The Development of an Integrated Molecular and Phenotypic Method for the Management of Urinary Tract Infections

Dave Baunoch, PHD
Urinary Tract Infections
Cost of Urinary Tract Infections (UTI)

Cost to the Healthcare System

- Responsible for \(\approx 10.5\) MILLION office visits/year\(^1\)
- UTI complications result in 9-11 DAYS longer for each hospital stay\(^2\)
- Urinary Tract Infections account for 50% of all Medicare admissions\(^5\)

Cost to Humanity

- Up to 1/3 of infections illustrate resistance to an antibiotic\(^3\)
- According to the CDC, antibiotic resistance gives rise to at least 2 MILLION INFECTIONS and 23,000 DEATHS/YEAR\(^4\)

Problem: Urinary Tract Infections cost the US healthcare system \$13B a year. Without efficacious diagnostic tests, ineffective antibiotics will continue to lead to more resistant microorganisms.

UroSepsis

Impact of Urosepsis

- **50%**
  - Of the sepsis cases in Nursing Homes originate from urinary tract infections

- **60%**
  - Of patients with sepsis caused by urinary tract infections die

- **$2.8 B**
  - Total cost of sepsis due to urinary tract infections in the US annually

Impact of an incorrect Diagnosis and Treatment

- **67.8%**
  - Of patients prescribed the wrong antibiotic on initial treatment died (as compared to 28.7% of patients receiving the right antibiotic)

- **1.98**
  - Initial treatment delay increases the odds of admission to ICU up to

Problem: Culture has significant limitations including the inability to detect gram-positive organisms.

- **57.1%**
  - of patients with gram-positive urosepsis die.

References:

4. J. Antimicrobial chemotherapy. 2008 61, 436-441
5. Supportive Care in Cancer. 2019. 27:4171-4178
The Problem with Routine Culture and Sensitivity
URINE IS NOT STERILE AND CONTAINS A MICROBIOME

- Urine has historically been viewed to be sterile with the presence of bacteria in the urine seen as an indicator of infection.
- Recent work has clearly demonstrated that the urinary tract contains a microbiome that is characterized by a preponderance of *Lactobacillales* in women and *Corynebacterium* in men.\(^1\)

  Often times, the bladder contains additional uropathogenic organisms (including E.coli) that can coexist in a persistent state of asymptomatic bacteriuria.\(^2\)

---

Traditional Method

Step 1: Identify Organism: 24-48 hours
Step 2: Susceptibility: 24-48 hours
Current Methodology - Developed in the 1950’s, the standard method involves applying 1ul of urine onto Blood and MacConkey Agar plates and incubating them at 35 degrees centigrade for 24 hours in the presence of oxygen.¹

What is the Fundamental Problem with culture as a detection method? From the composition of the agar, to the pH, gas ratio’s, and time of incubation culture is a methodology that has been biased for the detection of a subset of pathogens – primarily E. Coli.²

- The biased results developed using this methodology often creates findings that are not consistent with the clinical symptoms
- It is unable to detect slow growing organisms including fastidious and anaerobic organisms as well as most gram-positive organisms
- Time consuming process that can take up to 72 hours to complete
- Inherent methodology issues limit the number of organisms reported to no more then 2 with 3 or more considered indications of contamination

URINE CULTURE MISSES THE VAST MAJORIT OF POTENTIAL UROPATHOGENS
Problem Statement #3

THE ORGANISMS MISSED BY URINE CULTURE ARE CLINICALLY SIGNIFICANT

The Loyola Study- Pivotal study demonstrating limitations of standard culture in identifying the pathogens associated with UTI's.

- Followed 150 patients who were split into two groups based on whether they believed they were symptomatic for UTI.¹

- Compared the results obtained when they used standard culture to an enhanced version which had modified growth conditions including an increased incubation time.

- In the group who believed they were symptomatic standard culture detected only 57% of the uropathogens where the enhanced methodology detected 91%.

Why is this important?

IN SYMPTOMATIC PATIENTS, STANDARD CULTURE MISSED 2/3 OF ALL POSITIVE PATIENTS

• Outcome

  • Culture positive: 38.5%
    • Failed to see symptoms improve – with standard of care based upon culture results
    • Symptoms worsen

  • Culture negative 67%
    • Failed to see symptoms improve
    • Symptoms worsen
Problem Statement #4

URINE CULTURE MISSES POLYMICROBIAL INFECTIONS

• Wolfe and Brubaker have proposed moving from an E.coli-centric view of urinary tract infections.\(^1\)
  - With an increasing number of studies demonstrating that most urinary tract infections have multiple urinary pathogens present in the same sample, we should begin to shift our thinking away from a monocentric view of urinary tract infections.

