antibiotics in Gram-negative MDR Bacteria and Biofilms for Synergistic Antimicrobial Therapy

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S1. Materials

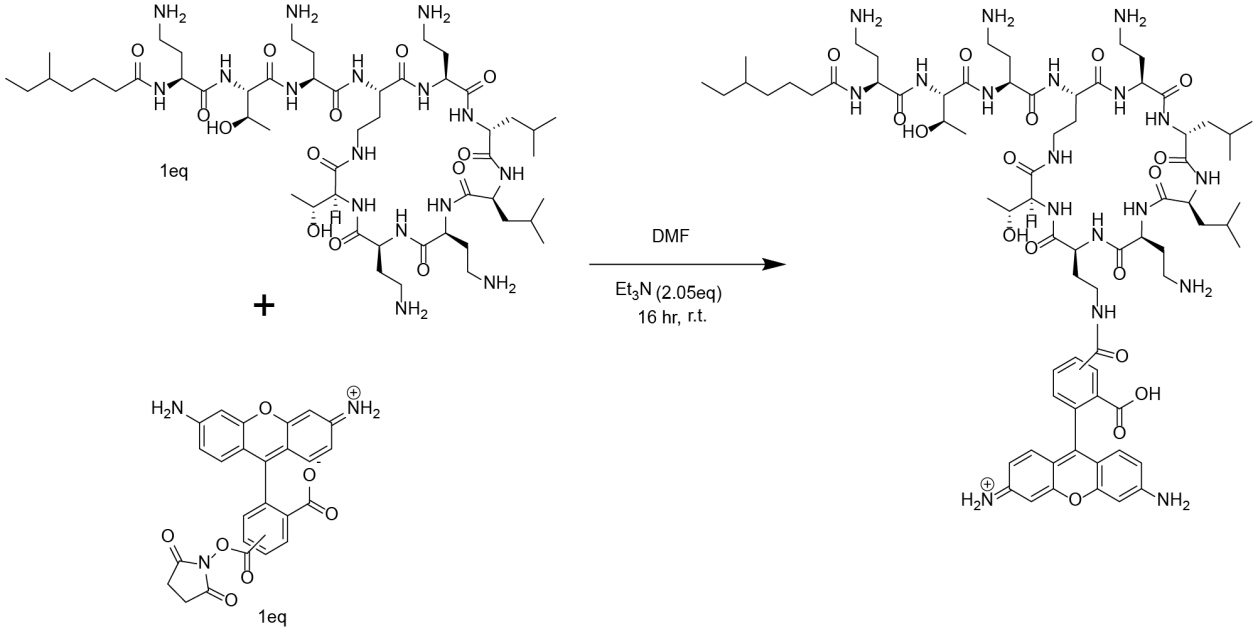
All chemicals and solvents for syntheses were purchased from Fisher Scientific and Sigma-Aldrich, and used without further purification, unless otherwise stated. The chemicals were used as received. Dichloromethane (DCM) and tetrahydrofuran (THF) used as a solvent for chemical synthesis and dried per standard procedures. All reagents/materials were purchased from Fisher Scientific and used as received. NIH-3T3 cells (ATCC CRL-1658) were purchased from ATCC. Dulbecco's Modified Eagle's Medium (DMEM) (DMEM; ATCC 30-2002) and fetal bovine serum (Fisher Scientific, SH3007103) were used in cell culture. The yields of the compounds reported here refer to the yields of spectroscopically pure compounds after purification. ^{1}H NMR spectra were recorded at 400 MHz on a Bruker AVANCE 400 machine.

S2. Oxanorbornene Polymer Synthesis

Oxanorbornene monomers featuring 2,6 and 11 bridging carbon chains were synthesized using our own previously reported protocol.[1] The oxanorbornene monomers were then polymerized through Ring Opening Metathesis Polymerization using Grubbs 3rd generation catalyst as reported previously.[2] Next, the oxanorbornene polymers were post-functionalized with necessary tertiary amines to generate a library of quaternary ammonium poly(oxanorbornene) derivatives using the methodology reported previously.[1,3,4] Finally, the polymers were added to 10,000 MWCO dialysis membranes and allowed to stir for 3 days, changing the water periodically. The polymers were filtered through PES syringe filters and freeze-dried to yield all the respective quaternary ammonium polymers and characterized using ^{1}H NMR.[1]

