W15: Bladders Under Siege: Could Bacteriuria be the key to understanding refractory urge incontinence?

Workshop Chair: Kate H Moore, Australia
12 September 2017 11:00 - 12:30

<table>
<thead>
<tr>
<th>Start</th>
<th>End</th>
<th>Topic</th>
<th>Speakers</th>
</tr>
</thead>
<tbody>
<tr>
<td>11:00</td>
<td>11:10</td>
<td>Introduction to the Workshop</td>
<td>Kate H Moore</td>
</tr>
<tr>
<td>11:10</td>
<td>11:25</td>
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</tr>
<tr>
<td>11:25</td>
<td>11:40</td>
<td>Intracellular Enterobacter in <em>in vitro</em> models of UTI</td>
<td>Harry Horsley</td>
</tr>
<tr>
<td>11:40</td>
<td>11:50</td>
<td>Questions</td>
<td>All</td>
</tr>
<tr>
<td>11:50</td>
<td>12:00</td>
<td>Culture independent study of recurrent Bacteriuria in refractory DO</td>
<td>Kate H Moore</td>
</tr>
<tr>
<td>12:00</td>
<td>12:15</td>
<td>Urinary Microbiome in urge incontinence and relation to treatment response</td>
<td>Elizabeth Mueller</td>
</tr>
<tr>
<td>12:15</td>
<td>12:20</td>
<td>Summary and Clinical significance</td>
<td>Kate H Moore</td>
</tr>
<tr>
<td>12:20</td>
<td>12:30</td>
<td>Questions</td>
<td>All</td>
</tr>
</tbody>
</table>

**Speaker Powerpoint Slides**

Please note that where authorised by the speaker all PowerPoint slides presented at the workshop will be made available after the meeting via the ICS website [www.ics.org/2017/programme](http://www.ics.org/2017/programme) Please do not film or photograph the slides during the workshop as this is distracting for the speakers.

**Aims of Workshop**

The overactive bladder (OAB) syndrome is the main cause of urge incontinence and urgency (generally associated with detrusor overactivity). Approximately 35% of patients with detrusor overactivity are unresponsive to current Antimuscarinic drugs. These “refractory” patients are a hard-core group of sufferers constantly expending health care resources in their search for relief. Recent studies find bacterial cystitis in approximately 30-50% of DO patients refractory to treatment. This workshop will bring together clinicians and scientists to discuss the recent findings on recurrent bacterial cystitis and reasons for antibiotic resistance in relation to the refractory state.

**Learning Objectives**

After this course the participants will be able to:

1. Critique the evidence linking bacteriuria with the aetiology of urge incontinence
2. Describe the interactions that occur between uropathogens and the urothelium
3. Predict the effect of antibiotics on the symptoms of urge incontinence

**Target Audience**

This workshop will be of interest to urogynaecologists, urologists, nurse continence advisors and basic scientists with an interest in the aetiology of urge incontinence and refractory detrusor overactivity, and the role of bacterial infection in these pr

**Advanced/Basic**

Advanced

**Conditions for Learning**

Interactive workshop, ideally restrict to 50 people (max 60)

**Suggested Reading**

Evidence for intracellular bacteria in urge incontinence.

Kylie J Mansfield, Ying Cheng, Samantha Ognenovska, Zhuoran Chen, Kate H Moore, School of Medicine, University of Wollongong, Australia

The role of subclinical infection in patients with urge incontinence has been largely ignored although recent evidence suggests that urinary tract infections (UTI) maybe involved in the aetiology of refractory Detrusor Overactivity (RDO) and several studies have reported that uropathogens such as E. coli may invade the urothelial cell layer using murine models and cell lines. Our aims were to 1) test for the presence of intracellular bacteria in the urine of patients with detrusor overactivity or mixed incontinence +/- a history of UTI, and compare this to a control group of patients with stress incontinence and no history of infection and 2) to examine cellular invasion as a pathogenic factor for three uropathogenic bacterial strains.

Mid-stream urine (MSU) specimens were collected from women: half was used for traditional microbiological diagnosis of UTIs, with the other half used for microscopic examination of exfoliated urothelial cells. Based on routine microbiology, bacterial cystitis was seen to be more common in patients with refractory DO.

Microscopy and Wright staining of concentrated urothelial cells demonstrated the presence of bacteria in the majority of samples. Filamentous bacterial cells, indicative of intracellular growth, were observed in 51% of patients and were significantly more common in patients with DO. On Wright staining, bacteria appeared intracellular at low-density in patient samples positive for each of the uropathogens examined, that is E. coli, E. faecalis and Group B Streptococcus, although this was seen more frequently in E. coli positive samples. Confocal microscopy revealed that both E. coli and E. faecalis were able to invade the urothelial cell. Due to technical difficulties relating to cross-reactivity of the antibodies used, the results for intracellular localisation Group B Streptococcus were inconclusive.

This study supports the possibility that a subset of patients with urge incontinence may have unrecognised chronic bacterial colonisation, maintained via an intracellular reservoir. In patients with negative routine microbiology, application of the techniques used in this study revealed evidence of infection, providing further insights into the aetiology of urge incontinence.

A urine-tolerant three-dimensional epithelial organoid from adult human bladder stem cells reveals novel aspects of host/pathogen interactions

Harry Horsley, Dhanuson Dharmasena, James Malone-Lee and Jennifer L. Rohn
Chronic UTI Group, Centre for Nephrology, University College London, United Kingdom

Urinary tract infection (UTI) constitutes an immense healthcare burden, not least because of its tendency to recur despite treatment, or to persist in a chronic form. Many questions still remain about the host/pathogen interactions during bladder infection, but current model systems have disadvantages. Traditional human bladder cell line monolayers bear no resemblance to the three-dimensional urothelium, and there is evidence that the mouse model of infection may not be entirely representative. Recent efforts using human organ- and stem-cell-derived organoid culture have yielded promising models, but none can withstand the presence of urine at the apical interface for more than a few hours. This is important because urine is the natural environment of UTI pathogens and may affect their behaviour, as well as the biological response of the epithelium to those pathogens. We therefore set out to create a human cell-based organoid culture with urine-tolerant properties.

