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UROPATHOGENS ARE CAPABLE OF INTRACELLULAR GROWTH IN EXFOLIATED UROTHELIAL CELLS ISOLATED FROM WOMEN.

<u>Hypothesis / aims of study</u>
Animal studies have consistently shown that uropathogens such as *Escherichia coli* (*E. coli*) are capable of intracellular growth. This intracellular growth may enable the bacteria to escape detection by the immune system and thus provide a reservoir for recurrent urinary tract infections. The aim of this study was to determine whether other uropathogens (*Enterococcus Faecalis* (*E. Faecalis*) and Group B streptococcus (GBS)) are also capable of intracellular colonisation of urothelial cells.

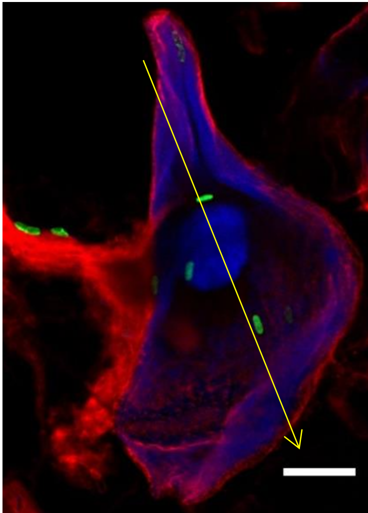
<u>Study design, materials and methods</u>
Mid-Stream Urine (MSU) specimens (n=121) were collected from women (n=94) attending a regional urogynaecology clinic. Half the sample underwent routine microbiology to identify uropathogens, the rest was used for evaluating bacterial colonisation in exfoliated urothelial cells. Exfoliated urothelial cells were concentrated onto a microscope slide and then stained using Wright staining and approximately 100 cells from each sample were counted by light microscopy and categorized according to the presence of bacteria, the location (attached to the outside of the urothelial cell or appears intracellular) and the bacterial density (low density (LD) or high density (HD)) The mean percentage (± SEM) of each category in relation to the total number of cells was calculated.
Based on the Wright staining results cells from samples that appeared to contain intracellular bacteria were stained by immunofluorescence, using specific antibodies to *E. coli* and *E. Faecalis*. The urothelial cell membrane was stained with Wheat-germ agglutinin (WGA) and the nucleus visualised with DAPI. These stained cells were imaged on confocal microscopy, to confirm intracellular localisation of the bacteria. Z-stack analysis included the top and bottom of the imaged urothelial cells. A single line was drawn through the urothelial cell on the X/Y axis, ensuring that the urothelial cell membranes and bacterial cells were included and the fluorescence intensity of each channel was measured.
<u>Results</u>
Uropathogens *E. coli* (n=19), *E. Faecalis* (n=10), and GBS (n=14) were detected in 35.5% (43/121) of MSU samples. Of these, cells from patients with GBS and *E. Faecalis* were more likely to have exfoliated urothelial cells that were clear of bacteria ($p<0.0001$, Table 1, no Bacteria). As expected low density intracellular growth was seen in a large proportion of urothelial cells from patients with *E.coli* (Table 1). However, GBS and *E. Faecalis* were also seen to localise intracellularly, although to a lesser extent (Table 1, $p<0.0001$).

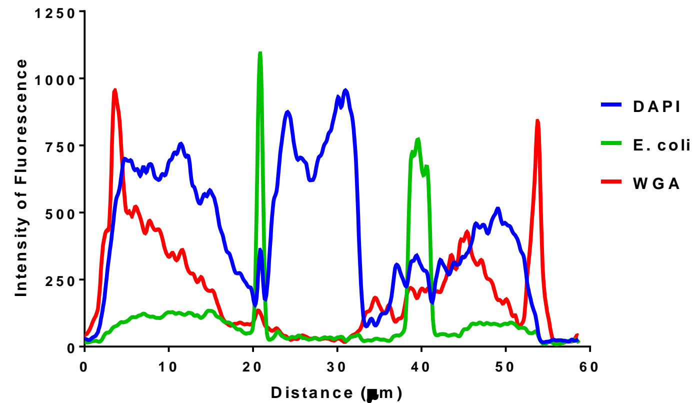
Table 1: The percentage of bacterial association to urothelial cells according to bacterial subtype.