Synthesis of RhodamineGreen-Colistin

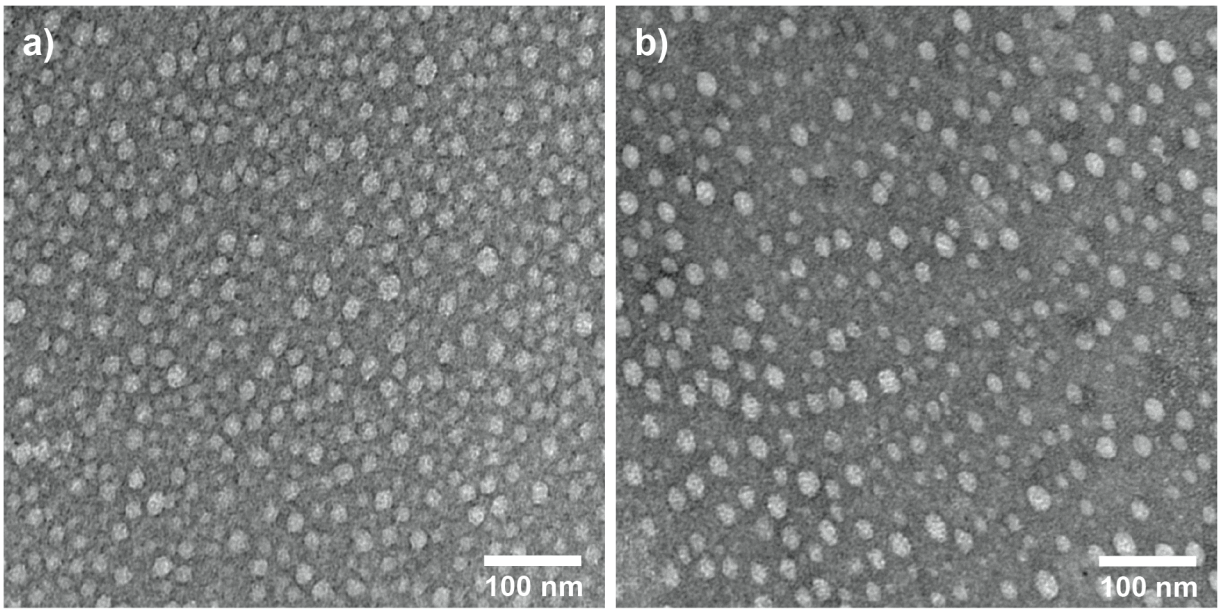
To a 7ml scintillation vial equipped with a stirbar was added Colistin (0.005 g, 0.004 mmol, 1 eq), triethylamine (1.2 µL, 0.0089 mmol, 2.05 eq) and DMF (0.5ml) and allowed to stir. Meanwhile, Rhodamine Green™ Carboxylic Acid, Succinimidyl Ester, Hydrochloride (5(6)-CR 110, SE) (0.0022 g, 0.004 mmol, 1 eq) was dissolved in 4 ml of DMF. At room temperature, the solution of Rhodamine Green was added slowly over the course of 2 hours. Once the addition was complete, the reaction mixture was covered with aluminum foil and allowed to stir overnight. Afterwards, the reaction mixture was transferred to the appropriately sized round bottom flask and rotovaped in the presence of toluene until all DMF was removed. Next, the residue was sonicated using THF and DCM and carefully decanted away. With all solvent removed, minimal amount of water was added and sonicated to dissolve the residue, filtered through a PES syringe filter and lyophilized to yield the RhodamineGreen-Colistin conjugate (red powder). MALDI analysis confirmed the M+1 species, 1512 g/mol



Supplementary Figure S1: Scheme for synthesis of RhodamineGreen-Colistin.

S3. TEM characterization of polymeric nanoparticles (PNPs)

TEM samples of polymers were prepared by placing one drop of the desired solution (10 μM) on to a 300-mesh Cu grid-coated with carbon film. These samples were analyzed and photographed using JEOL CX-100 electron microscopy.