Commercially available human bladder epithelial progenitor cell derivatives were grown and differentiated in 3D culture inserts for a maximum of 24 days, with specialized medium at the basal layer and sterile human urine at the apical liquid-liquid interface. A combination of confocal and electron microscopy showed this human urothelial organoid to be phenotypically similar to native human bladder tissue. Infection of the model with patient-isolated Enterococcus faecalis, a species common in chronic UTI cases, caused rapid apical live-cell shedding, which is one of the hallmarks of urine infection in human patients. Moreover, this common Gram-positive uropathogen invaded the intermediate and basal urothelial cells of the organoid, forming clear and numerous intracellular colonies. In contrast, a strain of uropathogen E. coli (UPEC) shown to be invasive in murine
models (UTI89) formed extensive biofilms on the organoid surface but did not exhibit an invasive phenotype. This result agrees with our previous published work with shed patient cells, which revealed superficial biofilms but again, no evidence for intracellular *E. coli* colonisation.

Considering the differences between the human and rodent bladder, we propose that further studies on patient material are needed before the question of uPEC's invasion behaviour can be settled. In conclusion, current advances in 3D tissue culture enabled us to grow physiologically relevant organotypic human models of the bladder. Human bladder biomimetics could be used as a reproducible test bed for chronic infectious disease formation, treatment and resolution in humans.

**Culture independent study of recurrent Bacteriuria in refractory DO**
Kate H Moore, Zhuoran Chen, Lucy Bates, Mark Schembri
Pelvic Floor Unit, St George Hospital, Sydney, Australia

Urinary tract infection (UTI) has become an increasing problem in women with refractory detrusor overactivity (DO), affecting at least 40% of such women. At the same time the high rate of infections caused by antibiotic resistant bacteria impacts our ability to successfully treat UTIs. This is especially true for uropathogenic *E. coli*, which is responsible for over 80% of all UTIs and is increasingly becoming multi-resistant. Our aim was to investigate women with refractory DO and co-existent recurrent UTI over an extended time period. We carried out periodic analysis of urine using a combination of routine microbial culture as well as using culture-independent methods (rRNA analysis) to determine the composition of bacteria present in the urine during the same time period.

Multiple MSU specimens were collected (with careful labial toilet) from 39 women over a two year period (Median age 75, range 57.81 years). Half of the urine sample was sent to the Microbiology Unit, cultured routinely at a threshold of >10^5 CFU/mL, to identify the major causative organism and antibiotic resistance. The results of routine culture, resistance patterns and isolates obtained from the agar plate were recorded. The remaining samples were stored in frozen aliquots (-20°C), from which total DNA was extracted. Genus-level characterization of the bacteria present in the urine samples was determined by employing 16S rRNA gene amplification and amplicon pyrosequencing (Willner et al 2014, mBio 5(2):1-10).

Symptoms were recorded at the time of urine collection and often the only UTI symptom was worsening urgency, frequency and urge leak. On average the women in the study experienced 8 UTI during the 6-24 months. Nine women with proven recurrent UTI and refractory DO provided multiple urine specimens (median 5 samples per patient; range 2-10, 42 specimens in total). Traditional microbiology culture results showed only 4 samples had no growth. 18 samples had a single dominant organism reported; 17 samples were reported as mixed perineal +/- Bowel flora. Three patients had documented changes in the bacterial flora on routine microbiology culture results over time. Of the 18 samples with confirmed bacteria on routine microbiology, only 4 were not resistant to multiple antibiotics.

Culture-independent 16S rRNA sequencing has revealed that a diverse array of organisms are present in the urine samples from individual patients. Each patient yielded an average of 26.7 different genera (SD 11.2, Median 25, IQR 21, 36). Further assessment of these populations will determine how the bacterial populations vary in each patient over time. This finding is important as most Microbiology laboratories do not routinely report all organisms grown, preferring to report only the dominant organism, especially when there is mixed growth. However, if multiple bacteria are actually colonising the bladder, then treatment with antibiotics (specific for the predominant organism), may encourage unreported organisms to proliferate and become resistant.

This study demonstrated that the organisms isolated from women with recurrent UTI and refractory DO alter over time, as does antibiotic resistance. In these patients, reporting all identified bacteria may help guide treatment. Culture independent 16S rRNA sequencing data will enable us to profile all of the organisms present in the urine over an extended time period, and enable us to link changes in the bacterial population to episodes of symptomatic UTI.

