Bacterial dynamics inside host cells during model Urinary Tract Infections

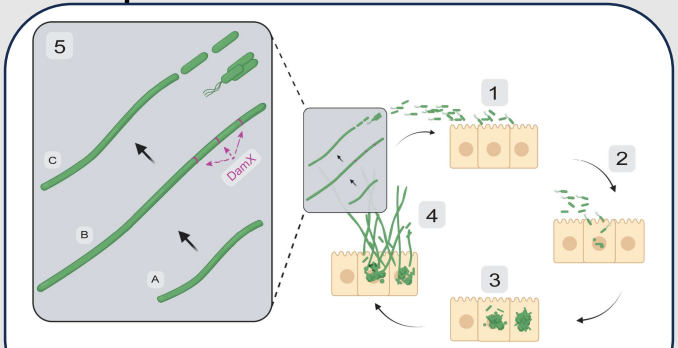
Lachlan Chisholm, Alaska Pokhrel, Charlotte Abell-king,
Ariana Costas, Rebecca von Essen & Bill Söderström,
Australian Institute of Microbiology and Infection, University of Technology Sydney

RES-021

Introduction

Urinary tract infections are one of the most commonly diagnosed infections world-wide today, where Uropathogenic *Escherichia coli* (UPEC) is the main causative agent. With the rising threat of antimicrobial resistance, there is need a to find alternative approaches combating these bacterial infections. However, one major bottleneck in this endeavour is that we still do not know enough fundamental microbial molecular biology to approach this issue in an effective manner. Here we have examined UPEC (model strain UTI89) growth, proliferation and protein dynamics (*e.g.,* division machinery) inside human urothelial bladder cells at a single cell level using a combination of live cell and high-resolution microscopy.

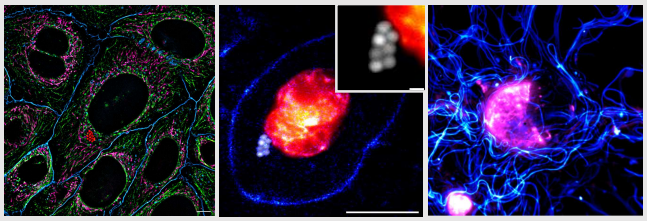
The UPEC infection cycle of epithelial bladder cells



1, UPEC adhesion. 2, Invasion. 3 Intracellular proliferation and IBC formation.
5, Filamentation and escape. 5, Reversal back to rod shape, guided by the DamX protein.

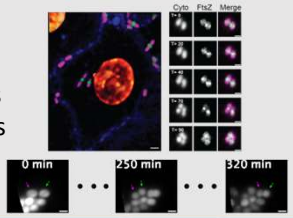
Results

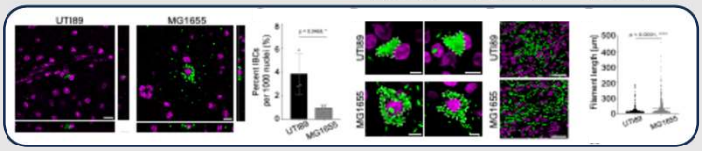
Using microfluidics based *in-vitro* or *ex-vivo* mouse infection models and high-resolution microscopy, we visualise what happens in the host when it is overrun by bacteria and how UPEC transition from rods to filaments inside live bladder cells prior to escaping from the hosts.



Typical images of infected bladder cells. The normally rod-shaped UPEC first transition into a coccoid morphology (left & mid) and then again transitions into long filaments (right) often several hundreds of micrometers in length.

The bacterial cell division machinery (FtsZ in the image) drives the transition from rods to coccoids, and the UPEC cells remain as coccoids for several generations.

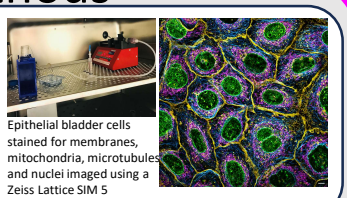




Comparison of infected bladder sheets by UTI89 and a 'non-pathogenic' lab strain (MG1655). Both strains can infect the sheets, form IBCs and subsequently filament when exposed to urine.

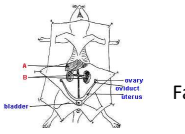
Methods

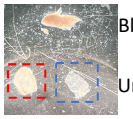
***In-vitro* microfluidics model for UTI infections.**
We continuously flow of nutrients and media/urine over infected human epithelial bladder cells during an infection cycle of 4 days to follow all aspects of UPEC morphology changes over time.

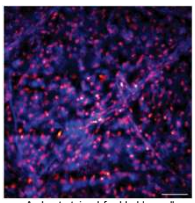


Epithelial bladder cells stained for membranes, mitochondria, microtubules and nuclei imaged using a Zeiss Lattice SIM 5 microscope.

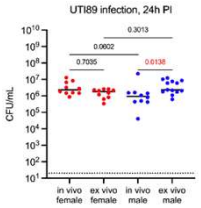
***Ex-vivo* bladder sheet infection model.**
Bladders are harvested from naïve C57BL/6J mice, the Urothelium layer separated from the fat layer then infected with UPEC. Essentially no differences are seen from *in-vivo* infection in terms of bacterial load 24-hour post infection







A sheet stained for bladder cell membranes and nuclei. Imaged by confocal.



Rixa-Mareike et al. *BioRxiv* 2024

Conclusions

By mapping single cell bacterial growth dynamics at high-resolution, we can begin to understand intracellular interactions and how bacteria are spatially localized inside hosts during infection. A better understanding of bacterial proliferation inside host cells could lead to the discovery of new targets for novel therapeutics going forward.

Further reading: Pokhrel & Chisholm et el, *PLoS Biology* 2025. Chisholm et al, *BioRxiv* 2025. Söderström et al, *Nat. Comms* 2022. Abell-King & Costas, *PLoS Pathogens* 2022.

Contact: Bill.Soderstrom@uts.edu.au
Lab webpage