Uropathogenic *Escherichia coli* target bladder cell junctions

Investigating the localisation of clinical Uropathogenic *Escherichia coli* strains to the tight junction regions of bladder cells

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Introduction

- **Urinary tract infections** (UTIs) are one of the most common bacterial infections with over 150 million cases reported annually[1].
- A **microbe** called **Uropathogenic *Escherichia coli*** (UPEC) is the main cause of these infections.
- Standard treatment for patients is **antibiotics** but the lack of antibiotic stewardship has increased the number of **antibiotic resistant UPEC strains**.
- **Alternative non-antibiotic treatments are required but** this requires knowledge of how UPEC infects bladder cells.
- Work has shown that a UPEC strain isolated from a patient with a recurrent UTI localised to specific regions of bladder cells called ***tight junctions* (TJ)[2]. TJ** connect bladder cells to each other.
- The **mechanisms** that UPEC uses to localise and attach to these bladder cell TJ regions may be **potential targets** for the development of new therapeutics to replace antibiotics.

Aim

- To investigate whether two **UPEC strains** also isolated from different patients suffering UTIs and called CAUTI 684 and AC3408 **also localise** to the tight junction regions of human bladder cells.

Methodology:

1) Human Bladder cells known as RT4 cells were cultured in the laboratory.

2) Each UPEC strain was engineered (transformed) to contain green fluorescent protein (GFP) so it fluoresced green and was easily detected.

3) Growth curves of transformed GFP UPEC strains were measured in the laboratory.

4) RT4 bladder cells were challenged with the GFP UPEC strains and the sites of attachment observed using a fluorescence microscope. (Figure 1)

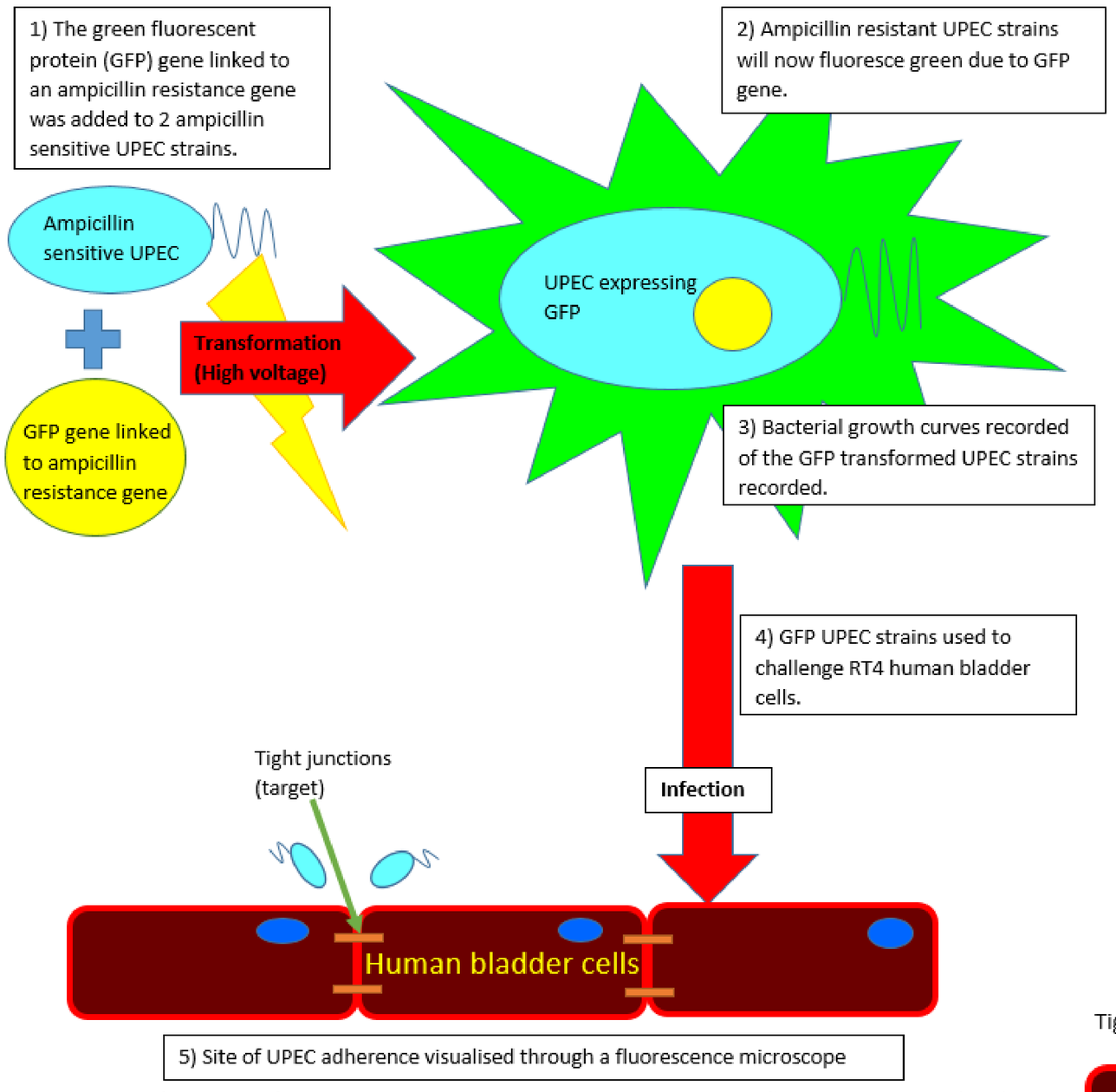

Figure 1: Transformation of ampicillin sensitive UPEC strains to express green fluorescent protein (GFP) so adherence location can be observed under a fluorescence microscope.