• Studies by Price et al, 2016, and Wolfe et al, 2012 have demonstrated that a significant number of cases identified as having only E.coli present actually have multiple species present.\(^2,3\)
  - In the Price et al, 2016 paper 35 of 43 patients had additional species
  - In 25 of 35 cases the additional species were uropathogens

• A simple truth: It is becoming increasingly clear that a significant number of urinary tract infections are polymicrobial in nature. Because of the polymicrobial nature of infections, efficacy of treatment is dropping significantly.

INTERACTION OF BACTERIA IN POLYMICROBIAL INFECTIONS RESULTS IN CHANGES TO ANTIBIOTIC SENSITIVITY

The sharing of metabolic products provides polymicrobial infections an advantage\(^1\)

- In the presence of antibiotics the sharing of metabolic products plays a protective role increasing resistance and virulence
- Testing isolated bacteria may over or under estimate the degree of antibiotic resistance

Problem Statement #5
Problem Statement #6

THE LENGTHY TURNAROUND TIME FOR STANDARD CULTURE AND SENSITIVITY SUPPORTS EMPIRIC TREATMENT AND ANTIBIOTIC RESISTANCE
Summary of the Problem

ROUTINE CULTURE AND SENSITIVITY

1. Urine is not sterile and contains a Microbiome
2. Urine culture misses that vast majority of potential uropathogens
3. The organisms missed by urine culture are clinically significant
4. Urine culture misses polymicrobial infections
5. Interaction of bacteria in polymicrobial infections results in changes to antibiotic sensitivity
6. The lengthy turnaround time for standard culture and sensitivity supports empiric treatment and antibiotic resistance
The Development of an Integrated Molecular and Phenotypic Method for the Management of Urinary Tract Infections
A unique rapid molecular test for both pathogen identification and antibiotic sensitivity providing personalized therapy options that work the first time.

- Detects 42 Specific Organisms
- Pooled Sensitivity for 19 Antibiotics
- Higher Sensitivity and Accuracy
- Turn Around Time of Less Than 24 Hours
- 38 Resistance Genes Tested
- Detects Polymicrobial Infections

Genotyping (Antibiotic Sensitivity Testing)

Phenotyping (Antibiotic Resistance)

Organism Identification (Bacterial, Viral, Fungal)

Personalized Treatment for the Patient
ORGANISMS DETECTED:

BACTERIAL/YEAST ORGANISMS

- Acinetobacter baumannii
- Actinobaculum schaalii
- Aerococcus urinae
- Alloscardovia omnicolens
- Candida albicans
- Candida glabrata
- Candida parapsilosis
- Citrobacter freundii
- Citrobacter koseri
- Corynebacterium riegelii
- Enterobacter aerogenes
- Enterococcus faecalis
- Escherichia coli
- Klebsiella oxytoca
- Klebsiella pneumoniae
- Morganella morganii
- Mycoplasma hominis
- Mycoplasma genitalium
- Mycobacterium tuberculosis
- Pantoea agglomerans
- Proteus mirabilis
- Providencia stuartii
- Pseudomonas aeruginosa
- Serratia marcescens
- Staphylococcus aureus
- Streptococcus agalactiae
- Ureaplasma urealyticum

BACTERIAL GROUPS

- Coagulase neg. staphylococci*
- Viridans group streptococci**

SEXUALLY TRANSMITTED ORGANISMS

- Chlamydia trachomatis
- Neisseria gonorrhoeae
- Trichomonas vaginalis
# Emerging Organisms