Supplementary Figure S2: TEM image of a) P8 and b) P9 Polymer nanoparticles.

S4. Checkerboard titration for synergy testing

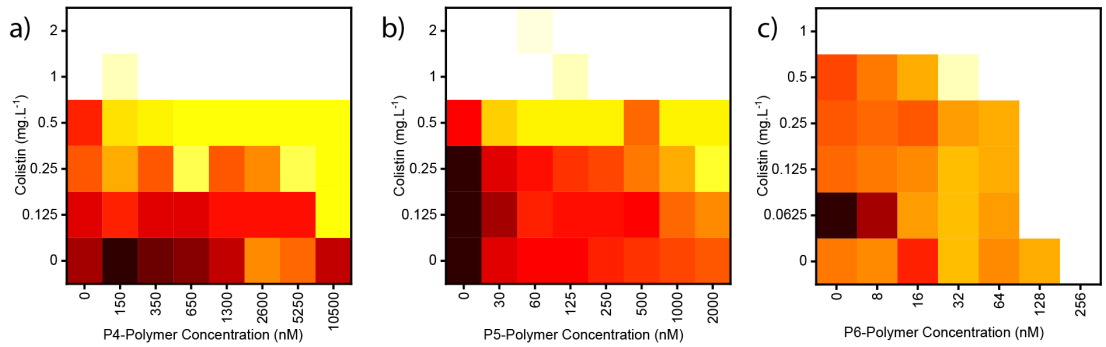


Figure S3. Checkerboard titration between colistin and a) P4, b) P5 and c) P6 polymer against uropathogenic *E. coli* (CD-2). Dark cells represent higher cell density. The combinations resulted in additive interactions.

A schematic of the checkerboard titration using colistin and polymers against planktonic bacteria and biofilms is described below:

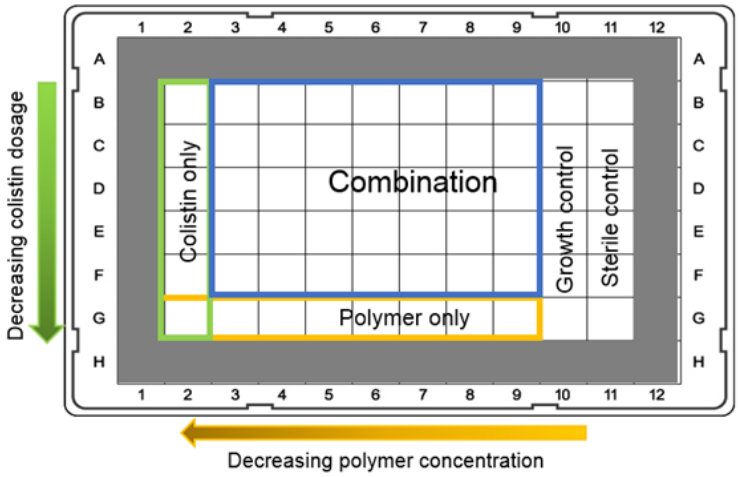


Figure S4. Sample of checkerboard titration plate with varied colistin and polymer concentrations. Concentration of colistin is decreasing with 2-fold dilutions from top to bottom while polymer concentration is decreasing from right to left.

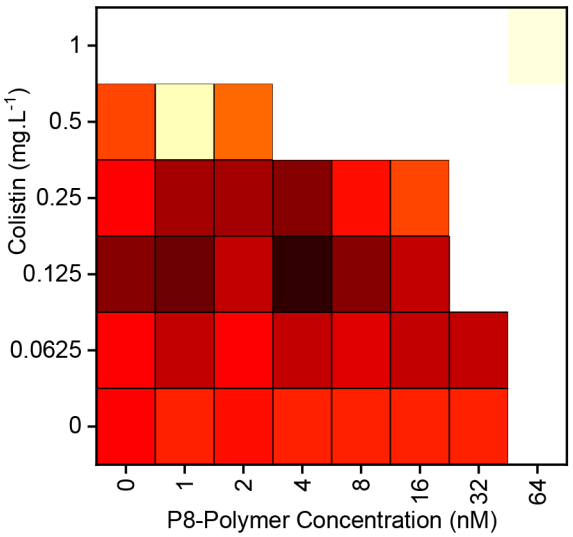


Figure S5. Checkerboard titration between colistin and P8 polymer against *P. aeruginosa* (CD-1006). Dark cells represent higher cell density.