**Urinary Microbiome in Urge Incontinence and relation to treatment response**
Elizabeth R. Mueller
Female Pelvic Medicine and Reconstructive Surgery, Loyola University Stritch School of Medicine, USA

The newly discovered female urinary microbiota has the potential to deepen our understanding of urinary tract health and disease, including common lower urinary tract conditions such as urinary incontinence and urinary tract infection. Studies using culture-independent techniques confirm prior reports of bacteria that reside in the female urinary bladder. These resident communities, the female urinary microbiota, possess characteristics that differ between women affected by urgency urinary incontinence and matched, unaffected controls. Enhanced urine culture techniques permit cultivation of organisms, including uropathogens, missed by standard urine culture, but detected by culture-independent sequencing techniques.
Based on the available data, it appears that the female urinary microbiota are similar to other human microbial niches in that there is no one “normal” state, but rather variable between individuals. However, there are distinct trends. Most urine samples studied to date are not rich and contain one or two microbes that are substantially more abundant than others. These samples can be categorized on the identity of that or predominant microbe. Each category has been termed a “urotype” similar to the “enterotype” used by many gut microbiome researchers. At the genus level, the most common urotype is Lactobacillus. The next most common urotypes are Gardnerella, Corynebacterium, Streptococcus and Staphylococcus; other less common urotypes exist. Notably, these are all Gram-positive bacteria, quite unrelated to the Gram-negative bacteria, such as E. coli, responsible for the vast majority of acute uncomplicated urinary tract infection (UTI). Some samples are not predominated by a single organism or even two; they are placed in a urotype called “diverse.” The biological significance of predominance by any specific organism or the lack of a predominant microbe is not yet known. However, female urinary microbiota diversity appears to have associations with the host’s hormonal status, body mass index and certain clinical conditions.

Despite hopes of finding a single “causative” organism (similar to H. pylori for stomach ulcers), community characteristics may be more important that the presence or absence of a particular microbe. This would be expected if the FUM play a protective role. For example, female urinary microbiota diversity appears to relate to the presence of urgency urinary incontinence (UUI). A recent report suggests that treatment response may be related to the number of unique organisms (richness) present prior to solifenacin treatment for UUI [1]. Following replication of this work, it may be possible to refine clinical estimates of treatment efficacy, based on a pre-treatment assessment of that individual patient’s urinary microbial community characteristics. Another report identified an association between UUI symptoms and several bacterial species, including a number of emerging Gram-positive pathogens; this report also found that Lactobacillus crispatus associates with the lack of symptoms, suggesting the possibility that L. crispatus may be beneficial to maintaining bladder health.

Workshop 15:
Bladders Under Siege: Could Bacteriuria be the key to understanding refractory urge incontinence?
Workshop Chair: Prof Kate H Moore, Australia

Workshop program
- Kate Moore Professor, Urogynaecology, UNSW, Sydney
- Kylie Mansfield, Assoc. Professor, Physiology, Graduate School of Medicine, University of Wollongong
- Harry Horsley, Cell Biologist, Chronic UTI Group, Centre for Nephrology, UCH, London
- Elizabeth Mueller, Female Pelvic Medicine and Reconstructive Surgery, Loyola University Stritch School of Medicine, Chicago USA

<table>
<thead>
<tr>
<th>Topic</th>
<th>Speakers</th>
</tr>
</thead>
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<td></td>
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</tr>
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</tr>
</tbody>
</table>

Overactive bladder syndrome
Patients suffer from urgency, frequency, nocturia, +/- urge incontinence; Affects 17% of age 40 years;

Urodynamics:
- reveals Detrusor Overactivity (DO)
- i.e. bladder spasms

Typical bladder spasms seen on cystometry

Treatment of OAB
Standard treatment:
- Antimuscarinic drugs with bladder training
Helpful in 50-60%
- Long-term cure in about 20%

Definition of Refractory = Failed response after 2 different drugs etc., for more than 1 year of Rx
About 30% of patients

A longitudinal study over 5 to 10 years of clinical outcomes in women with diabetic detrusor overactivity
Controversy

• Anecdotally, many women with DO/OAB report history of recurrent UTI, not always “proven UTI”
• However, patients often state that they had one or more classical symptoms, and antibiotics resolved these symptoms

PROBLEM:
• Kass’ traditional threshold for “significant” bacteriuria (≥10⁶ CFU/L) seemed unduly stringent
• UK + Australia: UTI = 10⁶ CFU/L
• USA + Europe: UTI = 10⁵ CFU/L

Experimental work examining the relationship between bacteriuria and detrusor overactivity

Colin Walsh
MB BCh BAO MRCP I MRCOG PhD
Fellow, Pelvic Floor Unit, 2010 – 2012

Common Microbiological Methods

• Collaboration with Department of Microbiology
• Specimens cultured on Horse Blood Agar (at 35°C in 7% CO₂ and MacConkey agar (at 35°C in air)
• Agar inoculated using larger 10µL quantitative loop – yields positive result at 10⁵ CFU/L

Significant pyuria defined as ≥10 white blood cells per mL on microscopy

1. Pilot Study

2-year study of MSU specimens
• “Refractory” idiopathic DO – (failed ≥2 anti-cholinergics etc for ≥ 1 yr with persistent disabling symptoms)
• invited to attend whenever urge symptoms became exacerbated and they provided MSU, careful clean catch with labial saline rinse
• Excluded: Dysuria, fever, malodorous urine
• Control MSUs: women without OAB

1. Pilot Study Findings!

50 women with “refractory” IDO 50 controls
At time of worse symptoms Age matched
168 MSU specimens 50 MSU specimens

Bacteriuria ≥10⁶/L Bacteriuria ≥10⁵ CFU/L
= 39% = 6% P<0.0001

Bacteriuria 10⁵-10⁶ CFU/L Bacteriuria 10⁵-10⁶ CFU/L
= 17% = 2% P=0.0091

39% of RDO urine specimens,
56% of RDO patients had bacteriuria

Difficulty with publication: MSU CRITICISED
2. Prospective Cross-Sectional Study of CSU

Purpose:
To address criticism regarding use of Mid stream urine Cultures in the Pilot Study

Hypothesis
1) Bacteriuria is more prevalent on CSU in incontinence versus continent controls
2) Bacteriuria on CSU is more common in DO compared to other diagnoses