## Gram Negative

<table>
<thead>
<tr>
<th>Organism</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>Known to cause several nosocomial infections of the urinary tract ($\dagger$). Wiping posterior to anterior following a bowel movement and normal intercourse can both bring about C. freundii UTIs.</td>
</tr>
<tr>
<td><em>Citrobacter koseri</em></td>
<td>Can cause UTI's more frequently in individuals with urinary diversions.</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>E. aerogenes is acquired by unintentional bacteria transmission in hospital settings, and E. aerogenes infections mainly occur when host immunity defenses are already suppressed.</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Uropathogenic E. coli (UPEC) is one of the leading root causes of UTIs.</td>
</tr>
<tr>
<td><em>Klebsiella oxytoca</em></td>
<td>Cause UTI's in individuals with catheters.</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>UTI's caused by multidrug-resistant K. pneumoniae isolates are a significant public health issue.</td>
</tr>
<tr>
<td><em>Morganella morganii</em></td>
<td>UTI's are typically connected with complicated features like indwelling catheters.</td>
</tr>
<tr>
<td><em>Pantoea agglomerans</em></td>
<td>Is associated with catheter-related bacteremia.</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>Can cause symptomatic infections of the urinary tract, including cystitis and pyelonephritis, and can appear in cases of asymptomatic bacteriuria.</td>
</tr>
<tr>
<td><em>Providence stuartii</em></td>
<td>Are among the most common cause of catheter-associated UTIs, particularly in the elderly with long-term indwelling urinary catheters.</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Is a common cause of UTIs, and patients with impaired immunity possess a greater risk for colonization by this organism.</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>Has now been implicated as an etiological agent in a wide range of infections, including UTIs.</td>
</tr>
</tbody>
</table>

## Gram Positive

<table>
<thead>
<tr>
<th>Organism</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Actinobaculum schaedi</em></td>
<td>Is a frequent cause for urinary tract infections amongst the elderly, in children, and in those with underlying urological conditions.</td>
</tr>
<tr>
<td><em>Aerococcus urinae</em></td>
<td>Can cause simple and complicated UTIs, bacteremia, and endocarditis in elderly adults who have multimorbidity, chronic urinary retention, or indwelling catheters.</td>
</tr>
<tr>
<td><em>Alloascardovia omniolens</em></td>
<td>Is hard to identify and one of the organisms identified by Enhanced Quantitative Urine Culture in women complaining of UTI symptoms.</td>
</tr>
<tr>
<td><em>Corynebacterium rigelesi</em></td>
<td>Have been reported to cause opportunistic infections in both immune compromised as well as immune competent patients.</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>Can trigger endocarditis and septicemia, UTI, meningitis, and other infections in people.</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Has been identified in children with urinary tract abnormalities and vesico-ureteral reflux.</td>
</tr>
<tr>
<td><em>Streptococcus agalactiae</em></td>
<td>Has been isolated in patients with UTI symptoms.</td>
</tr>
</tbody>
</table>
Real-time PCR workflow

DNA Extraction

- 2-10mL urine sample
- Samples undergo extraction
- DNA isolated and loaded

Real-time PCR

- Samples undergo Real-Time PCR
- Results are analyzed by CLS for detection of microbial DNA

ABR

- Based on results, samples are set-up for ABR testing.
- Antibiotic sensitivity results are obtained through a spectrophotometric assay.
- Finalized report is released to client.
Real-time PCR “CHIP”

• Ability to run high numbers of samples simultaneously, with large numbers of targets.

• Isolated DNA and Master Mix is plated onto a nanofluidic plate with 3,072 through-holes.

• Each chip can hold 21-24 samples (18 patient samples and 3 controls) 56 Targets in duplicate

• Estimated Assay Run Time: 2 hours
Software analysis

A. Raw data is gathered through the software for analysis.

- Amplification plots are displayed for individual targets, samples, and subarrays.

- CT's, Amplification scores, and Cq Confidence values are given for each subarray and its 64 wells.
B. Raw data is exported from the software as a (*.txt) file.

C. These files will then be transferred into the Excel Analysis Tool where the data will be interpreted.

D. The completed analysis is used to generate reports through the Excel Integra Report Tool.
GENOTYPE ANSWERS ONLY PART OF THE COMPLEX PROBLEM OF ANTIBIOTIC RESISTENCE

• Guidance tests for the presence of 38 genes known to be associated with resistance to certain antibiotics
• Does Not Provide the Complete Answer – Why?
  – Limited number of resistance genes that can be identified via molecular assay
  – Gene resistance continuously change
  – Resistance gene may not be active.

