Supplementary Information

Prevention of Encrustation and Blockage of Urinary Catheters by *Proteus mirabilis* via pH-Triggered Release of Bacteriophage

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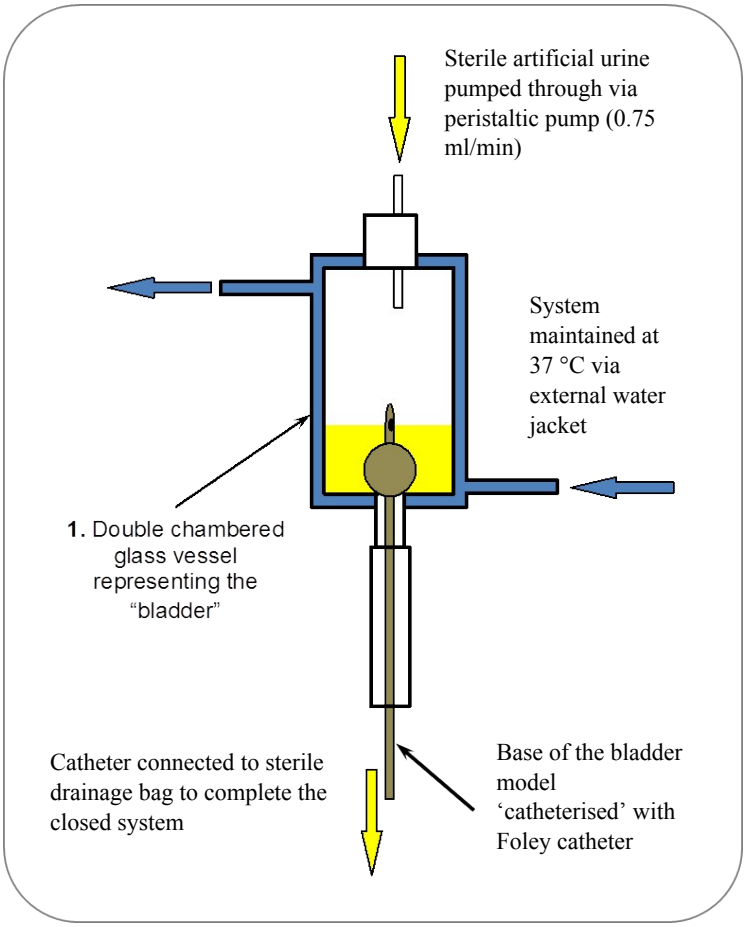


Supplementary Figure 1: Illustration of the *in vitro* bladder model system (modified from Stickler *et al*. 1999, Methods Enzymol 310: 494-501). Bacterial strains are inoculated directly into residual media within the glass bladder. Blue arrows indicate water flow through the outer chamber to maintain physiological temperature. Yellow arrows indicate flow of artificial urine media through the closed drainage system.

Double chambered glass 'bladder'

METHODS

Artificial Urine Preparation

Stock solutions were prepared at 5x working concentration, containing sodium disulphate (11.5 g/l), magnesium chloride hexahydrate (3.25 g/l), sodium oxalate (0.1 g/l), potassium dihydrogen orthophosphate (14 g/l), potassium chloride (8 g/l), ammonium chloride (5 g/l), gelatin (25 g/l), tryptone soya broth (5 g/l), urea (125 g/l) and calcium chloride dehydrate (3.25 g/l). Stock solutions of calcium chloride dehydrate and urea were filter sterilised by membrane filtration (0.22 μm; Sartorious, UK), whilst other components were sterilised by autoclaving. For use in bladder models, stock solutions were diluted using sterile deionised water, and the final pH adjusted to 6.1.

