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THE VAGINAL AND BLADDER MICROBIOMES IN WOMEN WITH LOWER URINARY TRACT SYMPTOMS

Hypothesis / aims of study

Overactive bladder (OAB) is a prevalent and chronic condition which is recognised to have a multifactorial aetiology. Acute urinary tract infection (UTI) may be associated with severe but transient lower urinary tract symptoms (LUTS), including pain/dysuria, frequency, urgency, and urgency incontinence (UUI). Whilst acute infections were not originally thought to contribute to OAB syndrome, incident urinary tract infection (UTI) may be more common among women with chronic LUTS, and bacteria is a frequent finding [1].

Historically the bladder was thought of as a sterile environment, but advancements in scientific techniques have enabled us to detect bacteria that evade conventional culturing techniques. The introduction of 16S RNA sequencing technology has helped identify the role that communities of bacteria play in many types of common non-communicable disease. Urine has its own unique microbiome, which may play a critical role in functional disease of the lower urinary tract [2,3].

Our aim was to assess the relationship between the urinary, urothelial and vaginal microbiomes in patients suffering from lower urinary tract symptoms, specifically those with overactive bladder syndrome.

Study design, materials and methods

We recruited women from a tertiary centre over a two year period. Symptomatic cases were identified from either Urogynaecology clinics or from urodynamic studies and included women over the age of 18 years who suffered with a variety of OAB symptoms and who were undergoing cystoscopic examination of the bladder. Controls were women undergoing surgery for stress urinary incontinence or prolapse surgery with no other LUTS. We excluded women with urinary calculi, urinary tract malignancy, neurological disease, current pregnancy or breast feeding. After informed consent women completed the 12 item ICIQ-FLUTS questionnaire assessed by recall over the previous one month period. From this we specifically looked at question 9a; "does urine leak before you get to the toilet?". Patients with a score of 2 or more would be included as symptomatic case participants. At the time of cystoscopy we collected a bladder biopsy, a catheter urine sample and a high vaginal swab sample. Samples were stored at -80°C prior to extraction. DNA from the samples were extracted using QiAMP DNA minikit, and they were sent to Research and Testing Labs in Texas for sequencing using 16S rRNA analysis. Data was analysed using STAMP statistical software.

Results

In total 103 women were recruited for this study with a mean age of 50 years (SD 16.4) and median parity of 2 (range 0-5). All 103 women had a bladder biopsy taken, 99 women had a urine sample taken, and 68 women had a high vaginal swab analysed. 964 individual species of bacteria were identified in the microbiomes, however 405 of these species were defined as "unclassified" or "unknown" at different points of their taxonomic ranks. All "unclassified" bacteria have been assigned a number in order to be included in the statistical analysis. Samples which were contaminated with common water and soil born pathogens were removed from analysis.

Table 1 summarises all the significant bacteria found in each individual microbiome in women with urgency urinary incontinence. Propionimicrobium lymphophilum was found significantly more often in the urothelium of women suffering from UUI compared to controls, whereas prevotella bivia was found significantly more in the urothelium of controls. Equally, Acidocella was found at higher levels in the urine of control patients compared to symptomatic women. No significant species were found in the vaginal microbiome.

Table 1. Results of individual microbiomes for women with urgency urinary incontinence.

Microbiome	Cohort	Bacterial Species	P value
Urothelium	Control	prevotella bivia	0.026
Urothelium	Case	Propionimicrobium	0.034
Urine	Control	Acidocella sp	0.042
Urine	Case	Nothing significant found	N/A
Vaginal	Control	Nothing significant found	N/A
Vaginal	Case	Nothing significant found	N/A

When considering abundance of bacteria within each microbiome we found Lactobacillus sp ($p=4.32e-3$) was associated with urinary microbiomes that had a high abundance of bacteria, whereas Acinobacter sp ($p=8.99e-4$) was associated with low bacterial abundance urinary microbiomes. Lactobacillus iners was also associated with individuals who had a high abundance of bacteria in their urothelium microbiome.

The microbiomes were then compared with each other to assess their relationship. We successfully sequenced: 65 pairs of urine and vaginal samples, 99 pairs of biopsy and urine samples, and 68 pairs of vaginal and biopsy samples. When comparing urinary and urothelial microbiomes, there was more bacteria present in the urine than the urothelium in patients with UUI. From the 68 patients who had both vaginal and urothelium microbiomes sequenced, we found that patients suffering from UUI had significantly increased amounts of *Propionimicrobium lymphophilum* ($p=0.026$) in their urothelium. Patients who had *Lactobacillus* dominant vaginal microbiomes also had a significantly increased amount of *L.crispatus* in their urothelium ($p=2.42e-3$) and urinary microbiomes.

Interpretation of results

Propionimicrobium lymphophilum is a gram positive bacteria associated with UTI so it was interesting to find the presence of *Propionimicrobium lymphophilum* in the urothelium of women suffering from UUI. This suggests a further bacterial component to the development of OAB. Interestingly, none of our patients had microbiological UTI at the time of bladder biopsy and the bacteria was only identified on sequencing.

We found statistically significant higher levels of *Prevotella bivia* in the urothelial biopsy of patients without LUTS. This is similar to findings from other studies which have indicated that this bacteria may have a protective role against development of LUTS. Similarly, in the urinary microbiome the presence of *Acidocella* sp. was also found to have a protective effect against urinary incontinence. This highlights that bacteria can have a protective as well as a pathological role within the bladder of patients with OAB symptoms.

Lactobacillus is often considered to have a protective effect against development of UTI. From our results it would seem that it can be either protective or causative depending on the species found.

Concluding message

Incidental bacteriuria is associated with a range of LUTS including nocturia, urgency, bladder pain, and daytime frequency, supporting a role for bacterial colonisation in the pathogenesis of OAB. This study also highlights that bacteria can have different roles depending on their environment. Sequencing can be used to identify a wide variety of specific bacteria. From our study this method of bacterial identification from urothelium samples seems to identify bacteria that a simple microscopic culture does not. Whilst this is unlikely to replace microscopic culturing this could become a useful test in the future in identifying causation of refractory OAB in patients where standard culturing fails to identify specific bacteria.

References

1. *Obstet Gynecol.* 2008 Feb.;111(2 Pt 1):317–323
2. *Journal of clinical microbiology* 2007;45(9): 2761-2764.
3. *Molecular Diagnosis* 2001;6(4): 313-321.

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