

## Supplementary Appendix 1

The following refers to the process by which we arrived at the precise composition of the ‘antibiotic cocktail’ used in experiments described in Figure 6. This is all described in fuller detail in Khasriya<sup>1</sup>.

1. We initially adapted our antibiotic assay from Mulvey et al.<sup>2</sup>. They used a known concentration of *E.coli* only and Gentamicin to kill extracellular bacteria. However, we knew from culturing patient urine samples that they contain multiple bacterial species. For this reason, we chose to use Gentamicin and Amoxicillin for a broad spectrum of antibiotic cover of both aerobic and anaerobic bacteria, both of which have been shown previously to demonstrate minimal intracellular accumulation<sup>3</sup>. At 200 µg/mL, we showed that each of these drugs individually could achieve efficient killing of appropriate target bacterial species (see Tables 9.9, 9.10 and 9.11 in Khasriya<sup>1</sup>, reproduced below).
2. We trialled this mixture on a patient sample; however, incomplete extracellular bacterial killing resulted (figure 9.1 in Khasriya<sup>1</sup>). Therefore, we decided to add a third antibiotic. We chose Linezolid, again for its low intracellular accumulation<sup>3</sup>. We used a concentration of 200 µg/mL, which achieved efficient killing of the appropriate targets in trials (see Tables 9.9, 9.10 and 9.11 in Khasriya<sup>1</sup>, reproduced below).
3. We tested the triple cocktail on selected patient bacterial isolates and found it to efficiently kill all of them (again, see the tables below). We therefore used them successfully in the experiments in Figure 6, which demonstrated that extracellular washes were devoid of live bacteria, whilst intracellular lysates could grow live bacteria.

### References:

1. **Khasriya, R.** 2011. Occult urine infection in the aetiology of the overactive bladder PhD Thesis UCL. University College London.
2. **Mulvey MA**, Schilling JD, Hultgren SJ. Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection. *Infect Immun* 2001 July;69(7):4572-9.
3. **Barcia-Macay M**, Seral C, Mingeot-Leclercq MP, Tulkens PM, Van BF. Pharmacodynamic evaluation of the intracellular activities of antibiotics against *Staphylococcus aureus* in a model of THP-1 macrophages. *Antimicrob Agents Chemother* 2006 March;50(3):841-51.

Figure 9.1 Total bacterial counts at different stages of the Intracellular bacterial isolation assay for sample 787 aerobic and anaerobic cultures. (Time zero=initial bacterial count at the start of the assay (cfu ml-1), Supernatant 24hrs= the bacterial count after 24hrs incubation of the urothelium with Gentamicin and Amoxicillin (cfu ml-1), Wash1= the bacterial count after washing the incubated cells in PBS for the first time (cfu ml-1), wash 2= the bacterial count after washing the incubated cells in PBS for the second time (cfu ml-1), wash 3= the bacterial count after washing the incubated cells in PBS for the third time (cfu ml-1), Triton x= the bacterial count after the addition of triton X. This corresponds to the intracellular bacterial count (cfu ml-1).

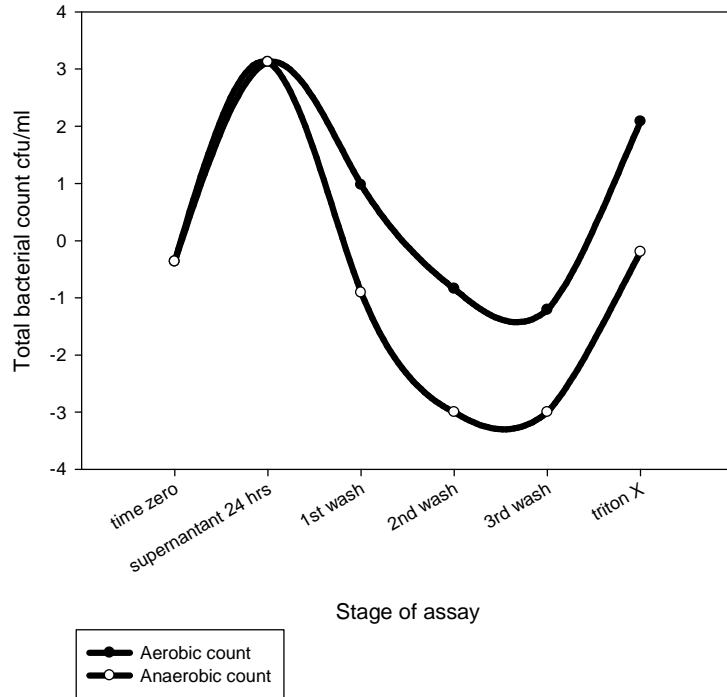


Table 9.9. Bacterial sensitivity at bacterial count of  $1 \times 10^8$  cfu/ml to combinations of gentamicin 200µg/ml, Linezolid 200µg/ml, amoxicillin 200µg/ml and mutanalysin 50µg/ml, Lysostaphin 50µg/ml and Lysozyme 50µg/ml. G= Gentamicin, L= Linezolid, A= Amoxicillin, E= Enzymes (Lysostaphin, Lysozyme, mutanalysin)