Results: Incontinent v Continent

<table>
<thead>
<tr>
<th>Urine culture result (CSU)</th>
<th>Incontinent (n=161)</th>
<th>Continent (n=75)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Low-count” bacteriuria 10^5-10^7 CFU/ml</td>
<td>12 (7.4%)</td>
<td>1 (1.3%)</td>
<td>5.9 (0.76 to 46.7)</td>
<td>0.044</td>
</tr>
<tr>
<td>“High-count” bacteriuria &gt;10^8 CFU/ml</td>
<td>8 (5%)</td>
<td>1 (1.3%)</td>
<td>3.9 (0.47 to 31.5)</td>
<td>0.161</td>
</tr>
<tr>
<td>Any bacteriuria &gt;10^9 CFU/ml</td>
<td>20 (12.4%)</td>
<td>2 (2.7%)</td>
<td>5.2 (1.2 to 22.8)</td>
<td>0.011</td>
</tr>
</tbody>
</table>

60% of the positive urine cultures were “low-count” bacteriuria
82% of positive specimens grew E.coli

Results by Urodynamic diagnosis

<table>
<thead>
<tr>
<th>Diagnosis (n=181)</th>
<th>Low count</th>
<th>High count</th>
<th>Any (%)</th>
<th>Odds ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Detrusor Overactivity</td>
<td>40</td>
<td>3</td>
<td>3</td>
<td>6 (15)</td>
<td>6.4 (1.24 to 33.6)</td>
</tr>
<tr>
<td>Pure Urodynamic Stress Incontinence</td>
<td>63</td>
<td>3</td>
<td>1</td>
<td>4 (8)</td>
<td>2.93 (0.53 to 16.9)</td>
</tr>
</tbody>
</table>

Conclusions
1) “Low-count” bacteriuria now known to be important in refractory DO
2) Women with refractory IDO have bacteriuria rates of 39% of MSUs, 56% of patients
   27% of CSUs, 28% of patients without acute dysuria – at time of acute exacerbation of urge
3) Newly diagnosed DO have OR 5.9 low count bacteriuria compared to those with a stable bladder

3. Cohort Study - Methods

Prospective CSU cohort study in women with “refractory” DO
Eligible women who were mailed a personal invitation to attend PFU when urgency symptoms acutely worsened
Patient catheterised, CSU taken

RESULTS:
Overall, 27% of 56 CSU results showed significant bacteriuria,
28% (9/32) with refractory DO had bacteriuria on CSU

Workshop program

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Summary and Clinical significance

Prof Kate H Moore, Australia

So what is the clinical significance?

Could prolonged bladder-specific antibiotics correct the problem?

There have been two open studies conducted by colleagues in London

Vik Khullar (St Marys Hospital London)
James Malone-Lee (UCL)

Positive results, but no controls

1. Trial of antibiotics in OAB patients

Patients = refractory OAB
Antibiotics = a 6 week course of rotating antibiotics
Three consecutive antibiotics were given for 2 weeks each
- Ciprofloxacin
- Doxycycline
- Cephalexin or co-amoxiclav

1. Trial of antibiotics in OAB patients

Table 3: Overactive bladder (OAB) symptoms at baseline and after a 6-week course of antibiotics

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Pre-treatment</th>
<th>After 6-week course of antibiotics</th>
<th>p</th>
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<tbody>
<tr>
<td>Daytime Frequency</td>
<td>12.4 (±3.5)</td>
<td>8.7 (±2.7)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Nocturia</td>
<td>2.0 (1.0 to 3.0)</td>
<td>1.0 (0.0 to 3.0)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PIPIC score</td>
<td>3.0 (1.0 to 5.0)</td>
<td>2.0 (1.0 to 4.0)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>PPIUS score</td>
<td>3.0 (1.0 to 5.0)</td>
<td>2.0 (1.0 to 3.0)</td>
<td>&lt;0.005</td>
</tr>
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PPIC Patients' Perception of Bladder Condition; PPIUS Patients' Perception of Intensity of Urgency Scale

*Values are expressed as mean (standard deviation)
*Values are expressed as median (25th to 75th interquartile range)

Significant improvement in symptom scores but not placebo controlled

2. Antibiotic treatment of OAB

Patients in two groups:
1. n=147, antibiotics given (nitrofurantoin or Cephalexin)
2. n=212, no antibiotics

Significant improvement in symptoms in both groups
But the antibiotic treated group improved over a shorter time course

THE ANTIBIOTIC TREATMENT OF OAB COHORT

Significant improvement in symptom scores but not placebo controlled
RCT of antibiotics in refractory DO

Phase IIB RCT of antibiotic therapy vs placebo at St George Hospital + Wollongong
Women with urodynamically proven refractory DO
n = 120, 2:1 ratio of antibiotics versus placebo (with darifenacin in both groups)
6 weeks of rotating antibiotics (2 weeks each)
- Augmentin Duo (or trimethoprim)
- Norfloxacin
- Nitrofurantoin

All patients will be followed for 6 months

RCT protocol

Other treatments for UTI

There are other treatments being discussed for UTI
- Mannosides
- Vaccinations
- Anti-inflammatory agents
- These could also apply for OAB/DO

Questions & discussion
Evidence for intracellular bacteria in urge incontinence.

Kylie J Mansfield, Ying Cheng, Samantha Ognenovska, Zhouran Chen, Kate H Moore

E. Coli can colonise the urothelium

Justice et al., 2004 PNAS

Detection of Intracellular Bacterial Communities in Human Urinary Tract Infection

AIM 1

- UTI is more common in patients with DO

To test for the presence of intracellular bacteria in the urine of patients with detrusor overactivity or mixed incontinence +/- a history of UTI, and compare this to a control group of patients with stress incontinence and no history of infection.