Table S1. Table showing fold-increase in antibiotic efficacy obtained for the combination of PNPs and antibiotics tested against multiple strains.

Species (Strain)	**Polymer**	**Fold-increase in antibiotic efficacy**
E. coli (CD-2)	P4	0
E. coli (CD-2)	P5	0
E. coli (CD-2)	P6	2
E. coli (CD-2)	P7	8
E. coli (CD-2)	P8	8
E. coli (CD-2)	P9	8
P. aeruginosa (CD-1006)	P7	16
En. Cloacae (CD-1412)	P7	16
E. coli (CD-549)	P7	8
Acinetobacter species (CD-575)	P7	8
P. aeruginosa (CD-1006)	P8	8

S5. Combination therapy against Gram-positive bacteria

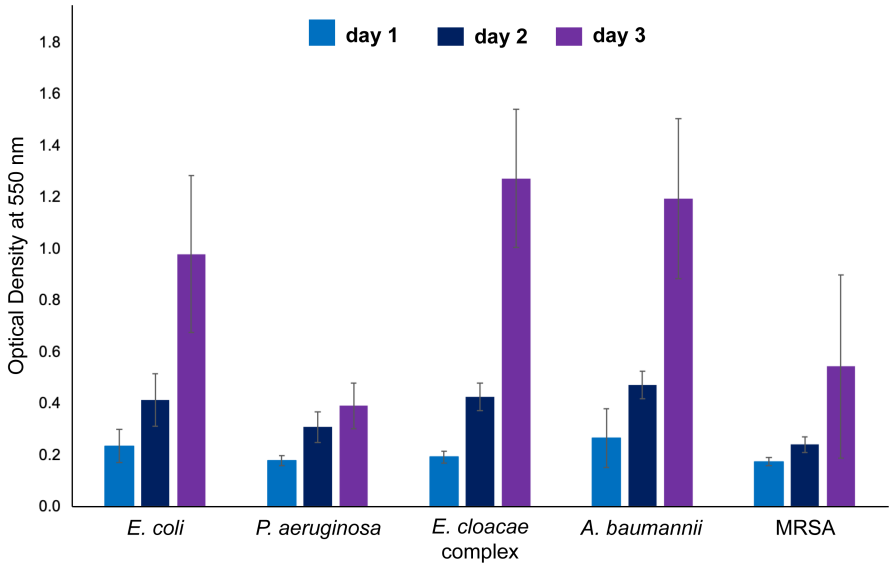


Figure S6. Biofilm quantification using Crystal Violet staining method at days 1, 2 and 3 of growth.

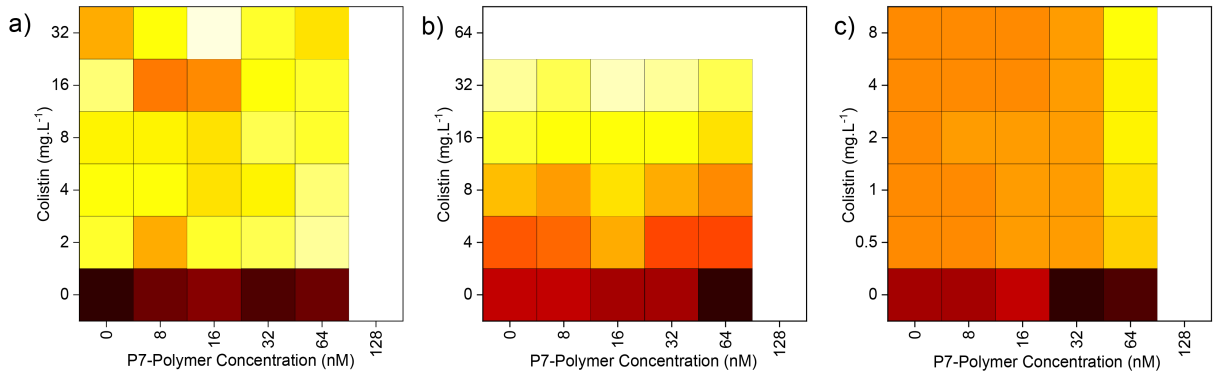


Figure S7. Checkerboard titration between colistin and P7 polymer against a) *Bacillus subtilis* (FD6 b) b) *S. epidermidis* (IDRL-7073) and c) methicillin-resistant *S. aureus* (CD-489). Dark cells represent higher cell density. The combinations did not show any significant increase in the efficacy of the antibiotics.

