Urinary Tract Infection

Antibiotic-sparing Therapeutic Revolution
CRE, a family of bacteria pictured here, is considered one of the deadliest superbugs because it causes infections that are often resistant to most antibiotics. (Centers for Disease Control and Prevention/Reuters)

For the first time, researchers have found a person in the United States carrying bacteria resistant to antibiotics of last resort, an alarming development that the top U.S. public health official says could mean “the end of the road” for antibiotics.

The antibiotic-resistant strain was found last month in the urine of a 49-year-old person.
Global epidemiology of fluoroquinolone resistance in UPEC
**Percentage of Antibiotics Prescribed Annually**
(United States 2007-9)

<table>
<thead>
<tr>
<th>Infection</th>
<th>% Antibiotic Prescribed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory</td>
<td>41%</td>
</tr>
<tr>
<td>Skin/Mucosal</td>
<td>18%</td>
</tr>
<tr>
<td>UTI</td>
<td>15%</td>
</tr>
</tbody>
</table>

Global epidemiology of fluoroquinolone resistance in UPEC

Women’s health is intricately intertwined with the spread of antibiotic resistance.
DRUG RESISTANCE INDEX

DRI provides an aggregate trend measure of the effectiveness of available drugs.

The index for UTIs shows the number of infections facing treatment difficulties has been increasing since the mid-2000s due to the rapid spread of resistance among Gram-negative organisms (such as E. coli) that are the primary cause of UTIs.
• Over 15M women suffer from UTIs per year with cost over $2.5 billion
• Chronic/Recurrent
• Multi-drug resistant bacteria
• Lead to inadequate treatment options
• CA-UTI adds $1 Billion to US healthcare costs
• Abx resistance is intricately intertwined with women’s health
UTI risk: matching urovirulence phenotypes with dynamic host susceptibility determinants

- UPEC occupies diverse habitats (gut, bladder, kidney, etc.) and each has unique sets of colonization requirements (“Locks”)

- UPEC strains contain variable sets of fitness factors (“Keys”) enabling colonization depending on the host

- Colonization and persistence occurs when a "Lock" is opened by the matching “Key.”

- The shape of Locks can change based on history, genetics, and behavior.

- UTI Complexity Results from Diversity at the Bacterial-Host Interface
Bacterial Attachment
Pili allow bacteria to stick around

- Plague
- Pneumonia
- Cystic Fibrosis
- Biofilms - Wound Infections
- Food-Borne Illness
- Ear Infections
- Whooping Cough
- Urinary Tract Infection
- Catheter Infections
- Heart Infections (Endocarditis)

Pili used by diverse human pathogens
FimH-Mediated binding of *E. coli* to bladder

Type 1 pili are tipped FimH

FimH-mannose
UPEC form Intracellular Bacterial Communities (IBC)
Bacterial Communities Escape Attack by Immune Cells
Uropathogenic *E. coli* (UPEC) infection of the urinary bladder has distinct acute and chronic phases.
History of UTI is among the most significant risk factors.

UTI pathogenesis has exclusively been studied in naive mice, but may not reflect rUTI pathogenesis.
Investigate how prior history of UTI impacts the pathogenesis of rUTI
Investigate how prior history of UTI impacts the pathogenesis of rUTI

Naive
Investigate how prior history of UTI impacts the pathogenesis of rUTI

Naive  Sensitized
Investigate how prior history of UTI impacts the pathogenesis of rUTI
History of infection sensitizes recurrent UTI

- Mock-Infected
- Chronic Cystitis
- Spontaneous Resolution

Naive | Sensitized | Resolved
History of infection sensitizes recurrent UTI

Bladders are remodeled through sensitization

Valerie O’Brien, Tom Hannan
Defect in Terminal Differentiation

Bladders are remodeled through sensitization.
Defect in Terminal Differentiation

Spenser Souza  
Cathy L. Mendelsohn
Defect in Terminal Differentiation
An infection can leave a molecular imprint on the bladder sensitizing it to

- Lipid metabolism
- Protease Inhibitors
- Cell-cell junctions/ECM
- Cytoskeleton
- Oxidative stress
- Tissue morphology
- Cellular development
- Cellular growth and proliferation

