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DIFFERENCES IN THE BLADDER MICROBIOME BETWEEN WOMEN WITH OVERACTIVE BLADDER AND HEALTHY CONTROLS.

Hypothesis / aims of study

The bladder was previously understood to be a sterile environment, however, recent advances suggest that like the skin or gut that the healthy bladder has a bacterial flora. Bacteria have both been grown (1) and bacterial DNA identified from (2) the urine of healthy individuals with no bladder symptoms. In addition, differences in the bladder microbiome have been shown in healthy individuals and in individuals with bladder symptoms (1,3). However, the number of urine samples previously studied has been relatively small making statistical interpretation uncertain. This study aims to capture and characterise the microbiome of the bladder of healthy controls and women with overactive bladder (OAB). We hypothesise that the there are differences in the resident bacterial flora between these two groups with some bacteria being protective and other associated with OAB.

Study design, materials and methods

A consecutive cohort of women with OAB attending a urogynaecology clinic between 2014 and 2015 were identified and invited to participate in the study. Controls were identified from women attending a general gynaecology clinic excluding those with bladder or prolapse symptoms (score=0 on ICIQ-UI short form). All women gave Informed consent to be included in the study. Demographic data was recorded and the ICIQ-FLUTS validated questionnaire was completed.

A clean catch mid-stream urine (MSU) was taken from both controls and women with OAB. Urinalysis was undertaken and patients with urine positive for nitrates were excluded from the study. A sample was sent for routine clinical microbiological assessment and patients with a positive MSU (> $1x\ 10^5$ cfu) for urinary tract infection (UTI) were also excluded.

A 5ml sample of urine was taken and centrifuged, the resulting sediment was resuspended and plated on to two chocolate agar plates. Both were incubated at 37ºC, one in 5% CO_2 for 48 hours the other in anaerobic conditions for 7 days. Each morphologically different colony was purity plated and incubated in the same conditions for a further 48 hours. Bacteria were then lysed and polymerase chain reaction undertaken using primers for the 16rRNA gene. The resultant DNA was purified and sequencing undertaken of the 16srRNA gene allowing identification of the bacterial genera.

Results

The urine samples of 152 women were analysed (70 from women with OAB and 82 from healthy female controls). The mean number of different bacterial genera was 5 in urine samples of both the control and OAB group (5.4 Controls v 4.8 OAB). 98.7% of all urine samples grew at least one isolate. The most commonly grown organisms in all samples were Staphylococcus in 62%, Streptococcus (49%), Lactobacillus (39%) and Corynebacterium (38%).

There are significant differences found in the prevalence of some organisms in the bladder of women with OAB and healthy controls (Fishers exact). Some “protective” organisms are more prevalent in bladders of controls whilst others feature more often in women with OAB (table 1).

Table 1 Significant differences identified in Microbiome

Bacteria	**Difference**	**P value**
Lactobacillus	Control > OAB	0.0001
Prevotella	Control > OAB	0.05
Peptoniphilus	Control > OAB	0.04
Fusobacterium	Control > OAB	0.005
Klebsiella	OAB>Control	0.05
Proteus	OAB>Control	0.04
E. coli	OAB>Control	0.05
Serratia	OAB>Control	0.05
Haemophilus	OAB>Control	0.05
Clostridium	OAB>Control	0.05

Interpretation of results

The most striking result is that as well as being one of the most prevalent bacteria found in 39% of all urine samples, Lactobacillus is more often found in the bladder of women with no significant bladder symptoms than those with OAB. This is highly statistically significant in our results (P value 0.0001). This result is in line with another study that found lactobacillus in 29% of control urine samples compared with 12% of urine samples from patients with OAB (1). This raises the possibility of Lactobacillus having a protective effect. A further study comparing women with urgency urinary incontinence (UUI) to controls found decreased Lactobacillus amongst the UUI group (3). This study found distinctions at the species level between Lactobacillus found in each group. The urine for both these studies were collected by transurethral catheter suggesting that lactobacillus is likely to be present in the female bladder and not present by contamination from the genital tract. These results raise the possibility of lactobacillus having a protective effect.

The other bacteria we have found to be statistically significantly more common in control group urine samples; Prevotella, Peptoniphilus and Fusobacterium in contrast were only found in the OAB urine samples in a different study (1). The numbers are very small in both studies and perhaps these results should be interpreted with caution.

This study shows pathogenic organisms commonly seen in patient with urinary tract infection are found more commonly in the urine of patients with OAB than controls; eg Klebsiella, Proteus, E.coli. These organisms may be responsible for lower urinary

tract symptoms by causing a chronic low grade inflammatory process similar but distinct from that seen in an acute UTI. Likewise, other studies have found some bacteria more common in women with bladder symptoms than controls (1,3).
This study with large numbers further adds to this growing body of evidence of a true background bladder microbiome with 98.7% or all samples growing bacterial species. The bacterial genera identified in this study overlaps with other studies (1-3). In this study we have shown significant differences in the microbiome between controls and women with OAB. Other studies have shown differences between women with and without bladder symptoms (1,3). These finding suggest that in common with other body systems alterations in the normal bacterial environment of the bladder is associated with disease processes.

Concluding message

Our results show the female human bladder has a diverse microbiome. There are significant differences in the prevalence of some organisms between controls and women symptomatic of OAB. Lactobacillus may have a protective effect for OAB.

References

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