

INVESTIGATION OF BACTERIAL SPECIES AND THEIR ASSOCIATION WITH UROTHELIAL CELLS IN REFRACTORY DETRUSOR OVERACTIVITY PATIENTS.

Detrusor Overactivity (DO) is a common condition (17% of patients >age 40). One third become refractory. Recent studies show that bacteriuria exacerbates symptoms. It has been reported bacteria such as *Escherichia coli* (*E.coli*) can invade and form colonization in bladder urothelial cells. Although two small studies have shown intracellular bacteria in human urothelium [1, 2], these employed the time consuming method of confocal microscopy. Wright stain is a well-established light microscopy technique that identifies bacterial DNA.

The purpose of this study was to: 1) to quantitate the levels (percentage) of urothelial cells associated with bacteria using microscopy on cytospin prepared and Wright-stained urine samples; 2) to compare the routine microbiology identification of so as to evaluate the detection sensitivity with Wright-microscopy method.

Study design, materials and methods

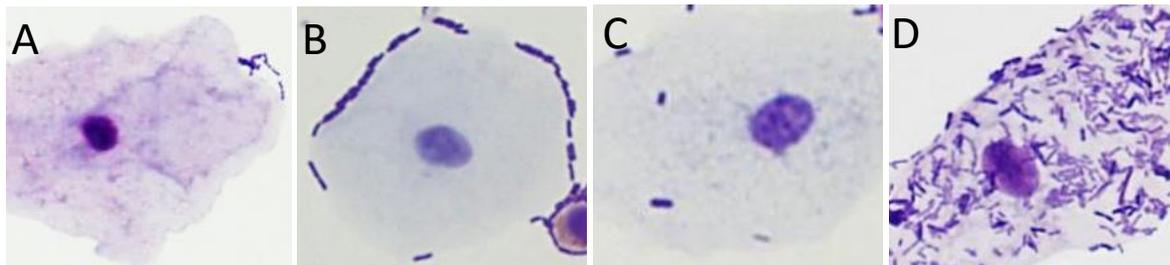
MSU specimens (n=115) were collected from women that presented with refractory DO at a regional urogynaecology clinic. Half of the specimen was sent for routine microbiological testing at the Microbiology Department and the remaining half was processed in the laboratory.

To examine the colonisation of bacterial and urothelial cells the urothelial cells within the sample were cytospun onto microscope slides and stained using Wright staining and visualised using light microscopy. To quantitate the degree of bacterial presence, a novel microscopic counting methodology was devised. Approximately 100 randomly selected urothelial cells were examined at 40x magnification and categorized according to: the presence of bacteria, the location of the bacteria (attached to cell or appears intracellular) and the bacterial density (low or high) (Figure 1). To be included, cells had to be large, spread in shape, intact and isolated with single or dual nuclei. Small, rounded cells and clustered groups were excluded. Low density was defined as occupying <50% of the cell surface. High density was occupation of ≥50%. If a cell appeared to have both adjacent and intracellular bacteria, the bacteria were recorded as “appears intracellular”. The overall percentage of urothelial cells associated with bacteria was counted.

Results

Association classification of bacteria with urothelial cells was revealed according to their location and quantitation of bacterial density: A) attached to cell, low density (<50% of cell surface occupied) (Fig 1A), B) attached to cell, high density (≥50% of cell surface occupied) (Fig 1B); C) intracellular, low density (Fig 1C); or D) intracellular, high density (Fig 1D).

Figure 1: Classification of bacteria adherent to urothelial cells according to their location and quantitation of bacterial density.



Urothelial cells associated with bacteria were observed on Wright stains in 97.4% (112/115) of MSU specimens from all 39 patients. In contrast, routine microbiological testing detected bacteria in only 76.5% (88/115) of samples. Of those, 61.4% (51/88) were reported as contamination and 38.6% (34/88) were reported as a UTI. The MSU specimens reported as UTI positive had a significantly higher proportion of urothelial cells associated with attached, high-density bacteria ($p < 0.05$).