Suggests that the “sensitized” bladder epithelium is more sensitive to neutrophil damage as a consequence of inflammation.
Sensitization (caused by prior infection) leads to long-lasting remodeling that increases vulnerability to subsequent infections.
Altered host-pathogen interactions - Colonization resistance
Enhanced COX-2 Expression in Sensitized Mice

**COX-2 Expression, 24 hpi**

- COX-2 expression (fold change vs. mock) vs. fold change vs. mock-infected
- Urine bacterial titer (CFU/ml)
- PMN score

**Urine neutrophils**

- PMN score over hours post-challenge
- 
  - Adult Naive
  - Sensitized
  - Resolved
Cox1 - Constitutively Expressed by many Cells/Tissues
Cox2 - Typically Induced by Inflammation

Pharmacological Intervention: NSAIDs
Clinical Data and Biomarkers Suggest that an Over-exuberant Inflammatory Response Predisposes to rUTI.

Dexamethasome Protected Against Chronic Cystitis

Question: Could Immunomodulatory Therapy Alone Alter the Outcome of rUTI?
COX-2 Inhibitors Protect against rUTI

Pyuria, 24 hpi

Bladders, 24 hpi
2 Inhibition Suppresses Epithelial Transmigration by Neutrophils and Bladder

Indomethacin (Indo): inhibits both COX1 & 2
SC-236 selectively inhibits COX-2
SC-560 selectively inhibits COX-1
Symptomatic treatment (ibuprofen) or antibiotics (ciprofloxacin) for uncomplicated urinary tract infection? - Results of a randomized controlled pilot trial

Although patients given only a 3 day course of drug, a similar clinical outcome was realized at days 4 and 7.

This suggests that NSAIDs do not just mask symptoms, but also alter the course of infection!
An infection can leave a molecular imprint on the bladder sensitizing it to
NSAIDs can protect against recurrent infections

By understanding the mechanisms underlying host susceptibility to recurrent infection we have discovered potential avenues for improved therapeutic approaches to prevent recurrence.
1. Uropathogens are shed from the gut...

2. ...colonize the periurethral area...

3. ...and then ascend the urethra to invade the bladder.
What are the population dynamics of UPEC in the gut before, during, and after UTI?

How does the gut microbiota influence UTI susceptibility?

2. ...colonize the periurethral area...

3. ...and then ascend the urethra to invade the bladder.
Role of the Human Microbiome

The makeup of the microbiota interacts with the host in such a way that it determines normal and/or abnormal nutrition abnormalities (disease, obesity, malnutrition, etc).

Our indigenous gut microbial communities endow us with physiological and metabolic attributes we have not had to evolve on our own.

A healthy microbiota in the gastrointestinal tract (GIT) serves an important function in the breakdown and absorption of essential dietary vitamins and nutrients. Additionally, it serves a role in the generation and maintenance of an immune balance that limits inflammation while combating colonization from unwanted pathogens. Antibiotic treatments are thought to expose individuals to an increased risk of opening up niches in the GIT which allows pathogens to expand.
UMB Cohort, Study Design, and Collections

Cohort Recruitment

14 x Women with frequent recurrent UTI (rUTI)
- >3 UTI in past year
- No recent Abx
- No chronic illnesses
- No urological abnormalities

14 x Demographically-matched Healthy Controls
- 0-1 UTI in lifetime
- No recent Abx
- No chronic illnesses
- No urological abnormalities

12 Month Longitudinal Study Design Collections at:

1. Enrollment

2. Monthly Time Points

3. UTI Episodes

4. Post Antibiotic Treatment

Total Collection:
- 387 fecal samples
- 47 urine samples
- 47 blood samples

19x UTI events
Thus, rUTI appears to be one of the growing number of human diseases associated with imbalance of complex microbial GIT communities.
E. coli blooms in the gut coincide with UTI
- The gut microbiotas of women with rUTI were significantly less rich (contain fewer species) than community- and age-matched healthy controls.
- Several bacterial species associated with “healthy guts” were depleted in rUTI, including:
  - *Faecalibacterium prausnitzii*
  - *Akkermansia municiphila*