Urothelial cells from women with acute cystitis were stained with antibodies against E. coli (green) and uroplakin III (red). Confocal microscopy revealed that these bacteria were intracellular.

Methods

- Urine sample were collected from women undergoing management for incontinence.
- Urine sample:
  1. Routine microbiology culture (10^6-10^8 PFU/L)
  2. Fixation (1% formalin) -> concentration -> cytopsin ->
     - Wright stain -> detect bacteria and filaments.
     - Immunocytochemistry (E. coli antibody)
     - Confocal Microscopy
- \( \chi^2 \) analysis: compare presence/absence of bacterial filaments in DO and controls.

Results – clinical history (UTI)

<table>
<thead>
<tr>
<th></th>
<th>Pure DO (n=47)</th>
<th>Mixed incontinence +/- UTI (n=21)</th>
<th>Control (n=20)</th>
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<tbody>
<tr>
<td>Recurrent UTI</td>
<td>10</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Previous proven UTI</td>
<td>7</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>No prior proven UTI</td>
<td>30</td>
<td>7</td>
<td>20</td>
</tr>
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</table>
Wright staining of urothelial cells

<table>
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<tr>
<th>Patient group</th>
<th>% patients with filament</th>
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<tr>
<td>Pure DO</td>
<td>82%</td>
</tr>
<tr>
<td>Mixed incontinence</td>
<td>23.5%</td>
</tr>
<tr>
<td>Control</td>
<td>27.3%</td>
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</table>

AIM 1: conclusions

Bacteria were commonly associated with urothelial cells
Intracellular bacteria were seen more commonly in patients with Detrusor Overactivity

AIM 2

To examine cellular invasion as a pathogenic factor for three uropathogenic bacterial strains.

Not all UTI is caused by E. coli

<table>
<thead>
<tr>
<th>Bacterial strain identified by Micro</th>
<th>E. Coli</th>
<th>Enterococcus faecalis</th>
<th>Streptococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>% specimens</td>
<td>38%</td>
<td>17%</td>
<td>26%</td>
</tr>
<tr>
<td></td>
<td>(13/34)</td>
<td>(6/34)</td>
<td>(9/34)</td>
</tr>
</tbody>
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We weren’t just interested in what bacterial species were causing the infection but in how the bacteria were associated with the urothelial cells
Methods

121 MSU specimens were collected from 94 women
- routine microbiology to identify uropathogens
- evaluating bacterial colonisation

Exfoliated urothelial cells were concentrated onto a microscope slide.

A. **Wright staining** and light microscopy to categorize according to the presence, location and density of bacteria.

B. **Confocal microscopy** was used to demonstrate intracellular localisation of bacteria.

Cells were stained 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.

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### CLASSIFICATION SYSTEM USED

Approximately 100 randomly selected urothelial cells were examined at 40x magnification and categorized according to the presence of bacteria, the location of the bacteria and the bacterial density

- Low Density
- High Density
- Appears Adjacent
- Appears Intracellular

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### RESULTS – Wright staining

<table>
<thead>
<tr>
<th>Bacterial strain identified by Micro</th>
<th>E. Coli</th>
<th>Enterococcus faecalis</th>
<th>Streptococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>% urothelial cells clear of bacteria</td>
<td>13%</td>
<td>52%</td>
<td>58%</td>
</tr>
<tr>
<td>% of urothelial cells classified as “Appears Intracellular – low density”</td>
<td>72%</td>
<td>36%</td>
<td>30%</td>
</tr>
</tbody>
</table>

---

### Confocal Results – *Escherichia coli*

Specific antibodies - *E. coli*
Urothelial cell membrane - Wheat-germ agglutinin (WGA)
Urothelial cell nucleus - DAPI

---

### Confocal Results – *Enterococcus faecalis*

Specific antibodies - *E. faecalis*
Urothelial cell membrane - Wheat-germ agglutinin (WGA)
Urothelial cell nucleus - DAPI

---

### AIM 2: Conclusions

The results of the current study demonstrate that all three uropathogens examined, *E. coli*, *E. Faecalis* and GBS are capable of intracellular growth

*E. Faecalis* and GBS demonstrated intracellular growth to a lesser extent that *E. coli*.

This suggests that intracellular growth might be a common characteristic of uropathogens

*Intracellular growth may increase the likelihood of UTI and lead to the development of bladder dysfunctions such as DO possibly through a change in afferent nerve activity as a result of the altered inflammatory response.*
Subversion of host defenses by invasive uropathogens: modeling intracellular infection with a novel 3D primary culture

Harry Horsley

Background

- *E. coli* invades mouse and human urothelial cells in acute UTI
- The innate immune response to infection is cell shedding, leaving a gap, allowing bacteria to form quiescent reservoirs responsible for recurrent infection
- *E. coli* is the foremost invasive pathogen in acute UTI
- However, *Enterococcus faecalis* is commonly isolated in chronic UTI
- We previously showed close association of *E. faecalis* with the urothelium of LUTS patients suggestive of intracellular colonisation
- The invasive properties of *E. faecalis* however has yet to be definitively proven

Cell shedding in 705 LUTS patients

- Log planktonic epithelial cell counts related to pyuria status
- Graphs show cell shedding in control and patient cells

Urine microscopy

- Images of urothelial cell shedding
- Control cells vs Patient cells

Affiliations to disclose:

Nothing to disclose

Funding for speaker to attend:

- Self-funded
- Institution (non-industry) funded
- Sponsored by:
**Patient urothelial cell – intracellular *E. faecalis***

- Infected T24 Cell Line

- **Results – Z-axis profile plot**

  E. Faecalis invaded the cell line but E. Coli produced extracellular biofilms

**Engineered human urothelium**

- Some LUTS may be generated by low-grade intracellular infection with *E. faecalis*

- All studies to date have relied on murine models and cancer cell lines to study the pathophysiology of UTI. Not physiologically relevant

- Produce a urine-tolerant organotypic culture using human bladder epithelial progenitor cells (HBEP) which mimics human urothelium.