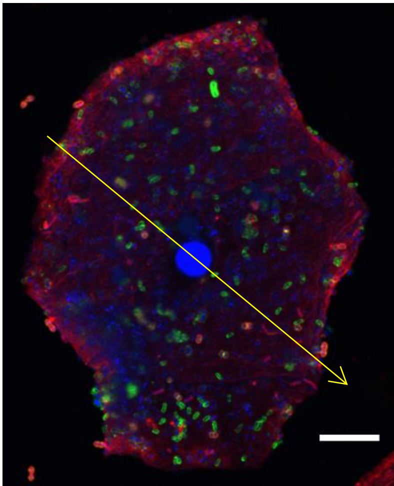
Bacterial Category	*E. coli*	*E. Faecalis*	GBS
Adjacent-LD	8.40 ± 2.07	7.63 ± 1.84	4.29 ± 0.96
Adjacent-HD	2.17 ± 1.12	0.58 ± 0.33	0.06 ± 0.06
Intracellular-LD	72.58 ± 4.12	36.29 ± 9.13^	29.66 ± 4.51^
Intracellular-HD	4.05 ± 1.65	3.61 ± 1.65	7.96 ± 2.18
No bacteria	12.81 ± 3.91	51.91 ± 11.29^	58.02 ± 5.42^

Data was analysed and percentages determined. ^$p<0.0001$, significant increase of urothelial cells with no bacteria and decrease of cells with intracellular-LD in both GBS and *E. Faecalis* positive MSUs compared to *E. coli*; two-way ANOVA (Tukey's multiple comparisons test).

Confocal microscopy confirmed the intracellular localisation of *E. coli* within exfoliated urothelial cells (Figure 1A and B). Similarly, confocal microscopy was able to demonstrate intracellular localisation of *E. Faecalis* within the urothelial cells (Figure 1C and D).







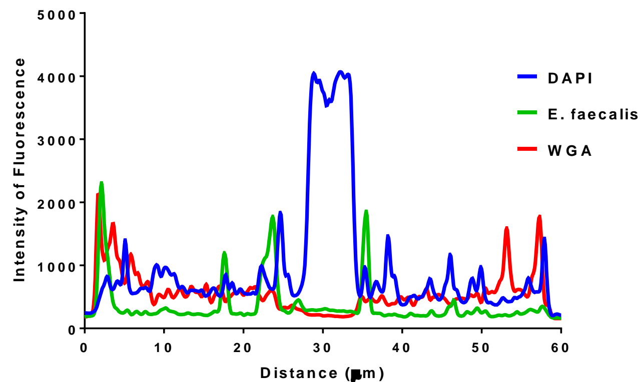


Figure 1: Confocal microscopy of exfoliated urothelial cells to demonstrate intracellular localisation of uropathogens. **A and C**: immunofluorescent staining with anti-*E. coli* (A, Green) or anti-*E. Faecalis* (C, green), wheat-germ agglutinin (red) and DAPI (Blue). Yellow arrows indicate line and direction of Z-stack analysis. **B and D**: Z stack analysis showing intracellular localisation of bacterial as the bacterial immunofluorescence (green) is encased within wheat-germ agglutinin staining (red). Scale bar indicates 10µm.

Interpretation of results

Previously only *E.coli* has been shown to grow intracellularly in exfoliated urothelial cells isolated from patient urine samples. The results of the current study demonstrate that both *E. Faecalis* and GBS are also capable of intracellular growth, although to a lesser extent that *E. coli*. This suggests that intracellular growth might be a common characteristic of uropathogens and may provide a mechanism for these pathogens to evade the bladders defence mechanisms, leading to an increased likelihood of urinary tract infections.

Concluding message

Intracellular growth may be a common strategy used by uropathogens to enhance their ability to cause urinary tract infections.

Disclosures

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