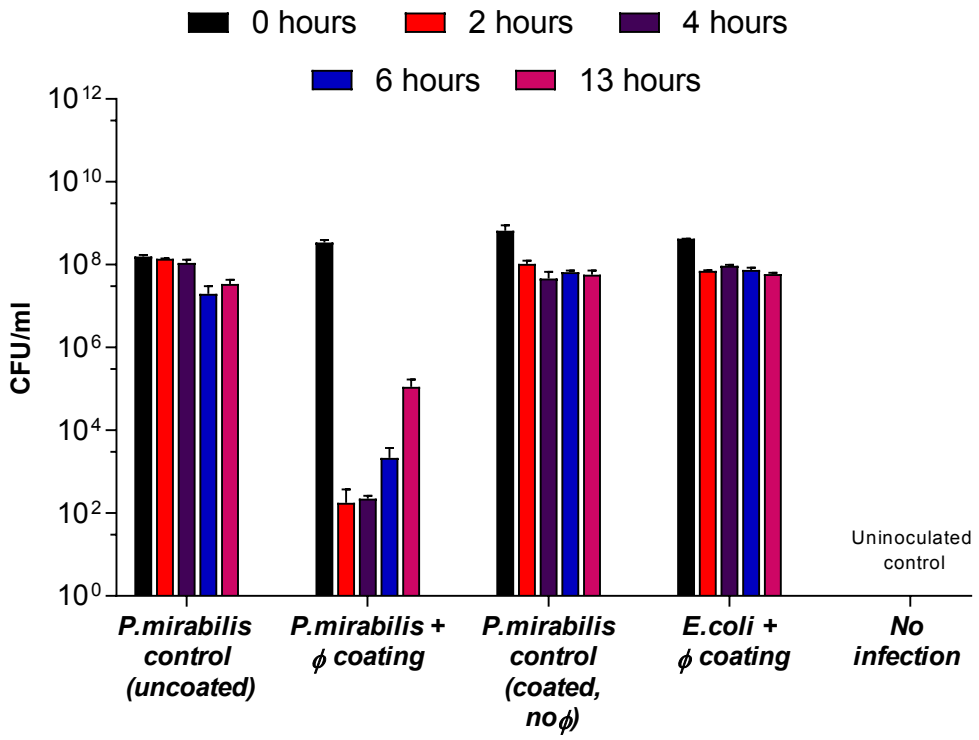
Bacteriophage Isolation and Purification

Bacterial enrichments with *P. mirabilis* isolates were performed to increase phage population. Actively growing *P. mirabilis* cells from overnight liquid culture (5 ml in LB media supplemented with 1 mM $MgSO_4$ and 1 mM $CaCl_2$) were incubated overnight at 37 °C with 150 ml crude sewage solution. 10 ml aliquots of the overnight enrichment were recovered and centrifuged (3000 xg, 30 minutes) after addition of NaCl (1 M) and chloroform (2%) to remove the bacteria. Supernatants were filter sterilised (0.22 μm; Sartorious, UK) to produce filtered phage lysate solutions. Double agar overlays were performed with filtered phage lysate (100 μl) and exponential-phase *P. mirabilis* B4 culture (100 μl), and plates were incubated statically at 37 °C for 18 hours. Plates displaying confluent lysis were selected for single plaque purification, whereby individual plaques were picked using Pasteur pipettes, and suspended in SM buffer (300 μl). The resulting suspensions were serially diluted in SM buffer (10^{-3} to 10^{-7}), and agar overlays repeated with strains used in initial isolations. To ensure clonality of phage types, single plaque purification was repeated a further 5 times until homogenous plaque purification was observed. Finally, an individual plaque was picked and resuspended in SM buffer for use in subsequent methods. The final clonal phage were stored at 4 °C until required.