- How do these cultures compare to native human bladder tissue?

- How does it respond to experimental infection?
Confocal Analysis – Animation

Cell layers
- Umbrella Cells
- Intermediate Cells
- Basal Cells

Reslice X/Y Maximum Projection. 3 Distinct layers

Characterisation

SEM / TEM

Urine dependence & GAG formation

infection?
Our recent work, along with other studies, shows differences between the largely *E. coli*-based acute UTI mouse model and the situation in human patients suffering from LUTS, suggesting the mouse model may not be physiologically relevant in all cases.

Given that the mouse urothelium is developmentally and functionally different to native human tissue, we have engineered and extensively characterised organotypic human urothelium *in vitro*.

We will use this model as a reproducible and standardised test bed for chronic infective disease formation and treatment using our novel drug delivery system.

Engineered human bladder models may prove to be an invaluable research tool in understanding the pathogenesis and resolution of infectious urinary tract disease.

Conclusion

Thank you for listening

Thank you to the MS Society for funding this work

Big thank you to Jennifer Rohn, Prof. James Malone-Lee and the chronic UTI group (CUTI) at UCL.

PNA-FISH: Culture-free visual identification

Under development (cell damage)
Workshop 15

Culture independent study of recurrent Bacteriuria in refractory DO

Z Chen1, L Bates1, M-D Phan2, M Schembri2, KH Moore1
1. Department of Urogynaecology, St George Hospital, Kogarah, Australia
2. School of Chemistry & Molecular Biosciences, University of Queensland

Background

Clinical observation in Refractory DO patients
• Up to 40% of patients have recurrent UTI
• Antibiotic resistance and difficulty in managing DO symptoms

Limitations of routine microbiology
• Reports limited to single organism

UTI Pattern

Flores-Mireles et al NRM 2015

Aim/ Methods

To investigate whether patients with Refractory DO have a persistent reservoir of bacteria in the bladder wall (via Culture independent methods)

Inclusion criteria
• Refractory DO
• History of recurrent UTI (≥ 2 infections/6months OR ≥ 3 infections/yr)

Methods
• MSU Routine Culture >10⁸ CFU/L (for comparison)
• Culture independent method

Results

9 rDO women with proven recurrent UTI (42 MSU) in 24months
Median age 75y (57-81)
Previous 6-24 months 3-20 confirmed UTI
Average 8 UTI/women

No significant voiding dysfunction
All had topical vaginal oestrogen
All had cystoscopy
• No urethral stenosis or mesh erosion
• Typically displayed cystitis cystica
Routine culture results

42 MSU (From 9 women)
5 samples “no growth”

20 samples had a reported single dominant organism
  13 E. Coli (65%)
  2 E. faecalis
  2 Klebsiella
  2 Streptococcus
  1 Citrobacter

17 reported as mixed growth

7/9 (78%) women had documented antibiotic resistance

Results of 16s rRNA Culture independent analysis

Diverse array of organism

25 median species per patient (IQR 21-36)

Changing proportion of organism in each urine sample

Results of 16s rRNA Culture independent analysis

Examples of mix growth on routine culture

Results of 16s rRNA Culture independent analysis

Patient 5 – E.coli

Patient 8 – E.coli

E.Coli FimH amplicon PCR sequencing

• Single persistent strain
• FimH43 predominant
• Clinically correlated because:
  (1) E.coli seen on culture
  (2) Antibiotic resistance to Amoxicillin + Trimethoprim
E. Coli FimH amplicon PCR sequencing

- E. Coli predominant, but constantly changing strain/resistance pattern
- FimH 43 same antibiotic resistance as Patient 5 (Amoxicillin + Trimethoprim)
- FimH 30 – ESBL

Conclusion

- First study of the microbiome of refractory DO patients with recurrent UTI
- Large number of bacteria detected in urine: median 25 species per patient (IQR 21-36)
- Organisms isolated on PCR change over time
- Further studies in this area may help link bacteria changes to exacerbation of DO symptoms
Girls Just Want to Have FUM
“Female Urinary Microbiome”

E.R. Mueller, MD MSME
Professor, Departments of Urology & Ob/Gynecology
FPMRS Division & Fellowship Director
Loyola University Chicago Stritch School of Medicine

GIRLS JUST WANT TO HAVE FUM
“Female Urinary Microbiome”

Elizabeth R Mueller, MD

Affiliations to disclose:
Astellas Pharma – Research Support

Funding for speaker to attend:

Dogma-based clinical care

Urgency Urinary Incontinence (UUI)
The bladder is sterile
based on negative standard urine culture

UUI does not have a bacterial contribution
caused by neuro-muscular imbalance

Perspectives
Recognizing the Problem
How we changed our paradigm

Is This Our Best?
Should we accept the dogma/assumptions that:
UUI is a chronic condition
Life-long treatment: pill, implant, etc.

UTI is caused by a single uropathogen
invading a “sterile” field

That the bladder (lower urinary tract)
is actually sterile?

Consider a team of basic and clinical investigators using the Urinary Microbiota project as a framework
Humans Are Superorganisms

2 integrated genomes
1. Genetically inherited human genome
   • (23,000 genes)
2. Environmentally acquired human microbiome
   • (over 1 million genes).