Results

Growth curves comparing the wild type and transformed UPEC strains showed that producing GFP had no effect on bacterial growth. (Figure 2)

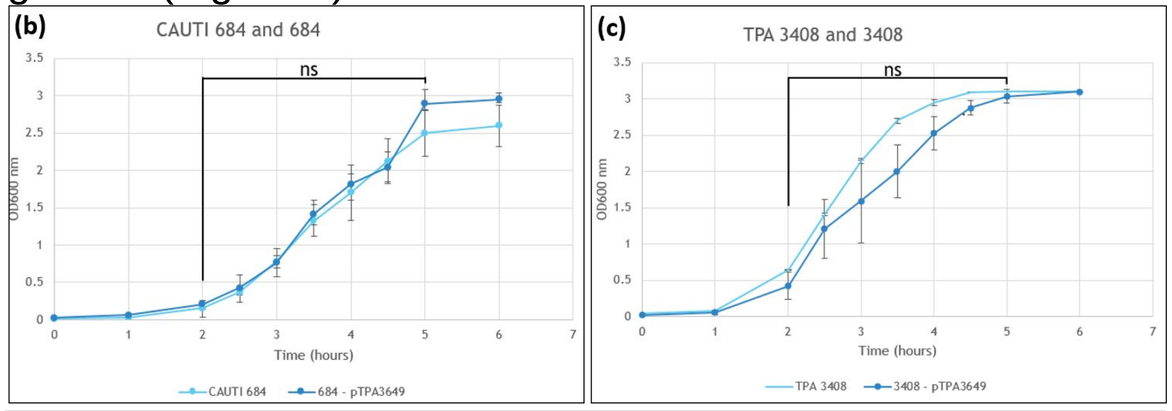

Figure 2. Growth curves of wild-type UPEC strains (light blue) plotted alongside growth curves of their transformed variants (dark blue) when grown in Luria-Bertani (LB) media. OD600 nm measurements were recorded at hourly intervals up to 6 h to generate the growth curves. Results are expressed as mean data points ±1SD (n=3). Non-significant (ns) = P≥0.05.

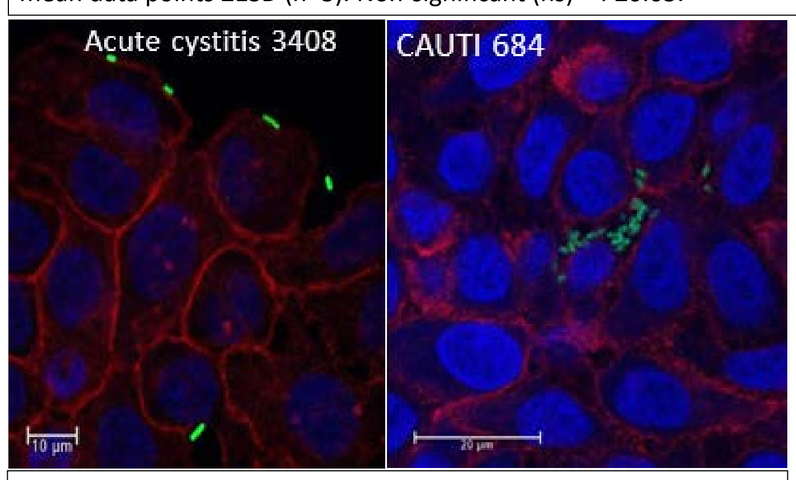

Figure 3: Adherence patterns on RT4 cells of 2 different UPEC strains (green). RT4 cells stained with phalloidin (red; actin cytoskeleton) and with DAPI (blue; nuclei)

Following challenge of the human RT4 bladder cells, the 2 transformed UPEC strains- CAUTI 683 and AC3408- were found to localise to the epithelial tight junction regions. (Figure 3)

Discussion

❖During this project we were **limited** to using **Ampicillin sensitive UPEC** strains. However, most clinical UPEC strains were already ampicillin resistant. This limited our choice of UPEC strains and may have influenced our results.
❖Therefore, a new plasmid that carried a **kanamycin** (a different type of antibiotic) rather than ampicillin resistance gene was engineered.
❖**Kanamycin resistance** is much **rarer** than ampicillin resistance among UPEC strains.
❖Using this GFP plasmid we can now transform **other UPEC strains** and check their localisation patterns in RT4 cells.

Conclusion

❖In this project, UPEC strains-CAUTI 683 and AC3408- exhibited **the same localisation pattern** to tight junction regions of human RT4 bladder cell as observed previously.
❖Further investigations are needed to check whether ampicillin resistance UPEC strains also show the same localisation patterns. This can now be tested using the **kanamycin resistant plasmid**.
❖The mechanism used by bacteria to attach to tight junctions may be a potential target for **future therapeutics** by preventing initial attachment and thus infection. (Figure 4)

Figure 4: Potential therapeutic mechanism.

References

1. Spaulding CN, Hultgren SJ. Adhesive Pili in UTI Pathogenesis and Drug Development. Pathogens (Basel, Switzerland). 2016;5
2. 2. Mowbray CA, Shams S, Chung G, Stanton A, Aldridge P, Suchenko A, et al. High molecular weight hyaluronic acid: a two-pronged protectant against infection of the urogenital tract. *Clinical & translational immunology.* 2018.

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