Bacterial Species and association with urothelial cells in urine samples of refractory DO:

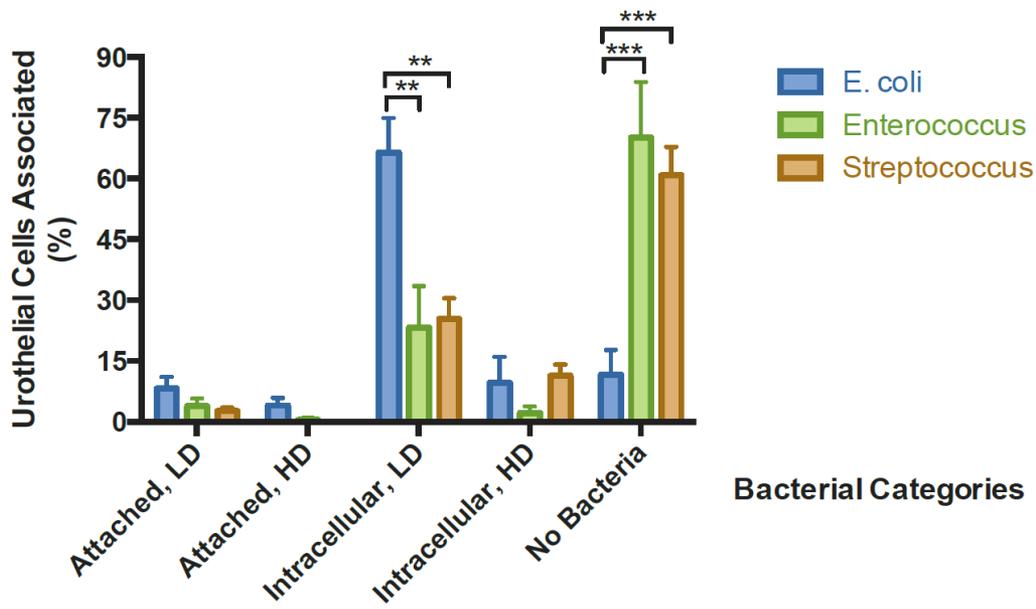
In the MSU specimens that were reported as UTI positive (n=34), the three most common pathogen responsible was *E.coli*, *Enterococcus faecalis* and *Streptococcus* (Table 1). These three pathogens were responsible for over 80% of all UTI reported by the microbiology department in this study. Samples infected with these pathogens were compared in regards to the ability of the pathogen to adhere to (Fig 1B) or invade urothelial cells (Fig 1D).

Urothelial cells isolated from patients with *E.coli* infections were more likely to be associated with bacteria (Table 1, Figure 2, $p < 0.001$). Similarly, while all three species appear to be capable of invading urothelial cells, *E.coli* was significantly more likely to have been seen as appearing intracellular than *Enterococcus* or *Streptococcus* (Table 1, Figure 2, $p < 0.01$). There was no significant difference in the proportion of cells with bacteria attached to the surface.

Table 1: % of Urothelial cell associated with bacteria uropathogens

Uropathogenic strain identified by the microbiology department	<i>E. Coli</i>	<i>Enterococcus faecalis</i>	<i>Streptococcus</i>
% specimens	38% (13/34)	17% (6/34)	26% (9/34)
% urothelial cells with bacteria	88.3%	29.9%	39.2%
% of urothelial cells classified as “Appears Intracellular – low density”	66.5%	23.3%	25.3%

Figure 2. Comparison of urothelial cells association with 3 major bacterial species detected.



Interpretation of results

Wright-microscopy has yielded a much higher rate (97.4%) of detecting bacterial presence in urine samples compared to routine microbiology culture tests (33.9%). Previously *E. coli* has been shown to be associated with forming intracellular bacteria *in vivo* in animal studies and in humans with an acute UTI. Enterococcus has been shown to be capable of invading urothelial cells *in vitro*. This study shows that in urothelial cells isolated from female patients all species are capable of appearing intracellular although *E. coli* are more likely to be intracellular.

Concluding message

Our results indicate that urothelial cell-associated bacteria are often intracellular, but may not be detectable with routine microbiology testing.

References

1. Rosen DA, et al., (2007) Detection of intracellular bacteria in human UTI. PLoS Med.4:e329.
2. Cheng Y, Chen Z, Mukerjee C, Varetas K, Mansfield KJ, Schembri MA, Moore KH (2014). Bacterial filaments are more common in DO than controls. Int. Urogyn J, 25: s90-91.

Disclosures

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