	Bacteria $1 \times 10^8$ cfu/ml	<i>E.coli</i>	<i>E.faecalis</i>	<i>Strep.anginosus</i>	<i>Corynebacteria.</i> <i>Sp.</i>	<i>Lactobacillus.</i> <i>gasseri</i>	<i>Proteus.</i> <i>mirabilis</i>
<b>Consistency of media</b>							
<b>Media+bacteria only</b>		Growth	Growth	Growth	Growth	Growth	Growth
<b>G</b>		No growth	Growth	No growth	No growth	No growth	No growth
<b>L</b>		Growth	faint growth	No growth	No growth	No growth	Growth
<b>A</b>		Growth	Growth	Growth	No growth	No growth	Growth
<b>GLA</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>GL</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>GA</b>		No growth	Growth	No growth	No growth	No growth	No growth
<b>AL</b>		Growth	faint Growth	No growth	No growth	No growth	Growth
<b>GE</b>		No growth	Growth	No growth	No growth	No growth	No growth
<b>LE</b>		Growth	faint growth	No growth	No growth	No growth	Growth
<b>AE</b>		Growth	Growth	Growth	No growth	No growth	Growth
<b>GLAE</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>GLE</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>GAE</b>		No growth	Growth	No growth	No growth	No growth	No growth
<b>ALE</b>		Growth	Growth	No growth	No growth	No growth	Growth

Table 9.10. Bacterial sensitivity at bacterial count of  $1 \times 10^6$  cfu/ml to combinations of gentamicin 200µg/ml, Linezolid 200µg/ml, amoxicillin 200µg/ml and mutanalsin 50µg/ml, Lysostaphin 50µg/ml and Lysozyme 50µg/ml. G= Gentamicin, L= Linezolid, A= Amoxicillin, E= Enzymes (Lysostaphin, Lysozyme, mutanalsin)

	Bacteria $1 \times 10^6$ cfu/ml	<i>E.coli</i>	<i>E.faecalis</i>	<i>Strep.anginosus</i>	<i>Corynebacteria.</i> <i>Spp</i>	<i>Lactobacillus.</i> <i>gasseri</i>	<i>Proteus.mirabilis</i>
<b>Consistency of media</b>							
<b>Media+bacteria only</b>		Growth	Growth	Growth	Growth	Growth	Growth
<b>G</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>L</b>		Growth	No growth	No growth	No growth	No growth	Growth
<b>A</b>		Growth	No growth	Growth	No growth	No growth	Growth
<b>GLA</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>GL</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>GA</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>AL</b>		Growth	No growth	No growth	No growth	No growth	Growth
<b>GE</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>LE</b>		Growth	No growth	No growth	No growth	No growth	Growth
<b>AE</b>		Growth	No growth	Growth	No growth	No growth	Growth
<b>GLAE</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>GLE</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>GAE</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>ALE</b>		Growth	No growth	No growth	No growth	No growth	Growth

Table 9.11. Bacterial sensitivity at bacterial count of  $1 \times 10^3$  cfu/ml to combinations of gentamicin 200µg/ml, Linezolid 200µg/ml, amoxicillin 200µg/ml and mutanalsin 50µg/ml, Lysostaphin 50µg/ml and Lysozyme 50µg/ml. G= Gentamicin, L= Linezolid, A= Amoxicillin, E= Enzymes (Lysostaphin, Lysozyme, mutanalsin)

	<b>Bacteria 1x10<sup>3</sup>cfu/ml</b>	<i>E.coli</i>	<i>E.faecalis</i>	<i>Strep.anginosus</i>	<i>Corynebacteria. Spp</i>	<i>Lactobacillus. gasseri</i>	<i>Proteus.mirabilis</i>
<b>Consistency of media</b>							
<b>Media+bacteria only</b>		Growth	Growth	Growth	Growth	Growth	Growth
<b>G</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>L</b>		faint Growth	No growth	No growth	No growth	No growth	No growth
<b>A</b>		Growth	No growth	No growth	No growth	No growth	No growth
<b>GLA</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>GL</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>GA</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>AL</b>		Growth	No growth	No growth	No growth	No growth	No growth
<b>GE</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>LE</b>		Growth	No growth	No growth	No growth	No growth	No growth
<b>AE</b>		Growth	No growth	No growth	No growth	No growth	No growth
<b>GLAE</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>GLE</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>GAE</b>		No growth	No growth	No growth	No growth	No growth	No growth
<b>ALE</b>		Growth	No growth	No growth	No growth	No growth	No growth