<table>
<thead>
<tr>
<th>Antibiotic Class</th>
<th>Gene</th>
<th>Antibiotic Class</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrolide resistance</td>
<td>ErmA + ErmB</td>
<td>Carbenem resistance</td>
<td>VIM</td>
</tr>
<tr>
<td></td>
<td>ermC</td>
<td></td>
<td>KPC</td>
</tr>
<tr>
<td></td>
<td>mefa</td>
<td></td>
<td>IMP-2 group</td>
</tr>
<tr>
<td>Extended-Spectrum-Betalactamase</td>
<td>TEM</td>
<td></td>
<td>IMP-1 group</td>
</tr>
<tr>
<td></td>
<td>CTX-M group 1</td>
<td></td>
<td>OXA-23</td>
</tr>
<tr>
<td></td>
<td>SHV</td>
<td></td>
<td>IMP-16</td>
</tr>
<tr>
<td></td>
<td>VEB</td>
<td></td>
<td>IMP-7</td>
</tr>
<tr>
<td></td>
<td>OXA-1</td>
<td></td>
<td>OXA-72</td>
</tr>
<tr>
<td></td>
<td>CTX-M group 2</td>
<td></td>
<td>OXA-40</td>
</tr>
<tr>
<td></td>
<td>CTX-M group 9</td>
<td></td>
<td>OXA-58</td>
</tr>
<tr>
<td></td>
<td>CTX-M group 8/25</td>
<td></td>
<td>OXA-48</td>
</tr>
<tr>
<td></td>
<td>PER-1</td>
<td></td>
<td>NDM</td>
</tr>
<tr>
<td></td>
<td>PER-2</td>
<td></td>
<td>biaOXA-48</td>
</tr>
<tr>
<td></td>
<td>GES</td>
<td></td>
<td>Tetracycline</td>
</tr>
<tr>
<td></td>
<td>biaNDM-1</td>
<td></td>
<td>TetM</td>
</tr>
<tr>
<td>Quinolone and fluoroquinolone resistance</td>
<td>QnrA</td>
<td>Aminoglycoside</td>
<td>aaC6-aph3</td>
</tr>
<tr>
<td></td>
<td>QnrB</td>
<td></td>
<td>anti-a-aph2</td>
</tr>
<tr>
<td>Meticillin resistance</td>
<td>mecA</td>
<td>Trimethoprim/Sulfamethoxazole</td>
<td>drf(A1, A5), sul (1,2)</td>
</tr>
<tr>
<td>Vancomycin resistance</td>
<td>vanA1</td>
<td>AmpC resistance</td>
<td>ampC, FOX, ACC</td>
</tr>
<tr>
<td></td>
<td>vanA2</td>
<td></td>
<td>DHA, MOX/CYM, BIL/LAT/CMY</td>
</tr>
<tr>
<td></td>
<td>vanB</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Developing a Methodology for Measuring Pooled Sensitivity
It is becoming increasingly clear that a significant number of urinary tract infections are polymicrobial in nature. Because of the polymicrobial nature of infections, efficacy of treatment is dropping significantly.

Increased human pathogenic potential of Escherichia coli from polymicrobial urinary tract infections in comparison to isolates from monomicrobial culture samples

Gamma Croswell, Vivienne Weston, Susan Joseph, Georgina Manning, Phil Cheetham and Alan McNally

1Pathogen Research Group, Nottingham Trent University, Clifton Lane, Nottingham NG11 8NS, UK
2Nottingham University Hospitals, Nottingham, UK

The current diagnostic standard procedure outlined by the Health Protection Agency for urinary tract infections (UTIs) in clinical laboratories does not report bacteria isolated from samples containing two or more different bacterial species. As a result many UTIs go untreated, particularly in elderly patients, who polymicrobial UTI samples are especially prevalent. This study reports the presence of the major uropathogenic species in mixed culture urine samples from elderly patients, and of resistance to frontline antibiotics, with potentially increased levels of resistance to ciprofloxacin and trimethoprim. Most importantly, the study highlights that Escherichia coli present in polymicrobial UTI samples are statistically more invasive (P<0.001) in vitro epithelial cell infection assays than those isolated from monomicrobial culture samples. In summary, the results of the study suggest that the current diagnostic standard procedure for polymicrobial UTI samples needs to be reassessed, and that E. coli present in polymicrobial UTI samples may pose an increased risk to human health.
Polymicrobial Infections are in the Community

Outpatient Polymicrobial Infection Rates

- Pain Clinic: 17%
- Primary Care Office: 36%
- Nursing Home: 39%
Resistance Higher For Polymicrobial Infections

Prevalence of E. coli antibiotic resistance in UTI isolates

- Piperacillin/Tazobactam: 6.2% Polymicrobial, 4.8% Monomicrobial
- Nitrofurantoin: 9.5% Polymicrobial, 17.1% Monomicrobial
- Trimethoprim/Sulfamethoxazole: 28.6% Polymicrobial, 44.2% Monomicrobial
- Ciprofloxacin: 9.5% Polymicrobial, 23.3% Monomicrobial
Increased human pathogenic potential of *Escherichia coli* from polymicrobial urinary tract infections in comparison to isolates from monomicrobial culture samples

