A 3D Infection Model to Determine How d-Mannose Could Reduce Recurrent Urinary Tract Infections.

s.ahmed47@edu.salford.ac.uk

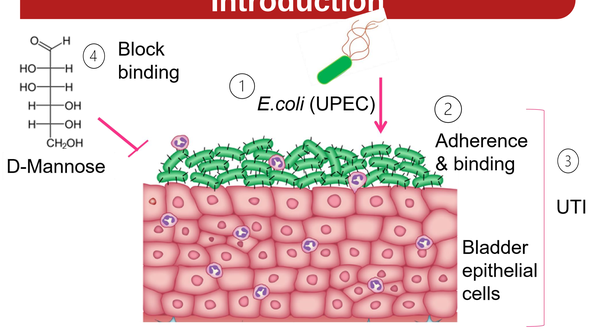
Sakina Ahmed[1], Sarah Withers[1], Rachel Floyd[2], Gail Hayward[3], Chloe James[1].

School of Science, Engineering and Environment, University of Salford, [2]Institute of Integrative Biology, University of Liverpool, [3]John Radcliffe Hospital, University of Oxford.

Abstract

Urinary tract infection (UTI) is the second most common bacterial infection worldwide, affecting millions of people annually, mainly women and primarily caused by Uropathogenic *Escherichia coli* (UPEC). D-mannose has been suggested as a prophylactic treatment to prevent UPEC binding to bladder epithelial cells and reduce the occurrence of recurrent (r) UTI. This project investigates the rates of UPEC adhesion to bladder epithelial cells in the presence of urine from women recruited to a double-blind trial of prophylactic D-mannose.

Introduction



1. UPEC = most common cause of rUTIs[1]
2. Adhesion is a key stage of UPEC pathogenicity[2],
3. rUTIs can lead to sepsis[2,3]
4. D-mannose may prevent UPEC binding & reduce rUTI[4]

We are using a 3D *in vitro* differentiated bladder-cell model[4] to investigate whether prophylactic D-mannose limits adherence of a well-characterised strain of UPEC (CFT073) to HBLAK cells using urine samples from the trial and artificial urine (AUM) controls

Methodology and Results

1. UPEC strains grow in the presence of artificial urine

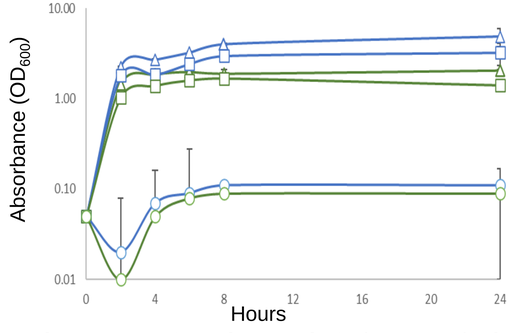


Figure 1: UPEC strain growth 24-hour growth of *E. coli* strains 536 (Triangles), CFT073 (Squares), and UT189 (Circles) in LB broth **(blue)** and artificial urine medium **(green)**.

2. Trans-wells enable differentiation of HBLAK cells

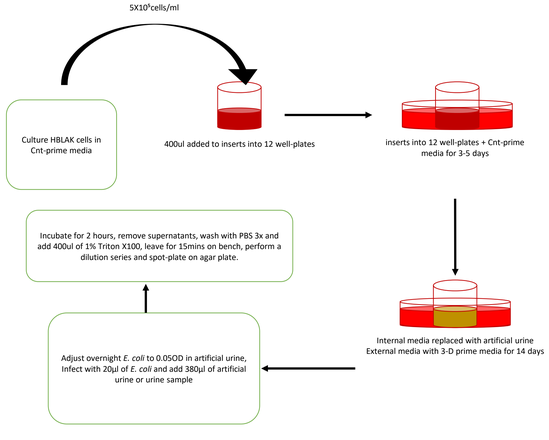


Figure 2: Infection Model Set Up and Adhesion Assay

3. Determined the confluence level with fluorescent staining

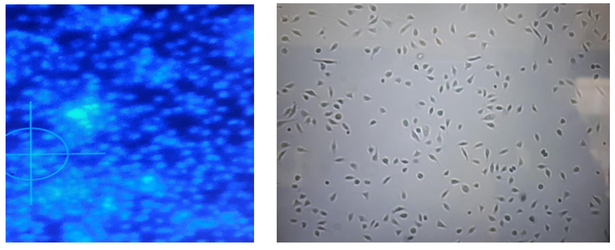


Figure 3: HBLAK cells
A: HBLAK cells stained with DAPI, incubated for 3 days in cnt-prime media, **B:** HBLAK cells growing in Cnt-prime media in T-75 flask, showing 20% confluence.

4. Survived in the presence of artificial urine

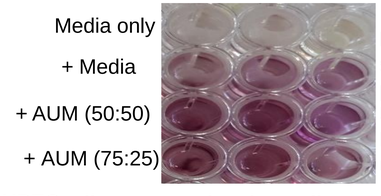
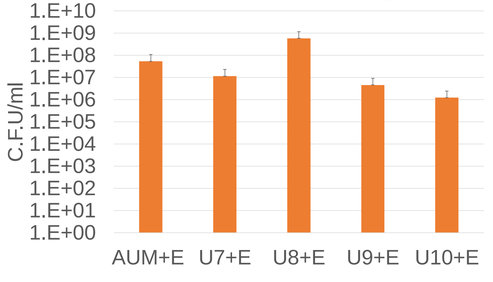


Figure 4: HBLAK cell survival in the presence of Artificial Urine. Cell viability of HBLAK cells in artificial urine (AUM)

5. UPEC adherence varies depending on urine sample used



Reduced binding was observed in the presence of urine samples 7, 9 and 10

Figure 5: *E. coli* binding to HBLAK cells. Dilution series and spot-plating performed to quantify number of CFT073 cells (E) binding to HBLAK cells in the presence of AUM and urine samples 7-10 from the double-blind trial (U) (n=3).

Discussion

Our data suggest the 3D HBLAK cell model is appropriate for investigating host-pathogen interactions in the presence of urine.

The adhesion assay showed that CFT073 can bind to the differentiated bladder cells and that both can withstand exposure to urine. The preliminary data suggest the binding of CFT073 is reduced in some urine samples.

Using this model we plan to screen more urine samples from the trial and further investigate the prophylactic effects of D-mannose treatment on both adherence to and invasion of the bladder epithelium by different strains of UPEC that express different surface adhesins.

Alternative therapeutics like D-mannose are essential to reduce antibiotic use that promotes antibiotic resistance.

Further studies are necessary to fully characterise the mechanisms by which prophylactic D-mannose effects host-pathogen interactions[4]

References

1. Conover, M, S., et al, (2016). Metabolic Requirements of *Escherichia coli* in Interacellular Bacterial Communities during Urinary Tract Infection Pathogenesis. *mBio,* 7(2). E00104-16.
2. Pannek, J. (2020). Prevention of Recurrent Urinary Tract Infections in Neurourology. *European Urology Focus.*
3. Rudd, K, E., et al, (2020). Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. *Lancet.* 395(10219). 200-211.
Barber, A, E. et al, (2016). Strengths and Limitations of Model Systems for the Study of Urinary Tract Infections and Related Pathologies. *Microbiol & Mol. Biol. Rev.* 80(2). 351-367.