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Appendix A:

Custom mold specifications:

A custom negative mold template for several bead sizes was created in Autodesk Fusion 360 (Boston, MA; Figure 1). To allow necessary mold filling and extraction, one surface of the bead is flat, accounting for loss of 20% of sphere volume (Figure 1). The mold was printed using stereolithography at high resolution through Quickparts Rapid Prototyping (3D Systems, Atlanta GA) and filled with a two-part fast cure silicone rubber (Dragon Skin 10 FAST, Smooth-On, Inc) that cured for 24 hours in a negative pressure chamber at room temperature.

Cured PMMA bead weights prior to elution experiment:

10mm bead: 0.56 ± 0.022g

6mm bead: 0.12 ± 0.012g

4.5mm bead: 0.045 ± 0.002g

Appendix B: Description of culture and broth micro-dilution techniques

S. aureus was cultivated on tryptic soy agar plates and tryptic soy broth containing kanamycin sulfate (200ug/ml, Sigma-Aldrich). *E.coli* and *A. baumannii* were cultivated on Luria-Bertani (LB) agar plate and LB broth. Bacterial isolates from overnight cultures were diluted 1:50 in PBS and incubated in their respective growth media until mid-logarithmic growth. Subculture concentrations were determined by optical density at 600nm (OD600; Ultrospec 2100 Pro, Amersham Biosciences).

For the broth microdilution assay, Mueller Hinton Broth 2 (CAMHB, Sigma-Aldrich), containing kanamycin sulfate (200ug/ml) broth was used for *S. aureus* and CAMHB without antibiotics was used for *E.coli* and *A. Baumannii*. 100ul of the appropriate broth was preloaded into all wells in a 96-well plate. 100ul of antibiotic solution or eluent (diluted to achieve an MIC90 in the mid-portion of the plate), were then added to the first column and serially diluted across the columns. An inoculation of $1x10^5$ CFU was then added to each well. The last two columns served as positive and negative controls.

Appendix C: High Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS) Parameters

1. Equipment

All analysis was performed on an Agilent 1290 Infinity II LC/MS/MS consisting of the following modules:

- Agilent 1290 Infinity II Flexible Pump
- Agilent 1290 Infinity II Multisampler, equipped with sample cooler
- Agilent 1290 Infinity II Multicolumn Thermostat
- Agilent 6495 Triple Quadrupole mass spectrometer

Software

MassHunter Acquisition Software, with Qualitative Analysis and Quantitative Analysis

Solvents

Solvent A = 20 mM HFBA in 95/5 Water/Acetonitrile

Solvent B = 20 mM HFBA in Acetonitrile

All solvents were of LCMS grade. Acetonitrile was obtained from Fisher Scientific. Heptafluorobutyric acid (HFBA) was obtained from Beantown Chemical and used as supplied. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 μm membrane point-of-use cartridge and LC-Pak point-of-use polisher.

2. Methods

Chromatographic Conditions

Parameter	Tobramycin		Amikacin	
Column	ACQUITY UPLC BEH C18 2.1 x 50 mm, 1.7 μ			
Flow Rate	0.5 mL/min		0.5 mL/min	
Gradient	time	%B	time	%B
	Initial	0	Initial	0
	0.50	20	0.50	20
	1.00	20	1.00	20
	2.00	40	2.00	40
	2.05	90	2.05	90
	2.50	90	2.50	90
	2.55	0	2.55	0
	3.00	0	3.00	0
Stop Time	4 min		4 min	
Post Time	1 min		1 min	
Injection Vol.	1 μL		1 μL	
Column Temp.	40 ˚C		40 ˚C	

Mass Spectrometer Conditions

Source Parameters

Parameter	Tobramycin	Amikacin
Gas Temp (˚C)	250	210
Gas Flow (L/min)	11	11
Nebulizer (psi)	20	20
Sheath Gas Temp (˚C)	400	350
Sheath Gas Flow (L/min)	10	10
Capillary (V)	2200	2400
Nozzle Voltage (V)	1200	600
High Pressure RF (V)	130	130
Low Pressure RF (V)	80	150

Scan Parameters

Analyte	Q1	Q3	Dwell	Fragmentor (V)	CE	CAV	Polarity
Tobramycin	468.27	162.9	275	380	22	3	Positive
Amikacin	586.30	163.0	275	380	38	3	Positive

3. Results

Analyte	Retention Time
Tobramycin	3.53 min
Amikacin	3.32 min