S6. Zeta Potential monitoring of bacterial membrane with PNP-colistin

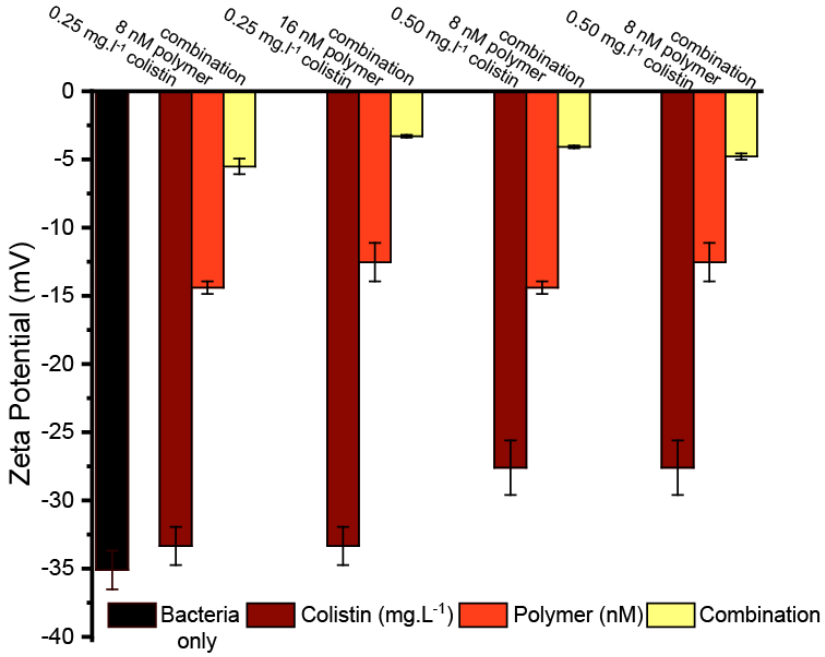


Figure S8. Change in bacteria membrane permeability assayed by zeta potential in presence of PNP, colistin and PNP-colistin combination. The figure shows different combination of PNP-colistin exhibiting increased ability to permeate bacterial membrane.