Gemma Croxall, Vivienne Weston, Susan Joseph, Georgina Manning, Phil Cheetham, and Alan McNally

1. Pathogen Research Group, Nottingham Trent University, Clifton Lane, Nottingham NG11 8NS, UK
2. Nottingham University Hospitals, Nottingham, UK

The current diagnostic standard procedure outlined by the Health Protection Agency for urinary tract infections (UTIs) in clinical laboratories does not report bacteria isolated from samples containing two or more different bacterial species. As a result many UTIs go unreported and untreated, particularly in elderly patients, where polymicrobial UTI samples are especially prevalent. This study reports the presence of the major uropathogenic species in mixed culture urine samples from elderly patients, and of resistance to front-line antibiotics, with potentially increased levels of resistance to ciprofloxacin and trimethoprim. Most importantly, the study highlights that *Escherichia coli* present in polymicrobial UTI samples are statistically more invasive (*P*<0.001) in in vitro epithelial cell infection assays than those isolated from monomicrobial culture samples. In summary, the results of this study suggest that the current diagnostic standard procedure for polymicrobial UTI samples needs to be reassessed, and that *E. coli* present in polymicrobial UTI samples may pose an increased risk to human health.

Table 1. Prevalence of antibiotic resistance in UTI isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th><em>Escherichia coli</em> (%)</th>
<th><em>Enterococcus faecalis</em> (%)</th>
<th><em>Proteus mirabilis</em> (%)</th>
<th><em>Staphylococcus aureus</em> (%)</th>
<th><em>Pseudomonas aeruginosa</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Poly (n=129)</td>
<td>Mono (n=21)</td>
<td>Poly (n=110)</td>
<td>Mono (n=4)</td>
<td>Poly (n=56)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>12.4</td>
<td>4.76</td>
<td>5.3</td>
<td>4.5</td>
<td>-</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>17.8</td>
<td>14.29</td>
<td>33.9</td>
<td>100.0</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloxacin (4)</td>
<td>18.6</td>
<td>9.52</td>
<td>35.7</td>
<td>100.0</td>
<td>-</td>
</tr>
<tr>
<td>Moxifloxacin (2)</td>
<td>0</td>
<td>0.35</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Piperacillin–</td>
<td>6.2</td>
<td>4.76</td>
<td>23.2</td>
<td>100.0</td>
<td>-</td>
</tr>
<tr>
<td>Oxacillin (16)</td>
<td>5.4</td>
<td>0</td>
<td>16.1</td>
<td>100.0</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloxacin (8)</td>
<td>44.2</td>
<td>20.87</td>
<td>89.0</td>
<td>22.2</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloxacin (4)</td>
<td>23.3</td>
<td>9.52</td>
<td>0</td>
<td>55.5</td>
<td>-</td>
</tr>
<tr>
<td>Co-amoxiclav (32)</td>
<td>28.7</td>
<td>19.05</td>
<td>55.3</td>
<td>50.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Nitrofurantoin (32)</td>
<td>17.3</td>
<td>9.52</td>
<td>11.0</td>
<td>27.5</td>
<td>50.0</td>
</tr>
<tr>
<td>Amoxicillin (32)</td>
<td>45.0</td>
<td>42.86</td>
<td>37.5</td>
<td>75.0</td>
<td>2.17</td>
</tr>
<tr>
<td>Gentamicin (10)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Piperacillin–</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oxacillin (85)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloxacin (1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Mutualism: Cross Feeding

**4 Examples of Stable Communities**

- **Positive Interactions (cross feeding)**
  - **Eco**: E. coli
  - **Ent**: Enterococcus spp.
  - **Ps**: Pseudomonas spp. (P. aeruginosa, P. fluorescens)
  - **Kl**: Klebsiella spp (K. pneumoniae, K. oxytoca)
  - **Pm**: P. mirabilis
  - **St**: Staphylococcus spp (S. aureus, S. haemolyticus, S. capitis)
Antibiotic Sensitivity Testing

Upon completion of UTI Molecular Testing, results are used to set up Antibiotic Sensitivity Testing for each sample.

Overview:

1mL of urine is processed by Micro Dept. Sample is incubated 6-16 hours.