The two genomes must work harmoniously to maintain health

Historical Perspectives

• Urine deemed sterile in mid-1800’s
  – vial of urine in a sealed container did not turn cloudy, while a vial of urine exposed to air or tap water did
  “...fresh and healthy urine is perfectly free from bacteria or other minute organisms”

1. Duclaux E. (1920),
2. Bloom DA J of U 1994;151(2)

Historical Perspectives

• In the 1950’s, a colony count of $10^5$ was the dividing line between contamination and pyelonephritis
  – Since then, this standard culture (SC) techniques has been adopted to LUT infections, despite several studies that suggest otherwise

• Jack Lapides suggested intermittent catheterization did not have to be sterile

2. Lapides J. Journal of Urology 1972

Human Microbiome Project

• There are 10 bacteria for every single cell in the human body
• National Institutes Health Initiative to map the human microbiome of 5 body sites:
  – GI tract, mouth, vagina, skin, nasal cavity using culture-independent methods.
  – Bladder not included due to belief it was sterile and complexities of sample collection.
What if our understanding of the female lower urinary tract has rested on an invalid assumption?

Dogma – Null Hypothesis: ‘Culture-negative’ urine is sterile

Alternative hypothesis: ‘Culture-negative’ urine is NOT sterile

16S Ribosomal RNA (rRNA) Gene Sequencing

- The 16S gene is a molecular chronometer
  - Permits classification to the family or genus level

Quick Classification Update

<table>
<thead>
<tr>
<th>Domain</th>
<th>Kingdom</th>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td></td>
<td>Proteobacteria</td>
<td>Gammaproteobacteria</td>
<td>Enterobacteriales</td>
<td>Enterobacteriaceae</td>
<td>Escherichia</td>
<td>Escherichia coli</td>
</tr>
</tbody>
</table>

GETTING AN ACCURATE SPECIMEN

The story begins

DNA evidence of bacteria in the bladder

- TUC & SPA resemble each other
- TUC & SPA do not resemble controls (voided)
- SPA bypasses vulvo-vaginal contamination

Wolfe et al. 2012. JCM. PMID: 22278835
Samples are often dominated by one organism
Clustered according to dominant organism (urotype)

Summary

Bacteria are present in women with and without lower urinary tract symptoms

*Lactobacillus* and *Gardnerella* are common members of the FUM

---

**LESSON #2**

**ARE THE BACTERIA REALLY ALIVE?**

The DNA evidence could be dead bacteria which would not be symptomatic

---

**Are “culture negative” urines truly negative?**

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Volume</th>
<th>Media</th>
<th>Atmospheric Conditions</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Urine Culture (SUC)</td>
<td>1 ul. urine</td>
<td>Blood Agar, MacConkey Agar</td>
<td>Aerobic</td>
<td>24 hrs 35°C</td>
</tr>
<tr>
<td>Enhanced Quantitative Urine Culture (EQUC)</td>
<td>100 ul. urine</td>
<td>Blood Agar, Chocolate Agar, CNA Agar, Anaerobic Blood Agar</td>
<td>Aerobic, CO₂ Anaerobic</td>
<td>48 hrs 35°C</td>
</tr>
</tbody>
</table>

Hilt et al. 2014. JCM. PMID: 24371246

---

**This urine is not sterile**

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Media</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUC</td>
<td>Blood agar, 1 ul., 24 hours</td>
<td></td>
</tr>
<tr>
<td>EQUC</td>
<td>Blood agar, 100 ul., 48 hours, CO₂</td>
<td></td>
</tr>
</tbody>
</table>

Hilt et al. 2014. JCM. PMID: 24371246

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**Quick Classification Update**

- **Bacteria**
  - **Eubacteria**
  - **Proteobacteria**
  - **Gammaproteobacteria**
  - **Enterobacteriales**
  - **Enterobacteriaceae**
  - **Escherichia**
    - **Escherichia coli**

Hilt et al. 2014. JCM. PMID: 24371246
URGENCY INCONTINENCE

The symptoms of UUI and UTI have so much overlap.

Baseline FUMs of UUI and non-UUI cohorts differ
Some species are more common in UUI cohort
One is more common in non-UUI controls

Response to UUI Treatment Study Design

<table>
<thead>
<tr>
<th>Adult women with UUI (Questionnaire eligibility)</th>
<th>Controls without UUI symptoms (Questionnaire eligibility)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticholinergic drug (solifenacin) treatment</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
</tr>
<tr>
<td>4 weeks</td>
<td></td>
</tr>
<tr>
<td>12 weeks</td>
<td></td>
</tr>
<tr>
<td>5 mg</td>
<td></td>
</tr>
<tr>
<td>Patient Global Symptom Control (PGSC) score</td>
<td>PSSC score</td>
</tr>
<tr>
<td>1-3 non-responder – 10 mg</td>
<td>1-3 non-responder</td>
</tr>
<tr>
<td>4-5 responder – 5 mg</td>
<td>4-5 responder</td>
</tr>
</tbody>
</table>

Cohort Comparison

- By design, UUI symptoms were significantly worse in UUI cohort than in non-UUI controls
- Similar with respect to race/ethnicity, diabetes, smoking
- The UUI population was:
  - *post-menopausal and not on hormone replacement therapy

<table>
<thead>
<tr>
<th>Category</th>
<th>UUI</th>
<th>Non-UUI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Older</td>
<td>61.5 (SD: 11.5)</td>
<td>49 (SD:14.7)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Heavier</td>
<td>32.7 (SD:8.4)</td>
<td>28 (SD:5.5)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Estrogen Negative</td>
<td>88%</td>
<td>43%</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>35%</td>
<td>18%</td>
<td>p=0.02</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>12%</td>
<td>2%</td>
<td>p=0.02</td>
</tr>
</tbody>
</table>