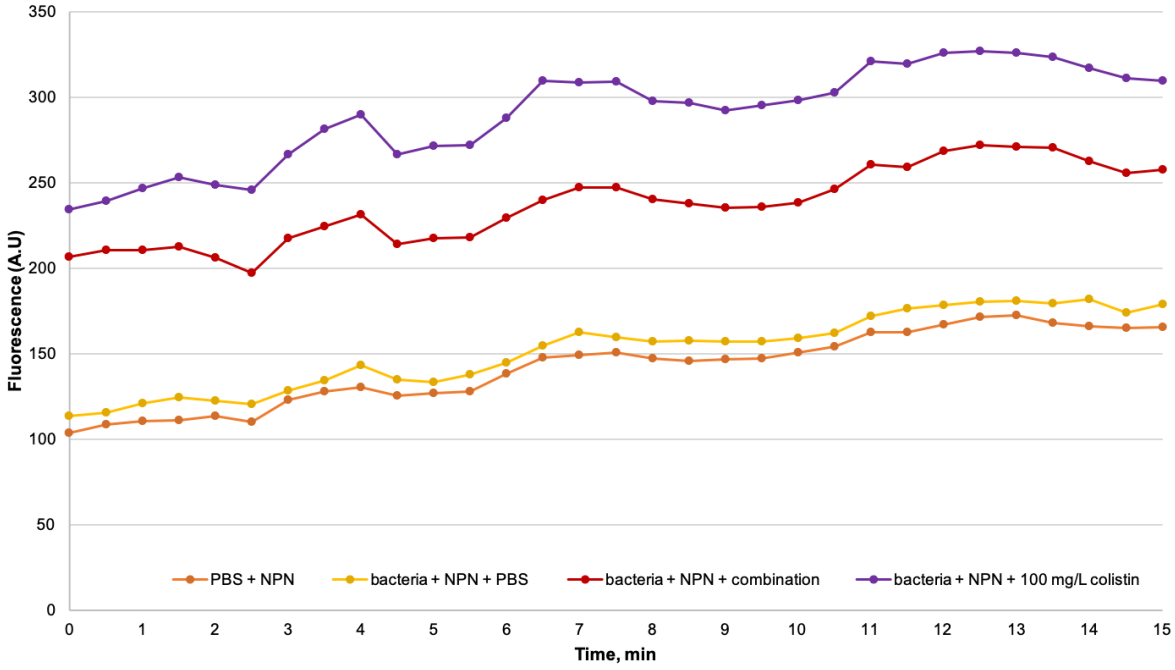


Figure S9. NPN fluorescence output to monitor bacterial membrane permeability.

S7. Combination therapy against biofilms

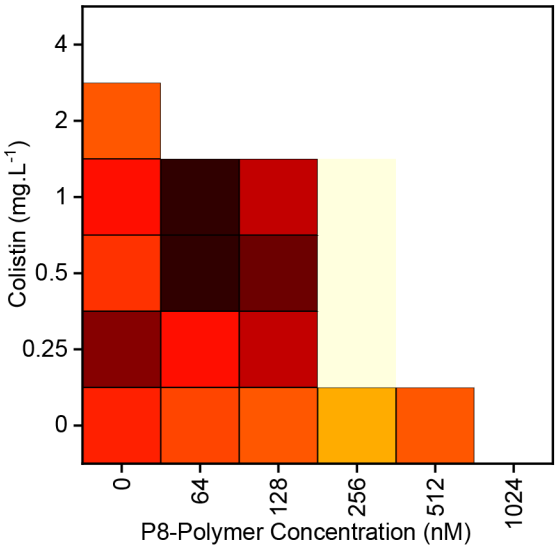


Figure S10. Checkerboard broth microdilution assays between colistin and P8 PNPs against uropathogenic biofilm *E. Coli* (CD-2). The combination shows upto 16-fold increase in the efficacy of colistin at sub-MBEC dosage of P8 PNPs.

S8. Uropathogenic Strain Information

Table S1 Uropathogenic *E. coli* strain information. All strains were harvested and tested for susceptibility in Cooley Dickinson Hospital Microbiology Laboratory (Northampton, MA). S: Susceptible; I: Intermediate; R: Resistant.

	Riley Strain Name	CD-2	CD-549
	Species	*E. coli*	*E. coli*
	Date Isolated	9/11/2011	1/9/12
	Specimen	UCC	UCC
	CFU/mL	>100,000	>100,000
	Note		
Aminoglycosides	**Amikacin** (Amikin)		S
	Gentamicin (Garamycin)	S	R
	Kanamycin High Level		
	Tobramycin (Nebcin)		R
Beta-Lactam	**Ampicillin** (Omnipen, Polycillin)	R	R
	Ampicillin/sulbactam (Unasyn)	I	R
	Amoxicillin/CA (Augmentin)		I
	Aztreonam (Zithromax)		R
	Oxacillin (Prostaphlin)		

	Penicillin		
	Piperacillin (Pipracil)		R
Cephalosporin	**Ceftazidime** (Fortaz, Tazicef)		
	Cefaclor (Ceclor, Ceclor CD)		R
	Ceftriaxone (Rocephin)	S	R
	Cefotaxime		R
	Cefazolin (Ancef, Kefzol)	S	R
	Ceftizoxime		R
	Cefepime (Maxipime)	S	R
	Cefoxitin (Mefoxin)	S	R
	Cefuroxime-Sodium		
	Cefuroxime-Axetil (Ceftin)		R
Carbapenem	**Ertapenem**		
	Imipenem (Primaxin)		S
	Meropenem (Merrem)		
Macrolides	**Azithromycin** (Azactam)		
	Erythromycin		
Fluoroquinolone	**Ciprofloxacin** (Cipro)	S	R
	Levofloxacin (Levaquin)	S	R
	Ofloxacin (Floxin)		
Lincosamides	**Clindamycin** (Cleocin)		
Oxazolidinones Folate pathway inhibitors	**Linezolid** (Zyvox)		