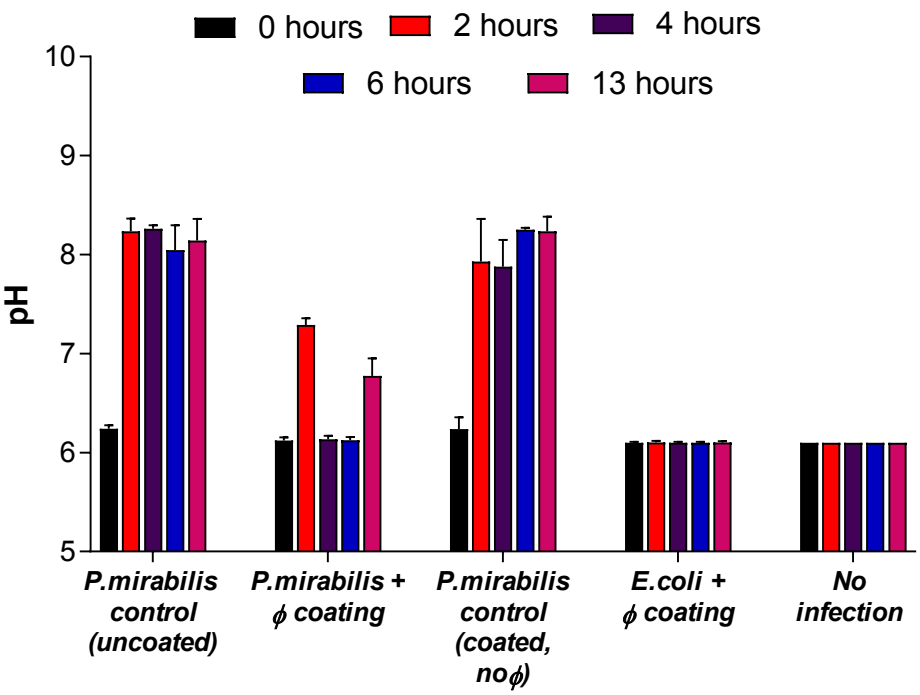
EXPERIMENTAL CONTROLS

Experimental controls were undertaken throughout all triplicate repeats of the bladder model experiments in order to:

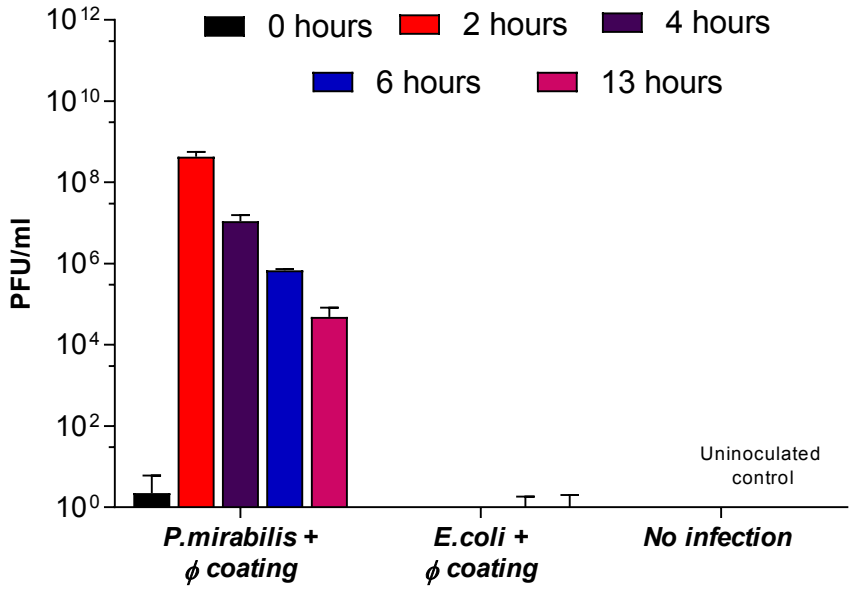
1. Assess time to blockage in the absence of a bacteriophage (ϕ) catheter coating
2. Assess bacterial health in the presence of an unimpregnated control model
3. Assess coating stability in the presence of a non-urease producing species (*E. coli)*
4. Assess the sterility of the closed drainage system in the absence of infection



Supplementary Figure 2: Analysis of *P.mirabilis* population within in vitro bladder models 0,2,4,6 and 13 hours after model start including full experimental controls. No significant differences were observed in viable cell count in the presence of an unimpregnated coating, or the *E.coli* infection models. Uninoculated control models confirm the sterility of the closed drainage system.



Supplementary Figure 3: Analysis of residual urine pH within bladder models 0,2,4,6 and 13 hours after model start including full experimental controls. No significant differences detected between the uncoated control and the unimpregnated control, or between the E. coli containing, and the uninfected models.



Supplementary Figure 4: Analysis of bacteriophage release within residual bladder model urine 0,2,4,6 and 13 hours after model start including full experimental controls. No significant differences in phage count was observed between the uncoated control and the *E. coli* models.