#342 Uropathogens: Intracellular localisation and virulence mechanisms

Ognenovska S[1], Cheng Y[1], Mukerjee C[2], Sanderson-Smith M[3], Mansfield K[3], Schembri M[4], Moore KH[1]

[1]Department of Urogynaecology, St George Hospital, [2]Department of Microbiology, St George Hospital, [3]Illawara Health and Medical Research Institute, University of Wollongong, [4]School of Chemistry and Molecular Biosciences, University of Queensland

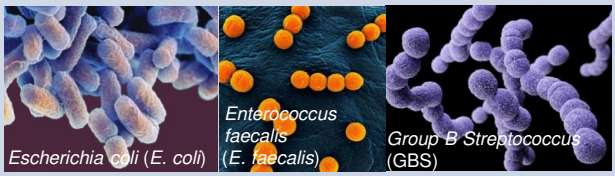
INTRODUCTION

With increasing awareness of the urinary microbiome in refractory Detrusor Overactivity, the mechanism of intracellular localisation has received much interest.

When bacteria are localised within the urothelium, then antibiotic efficacy is reduced and immune cell penetration is limited, thus promoting survival of the uropathogen within the bladder.

AIMS

1. To examine the capacity for common uropathogens to localise within the urothelium;
2. To determine what virulence factors uropathogens may possess to enable penetration.

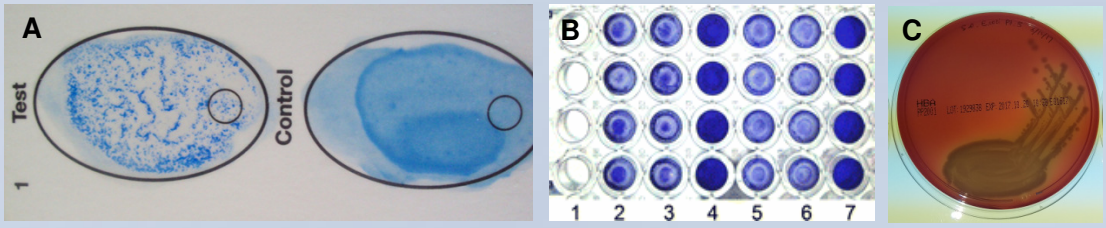


METHODS

Three strains of uropathogenic *E. coli*, *E. faecalis* and GBS were isolated from the urine of infected patients, while control strains (UTI89 + UTI89 FimH K/O) were provided by Prof. M. Schembri. Assays were as follows (**Figure 1**):

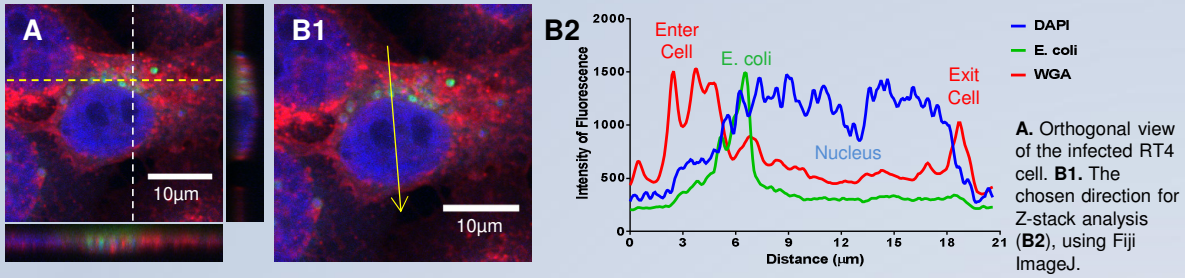
A. Yeast cell agglutination to test for FimH, an adhesin required for *E. coli* adherence to the urothelium[1];
B. Biofilm assay using crystal violet, as increased biofilm formation can enhance *E. faecalis* infection[2];
C. Haemolytic activity (using horse blood agar), as increasing toxicity is important for GBS penetration of cell membranes[3].

Figure 1: Assays performed to determine the presence of specific uropathogen virulence factors.



Intracellular localisation was confirmed by co-culturing each uropathogenic strain with RT4 urothelial cells for 2hours, followed by a 2hour gentamycin assay. The cells were stained using DAPI, Wheat-germ Agglutinin (WGA), and specific anti-bacterial antibodies to visualise the location of the uropathogen by confocal microscopy using orthogonal views and Z-stack analyses (**Figure 2**).

Figure 2: Confocal microscopy of *E. coli* and subsequent image analyses showing intracellular localisation.



A. Orthogonal view of the infected RT4 cell. **B1.** The chosen direction for Z-stack analysis (**B2**), using Fiji ImageJ.

RESULTS

Table 1: Summary of assay across the isolated bacterial strains.

Bacterial Strain	Agglut.	Haemolysis	Biofilm Formation	Confocal evidence for intracellular localisation
E. coli 1	✓	✓	weak	✗
E. coli 2	✓	✓	non	✓
E. coli 3	✗	✓	weak	✗
E. faecalis 1	✗	✓	v. strong	✓
E. faecalis 2	✗	✓	v. strong	✓
E. faecalis 3	✗	✓	strong	✓
GBS 1	✗	✓	strong	To be confirmed
GBS 2	✗	✓	v. strong	To be confirmed
GBS 3	✗	✓	strong	To be confirmed

Ticks indicate a positive result for the corresponding test, while crosses indicate a negative. Biofilm formation: OD_{540} <0.5 is non-biofilm forming, 0.5 – 1.5 is weak, 1.5 – 2.5 is intermediate, 2.5 – 3.5 is strong, OD_{540} >3.5 is very (v.) strong.

CONCLUSIONS

Uropathogens possess a variety of virulence mechanisms which may allow for their localisation in the urothelium and their continued survival within the urinary tract.

For the first time, these studies clarify these potential mechanisms of penetration for three common uropathogens in the *human urothelium*.

REFERENCES

1. Rosen et al., *Infect Immun* (2008) 76:3346-3356.
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3. Rajagopal, *Future Microbiol* (2009) 4:201-221.