Proteus mirabilis involvement in Recurrent Urinary Tract Infections (rUTIs)

Aim: To investigate *in vitro* the adherence of *Proteus mirabilis* clinical isolates to bladder cells

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Introduction

- **Urinary tract infections (UTI) - The clinical problem**
- UTIs are the second most common bacterial infection with over 150 million cases reported annually (1).
- 40-50% of women will develop an UTI at least once in their life-time.
- The main cause of infection is uropathogenic *Escherichia coli* (UPEC) (Figure 1).
- **Treatment of UTIs & Antibiotic resistance: A global problem**
- The current treatment for UTIs is antibiotics.
- BUT ..1 in 4 patients experience recurrence of the UTI after treatment (rUTI).
- The use of antibiotics to treat UTIs is leading to resistant UPEC strains forming (2).
- Figure 2 shows the increase in the number of antibiotic resistant strains in the UK.
- Non-antibiotic treatment for rUTIs is needed urgently.

Study Background

- A study involving 30 uncomplicated rUTI patients identified four patients who presented with **no symptoms** over 6 months but whose urine contained a significant amount of a microbe called ***Proteus mirabilis***.
- These bacteria seemed to protect the patients from UPEC infection and are a **potential target** for therapeutic development.
- Studies have shown that the four isolated bacterial strains called p100, p139, p337 and p755, out-compete and kill UPEC (Figure 3) again supporting the development of *P. mirabilis* as a potential treatment for UTIs.
- The aim of this project was to explore how *P. mirabilis* interacts with bladder cells.

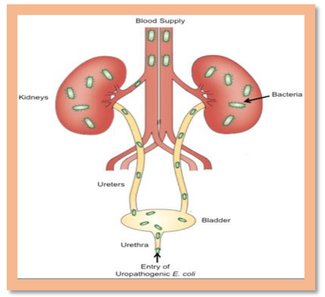


Figure 1. UPEC involvement in causing UTI
Kaper et al. (2004)

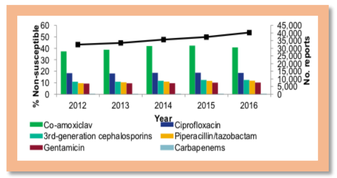


Figure 2. Increase in the number of antibiotic resistant UPEC strains in the UK.
ESPAUR, Public Health England (2017)

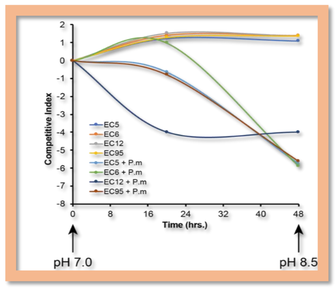


Figure 3. UPEC death in the presence of *Proteus mirabilis*.
P. Aldridge

Methodology

1) Human Bladder cells were cultured in the laboratory in a monolayer.
2) Each *Proteus* strain was engineered (transformed) to contain green fluorescent protein (GFP) meaning the bacteria glowed green under a fluorescent microscope (Figure 4) and could be visualised easily.
3) Bladder cells were challenged with UPEC and *Proteus* expressing GFP separately to observe the amount of bacterial attachment to the bladder cells (Figure 5).
4) UPEC and *Proteus* were added to bladder cells in co-competition for 3 hours and then stained with Trypan Blue to observe the integrity of the bladder cells after infection (Figure 6).

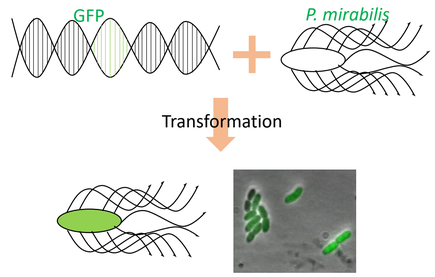
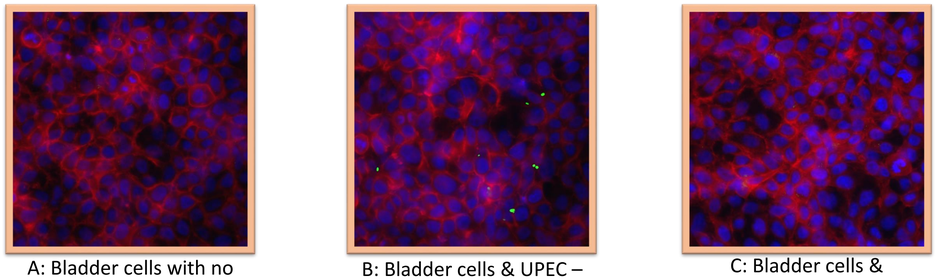


Figure 4. Transformation of *Proteus mirabilis* with GFP. Under a fluorescent microscope *P. mirabilis* now glows green.

Results

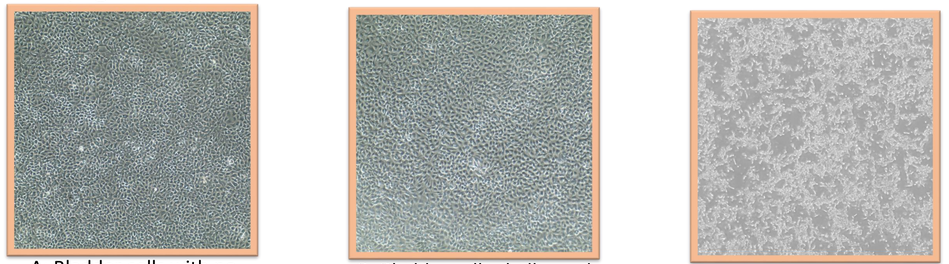
- UPEC attached to the bladder cells (Figure 5B) modelling UTI, but *Proteus mirabilis* did not attach to the human cells (Figure 5C).



A: Bladder cells with no bacteria | B: Bladder cells & UPEC – bacteria attaches to cells | C: Bladder cells & *Proteus* – no attachment

Figure 5. Bladder cells viewed under a fluorescence microscope to check bacterial adherence. Bladder cells have been stained, red: cell membrane, blue: cell nucleus, green: bacteria.

- Challenging the bladder cells with UPEC and *P. mirabilis* in co-competition did not affect the integrity of the human cells (Figure 6B) but UPEC alone killed the cells (Figure 6C).



A: Bladder cells with no bacteria | B: Bladder cells challenged with UPEC and *P. mirabilis* | C: Bladder cells challenged with UPEC

Figure 6. Bladder cells viewed under a light microscope to check bladder cell integrity.

Conclusions

- *P. mirabilis* did not attach to human bladder cells, did not affect cell viability but **protected** the cells from UPEC infection.
- Observations suggest *P. mirabilis* has the potential to function as an **alternative therapeutic** to antibiotics for treating rUTIs.
- Idea is to harness the protective properties of *Proteus* & seed urobiomes that function to prevent UPEC infections

Acknowledgements

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References

1. Spaulding CN, Hultgren SJ. Adhesive Pili in UTI Pathogenesis and Drug Development. Pathogens (Basel, Switzerland). 2016;5
2. Lee DS, Lee SJ, Choe HS. Community-Acquired Urinary Tract Infection by *Escherichia coli* in the Era of Antibiotic Resistance. Biomedical Research International. 2018; 2018: 7656752