**Allows UPEC Expansion: Seeds rUTI**
Gut Reservoir
Chaperone-usher pathway pili (CUPs)

Gut colonization
STM Model of UPEC GIT Colonization

A. STM (PO) UT189 (PO)

Days Post UT189 Inoculation

B. CFU/g feces

C. CFU/g organ

Untreated

Streptomycin treated

Tissue type

_days Post Inoculation

Ileum

Cecum

Colon

Caitlin Spaulding
UPEC Fitness Factors in GIT Colonization

Yfc  Yeh  Yad
P  Yqi  S
Mat  Type 1  F17-like
FimH binds N-linked Oligosaccharides of the Upper Crypts

Segolene Ruer
UclD binds O-linked Oligosaccharides of the Lower Crypts

Crypt colonization provides a less competitive environment with regards nutrient competition with the microbiota in the lumen.
F17-like Pili Restricted to Extra-intestinal E. coli

- 11% of all E. coli but 50% of B2 strains
B2 UPEC acquired F17-like pili from intestinal pathogens
UclD has same structure as F17G

Spaulding et al. (2017) Nature
Carriage of F17-like pili in *E. coli*
11% of *E. coli* strains encode F17-like pili
50% of B2 strains encode F17-like pili

Carriage of F17-like pili in UPEC
13/14 women with rUTI caused by a B2 *E. coli* strain encode F17-like

F17-like pili might be associated with UPEC persistence in women with rUTI by promoting the maintenance of the UPEC intestinal reservoir.
• Translate basic science advances into new and better antibiotic-sparing therapeutics

• Antibiotic resistance rising at an alarming rate

• Reaching a tipping point
Development of Anti-Virulence Therapeutics

- Mannosides
- UTI vaccine
- FmlD inhibitors
- PapG inhibitors
- Pilicides (Assembly)
- CAUTI vaccine

Need Antibiotic-Sparing Agents
**Molecular Basis of FimH Vaccine**

**FimH Lectin**
- Is a known UPEC virulence factor
- Plays an important role in all aspects of the infection cycle
- Mannose-specific

![Diagram of the infection cycle involving FimH Lectin and Type 1 pili](image)
Molecular Basis of FimH Vaccine

Anti-FimH Antibodies Inhibit Function of FimH

- prevents UPEC from causing further infection or from propagating an existing infection
Anti-FimH Antibodies Inhibit Function of FimH

- prevents UPEC from causing further infection or from propagating an existing infection
Phase 1A/1B FimH Vaccine Study

Objectives of this study were to:
- Assess safety and tolerability
- Measure the serum IgG response to the FimH lectin domain
- Measure the duration and sustainability of the IgG response

Study design:
- Included 67 women, ages 21-64, in 6 cohorts; dose escalation design
- Conducted at 5 clinical sites with monitoring by a Safety Review Committee
- Subjects in cohorts 1 to 4 (Phase 1A) did not have a history of UTI in the previous 24 months prior to enrollment into the study. Subjects in cohorts 5 and 6 (Phase 1B) had ≥ 5 documented UTI in the last 24 months, including at least 1 with *E. coli*. Doses for cohorts 5 and 6 were based on safety data and antibody responses from cohorts 1 to 4.
- Intramuscular (IM) dosing on days 0, 30, 90 and 180; end of study was 12 months after last vaccination

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Subjects</th>
<th>FimCH (µg)</th>
<th>Adjuvant (µg)</th>
<th>UTI Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>107</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
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<td>50</td>
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<td>4</td>
<td>8</td>
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<td>5</td>
<td>16</td>
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<td>40</td>
<td>Recurrent</td>
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<td>6</td>
<td>14</td>
<td>107</td>
<td>43</td>
<td>Recurrent</td>
</tr>
</tbody>
</table>
Incidence of Recurrent UTI among the 13 Subjects of Cohort 5
First 8 study months compared to the last 8 study months

The Phase 1B results show trends of a 74% reduction in total UTI and 70% reduction in E. coli and Klebsiella spp UTI. 6 of these subjects did not have any UTI during the last 8 months of the study. These preliminary data support conducting a randomized, placebo-controlled Phase 2 study.
The Phase 1B results show trends of a 70% reduction in total UTI and 87% reduction in *E. coli* and *Klebsiella* spp. UTI. 8 of these subjects did not have any UTI during the last 8 months of this study. These preliminary data support conducting a randomized, placebo-controlled Phase 2 study.
UTI History of a 73-year old woman