Use EQUC to compare women with and without UUI

<table>
<thead>
<tr>
<th>UUI + non-UUI</th>
<th>EQUC positive</th>
<th>EQUC negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUC positive</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>SUC negative</td>
<td>64</td>
<td>19</td>
</tr>
</tbody>
</table>

Standard urine culture had false-negative rates of
- 90.1% for total
- 90.3% for UUI
- 90.0% for controls

Pearce et al. 2014
**Response to UUI Treatment Study Design**

<table>
<thead>
<tr>
<th>Adult women with UUI (Questionnaire eligibility)</th>
<th>Controls without UUI symptoms (Questionnaire eligibility)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 responder groups</td>
<td></td>
</tr>
<tr>
<td>5 mg responder</td>
<td></td>
</tr>
<tr>
<td>10 mg responder</td>
<td></td>
</tr>
<tr>
<td>Non-responder</td>
<td></td>
</tr>
<tr>
<td>4-5 responder = 5 mg</td>
<td></td>
</tr>
<tr>
<td>PGSC score</td>
<td></td>
</tr>
<tr>
<td>4-5 non-responder</td>
<td></td>
</tr>
<tr>
<td>4-5 responder</td>
<td></td>
</tr>
</tbody>
</table>

**Clinicaltrials.gov Registry**

NCT01642277

**Figure**: Baseline FUMs of responders and non-responders differ

**Summary**

The baseline FUM of women with and without UUI differ

Some bacteria are associated with UUI

*Lactobacillus crispatus* is associated with controls

The baseline FUM is associated with response to oral UUI treatment

**What We Learned Thus Far**

- The Female Urinary Microbiota (FUM) exist and they are alive
  - Wolfe et al., 2012
  - Wei et al., 2013
- Some FUM members associate with lower urinary tract symptoms (UUI)
  - Others associate with the lack of UUI symptoms
  - The FUM can be associated with response to medication
    - Pearce et al., 2014
    - Pearce et al., 2015
    - Thomas White et al., 2015
  - The FUM is associated with post-instrumentation UTI
    - Brubaker et al., 2015
  - The FUM is associated with post-surgery UTI
    - Fok et al., 2013
  - The FUM influences the innate immune system of the urothelium
    - Nienhouse et al., 2014
    - Le et al., 2014
  - The microbiota of calcium oxalate kidney stones
    - Barr et al., 2015

**We Also Learned That**

Standard Urine Culture protocol is limited even in the context of conventional UTI diagnosis

Hilt et al., 2014
PMID: 24371246

Pearce et al., 2014
PMID: 25006228

Price et al., 2015
PMID: 26962083

**PMID**

- 24371246
- 25006228
- 26962083
MORE QUESTIONS THAN ANSWERS

- In adult women, especially those with urinary symptoms, what is the gold standard for UTI?
- How should we detect/treat of FUM dysbiosis?
- What causes UTI symptoms in patients with no known uropathogen?
- We must change our assumptions/language
  - If ‘normal’ is asymptomatic bacteriuria, what does the term mean?

TAKE HOME MESSAGE:

THERE IS A URINARY MICROBIOTA IN WOMEN

TAKE HOME MESSAGES:

As awareness of the Female Urinary Microbiota grows, we must avoid antibiotic overuse.

Must change the paradigm from “kill everything” to “modulate to optimize health”

QUESTIONS?
Summary and Clinical significance
Prof Kate H Moore, Australia

So what is the clinical significance?
Could prolonged bladder-specific antibiotics correct the problem?

There have been two open studies conducted by colleagues in London
Vik Khullar (St Marys Hospital London)
James Malone-Lee (UCL)

Positive results, but no controls

1. Trial of antibiotics in OAB patients

| Table 5: Overactive Bladder (OAB) symptoms at baseline and after 6 weeks of antibiotic therapy |
|---------------------------------|-----------------|-----------------|
|                                | Pre-treatment   | After a 6-week course of antibiotics | \( \Delta p \) |
| Daytime frequency              | 12.8 (c.5.5)    | 8.7 (c.5.7)     | <0.005 |
| Nociceptor                    | 2.0 (1.0)       | 1.0 (0 to 3.0)  | <0.05  |
| PPIES scores                   | 5.0 (4.0 to 8.0) | 2.0 (0.0 to 4.0) | <0.005 |
| PPIES scores                   | 3.0 (1.0 to 5.0) | 2.0 (0.0 to 3.0) | <0.005 |

PPIES Patients’ Perception of Bladder Condition, PPIES Patients’ Perception of Intensity of Urgency Scale

Values are expressed as mean (standard deviation)

Significant improvement in symptom scores but not placebo controlled

2. Antibiotic treatment of OAB

Patients in two groups:
1. n= 147, antibiotics given (nitrofurantoin or Cepalexin)
2. n= 212, no antibiotics

Significant improvement in symptoms in both groups
But the antibiotic treated group improved over a shorter time course

RCT of antibiotics in refractory DO

Phase IIb RCT of antibiotic therapy vs placebo at St George Hospital + Wollongong

Women with urodynamically proven refractory DO
n = 120, 2:1 ratio of antibiotics versus placebo (with darifenacin in both groups)
6 weeks of rotating antibiotics (2 weeks each)
- Augmentin Duo (or trimethoprim)
- Norfloxacin
- Nitrofurantoin

All patients will be followed for 6 months
There are other treatments being discussed for UTI:
- Mannosides
- Vaccinations
- Anti-inflammatory agents

These could also apply for OAB/DO

Questions & discussion