Antimycobacterial	**Rifampin** (Rifadin, Rimactane)		
Folate pathway inhibitors	**Trimethoprim /Sulfa** (Gantanol)	S	R
Tetracycline	**Tetracycline**		R
Glycylcyclines	**Tigecycline**		S
Glycopeptides	**Vancomycin** (Vancocin)		

Table S2. Uropathogenic *E. cloacae complex, P. aeruginosa,* and *S. aureus* strain information. All strains were harvested and tested for susceptibility in Cooley Dickinson Hospital Microbiology Laboratory (Northampton, MA). S: Susceptible; I: Intermediate; R: Resistant.

	Riley Strain Name	CD-1412	CD-1006	CD-489
	Species	*E. cloacae complex*	P. aeruginosa	*S. aureus - MRSA*
	Date Isolated	6/7/12	4/23/12	1/3/12
	Specimen	UCC	UCC	UCS
	CFU/mL	>100,000	>100,000	>100,000
	Note	Urine from nephrostomy tube		
Aminoglycosides	**Amikacin** (Amikin)	S		
	Gentamicin (Garamycin)	I	S	S
	Kanamycin High Level			
	Tobramycin (Nebcin)	R		

Beta-Lactam	**Ampicillin** (Omnipen, Polycillin)		S	
	Ampicillin/sulbactam (Unasyn)		S	R
	Amoxicillin/CA (Augmentin)			R
	Aztreonam (Zithromax)			
	Oxacillin (Prostaphlin)			R
	Penicillin			R
	Piperacillin (Pipracil)			
Cephalosporin	**Ceftazidime** (Fortaz, Tazicef)			
	Cefaclor (Ceclor, Ceclor CD)			R
	Ceftriaxone (Rocephin)	S	S	R
	Cefotaxime			R
	Cefazolin (Ancef, Kefzol)	R	S	
	Ceftizoxime			
	Cefepime (Maxipime)	S	S	
	Cefoxitin (Mefoxin)	R	S	
	Cefuroxime-Sodium			R
	Cefuroxime-Axetil (Ceftin)			
Carbapenem	**Ertapenem**			
	Imipenem (Primaxin)		S	R
	Meropenem (Merrem)			

Macrolides	**Azithromycin** (Azactam)			
	Erythromycin			
Fluoroquinolone	**Ciprofloxacin** (Cipro)	S	S	
	Levofloxacin (Levaquin)	S	S	R
	Ofloxacin (Floxin)			
Lincosamides	**Clindamycin** (Cleocin)			
Oxazolidinones Folate pathway inhibitors	**Linezolid** (Zyvox)	S		S
Antimycobacterial	**Rifampin** (Rifadin, Rimactane)			S
Folate pathway inhibitors	**Trimethoprim /Sulfa** (Gantanol)	R	S	S
Tetracycline	**Tetracycline**			S
Glycylcyclines	**Tigecycline**			
Glycopeptides	**Vancomycin** (Vancocin)			S

References

[1] A. Gupta, R. F. Landis, C. H. Li, M. Schnurr, R. Das, Y. W. Lee, M. Yazdani, Y. Liu, A. Kozlova and V. M. Rotello, *J. Am. Chem. Soc.*, 2018, **140**, 12137–12143.

[2] A. E. Madkour, A. H. R. Koch, K. Lienkamp and G. N. Tew, *Macromolecules*, 2010, **43**, 4557–4561.

[3] R. F. Landis, C. H. Li, A. Gupta, Y. W. Lee, M. Yazdani, N. Ngernyuang, I. Altinbasak, S. Mansoor, M. A. S. Khichi, A. Sanyal and V. M. Rotello, *J. Am. Chem. Soc.*, 2018, **140**, 6176–6182.

[4]W. J. Peveler, R. F. Landis, M. Yazdani, J. W. Day, R. Modi, C. J. Carmalt, W. M. Rosenberg and V. M. Rotello, *Adv. Mater.*, 2018, **30**, 1800634.