- Recurrent UTI caused by *E. coli* resistant to the standard of care
- Exhausted all therapeutic options requiring the last-line of defense carbapenem antibiotics
  - *E. coli* identified in her urine during UTI symptoms
    - February, 2016 – failed prophylaxis with oral ampicillin
    - March, 2016 - resistant to fluoroquinolones and trimethoprim-sulfamethoxazole
    - March, 2016 - failed prophylaxis with amoxicillin / clavulanate
    - April, 2016 - failed prophylaxis with nitrofurantoin
    - May, 2016 - resistant to nitrofurantoin
    - May, June, and August, 2016 – identified extended-spectrum β-lactamase (ESBL)
    - Final option used throughout failures in 2015 to 2016 has been intravenous ertapenem for seven to twelve days to achieve clinical response

Based on the first compassionate use experience above, Thomas Hooton, MD received approval to expand the compassionate use program in collaboration with Sequoia.

“First compassionate use patient: Prior to achieving FimH-Immunity, she had >20 recurrent UTI in about
Mannosides Target Attachment, the First Step in the Pathogenic Cycle

Rationally Designed Mannosides to Make High Affinity Interactions with FimH

Lead Mannosides have >1,000,000 Times Increased Affinity for FimH over Mannose

Mannosides Effectively Block Bacterial Binding to the Bladder
The Opportunity: Mannosides as Therapeutics

Bacteria Bind to Cells, Causing Infection

Mannosides Block FimH Mediated Binding, Preventing Adherence and Invasion into Bladder Epithelium

Moving our lead compounds into clinical trials
Mannosides bind with high affinity to FimH to block colonization and invasion.

**Treating Bladder Infections with FIM-4269**

**Prophylactic Treatment**
3 Hours Prior to Infection

FIM-4269 Shows Protection 3 hours Prior to Infection, Reducing CFUs in the Bladder ~100 fold.

**Treatment of UTI Infection**

- **Single Dose**
  - Bladder CFUs 6 hrs After Treatment

- **“Multiple” Doses**
  - Bladder CFUs 24 hrs After Treatment

Treatment of *E. coli* Infected Mice with Mannosides Clears Bacteria from Bladder More Quickly Than TMP-SMZ.

Treatment of an Infection Every 8 hrs Eliminates Bacteria from the Bladder and is Equivalent to TMP-SMZ.
Prevention of Multi-Drug Resistant ST131 Strain

BLADDER CFUs 6 HRS AFTER TREATMENT

Mannoside Treatment of ST131 Strain

Antibiotic Treatment of ST131 Strain

Treatment of Mice Infected with Multi-Drug Resistant E. coli Strain

Much More Effective with Mannosides

Mice treated with TMP/SMX (54/270 ug/mL) for 3 days prior infection or with 50 mg/kg of 4269 30 min. prior infection.
The Opportunity: Mannosides as Therapeutics

Bacteria Bind to Cells, Causing Infection

Mannosides Block FimH Mediated Binding, Preventing Adherence and Invasion into Bladder Epithelium

Moving our lead compounds into clinical trials

Caitlin Spaulding

Mannoside treatment reduces UTI89 intestinal colonization

Graphs illustrating the reduction of UTI89 colonization in different tissues (feces, cecum, colon) with and without M4284 treatment.

Spaulding et al. (2017) Nature
M4284 treatment minimally alters the microbiota community structure of naïve C3H/HeN mice
Mannosides Selectively Deplete Reservoir while Simultaneously Treating UTI
We are hoping to revolutionize the way bacterial infections are treated through dissection of the host-pathogen interface to produce antibiotic-sparing therapeutics.
Studying sex differences in UTI susceptibility and outcomes

Disclosure

I am a part owner of Fimbrion and may financially benefit if the company is successful in marketing the mannosides.
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**NIDDK**

**MIST**

Mucosal Immunology Studies Team
The type 1 pilus has been “fine-tuned” through evolution to balance conservation of its “spring-like” function with diversification of its exterior surface.