Spec plate containing antibiotics is inoculated with sample/growth media mixture. If gram-negative organism is detected, sample is also plated on CHROMagar for ESBL testing.

Spec plate is incubated for 12-16 hours. CHROMagar is incubated for 12-18 hours.

Spec plate is loaded into the plate reader and the OD measurements for each well are taken.

Results are analyzed by CLS to determine sensitivity or resistance to antibiotics present in designated wells.
**GUIDANCE UTI**

**Patient:** First Last  
**DOB:** 10-10-1989  
**Gender:** F  
**Phone:** (123) 456-0123  
**Medicare:** 123-456-7890  
**Caregiver:** John Doe  
**Date Collected:** 06-25-2021  
**Date Reported:** 09-20-2021

**POOLED PHENOTYPIC SENSITIVITY DETECTED (X):**
- Ceftriaxone (S)
- Piperacillin (S)
- Tobramycin (S)
- Vancomycin (R)

**ETIOLOGIC AGENT(S) DETECTED:**
- Escherichia coli
- Klebsiella pneumoniae

**POOLED PHENOTYPIC RESISTANCE DETECTED (X):**
- Ampicillin (R)
- Cefotaxime (R)
- Ceftriaxone (R)

**IN VITRO RESISTANCE GENES DETECTED:**
- 

**INTERPRETATION:**
- 

**RESULTS: PATHOGENIC DNA DETECTED**

**ORGANISM(s) TESTED: DETECTED:**
- Escherichia coli
- Klebsiella pneumoniae

**PATIENT'S MEDICATIONS:**
- 

**LENC**

**Formulations**
- 

**Organism(s) Tested: Detected**

**Antibiotic Testing:**
- 

**CONFIDENTIAL HEALTHCARE INFORMATION**

**First Last**  
**Pages 2 of 2**

**CONFIDENTIAL**
## RESULTS: PATHOGENIC DNA DETECTED

**ORGANISM(S) TESTED - DETECTED:** (See last page for Organism(s) Tested - Not Detected)

- **Citrobacter freundii >100,000 cells/mL**
- **Enterococcus faecalis >100,000 cells/mL**
- **Escherichia coli >100,000 cells/mL**
- **Viridans Group Strep >100,000 cells/mL**
- **Actinotignum schaallii 50,000-99,999 cells/mL**
- **Alloscardvia omnicolens 50,000-99,999 cells/mL**

### LEGEND

- **S** = Pooled Phenotypic Sensitivity Detected
- **R** = Pooled Phenotypic Resistance Detected
- **RGD** = Resistance Gene(s) Detected

### Formulations

<table>
<thead>
<tr>
<th>Levofloxacin</th>
<th>Tetracycline</th>
<th>Ciprofloxacin</th>
<th>Piperacillin / Tazobactam</th>
<th>Fluoroquinolone</th>
<th>Sulfamethoxazole / Trimethoprim</th>
<th>Gentamicin</th>
<th>Nitrofurantoin</th>
<th>Ampicillin</th>
<th>Cefadroxil</th>
<th>Cefuroxime</th>
<th>Vancocin</th>
<th>Ampicillin / Sulbactam</th>
<th>Cefepine</th>
<th>Cefazolin</th>
<th>Ceftriaxone</th>
</tr>
</thead>
<tbody>
<tr>
<td>PQ/IV</td>
<td>PQ/IV</td>
<td>PQ/IV</td>
<td>PQ</td>
<td>IV</td>
<td>PQ/IV</td>
<td>IV</td>
<td>PQ/IV</td>
<td>PQ/IV</td>
<td>PQ/IV</td>
<td>PQ/IV</td>
<td>PQ/IV</td>
<td>PQ/IV</td>
<td>PQ/IV</td>
<td>PQ/IV</td>
<td>PQ/IV</td>
</tr>
</tbody>
</table>

### Pooled Phenotypic Sensitivity

- **S**
- **R**
- **RGD**

### Resistance Gene(s) Detected

- **RGD**
- **RGD**
- **RGD**
- **RGD**
- **RGD**
- **RGD**
- **RGD**
- **RGD**
- **RGD**

### MIC Results (µg/mL)

- 1
- 2
- 1
- 32
- 16/4

---

Organism(s) Tested - Detected: ✓ = Check marks are supportive data and are NOT patient specific.

- **Citrobacter freundii**
- **Enterococcus faecalis**
- **Escherichia coli**
- **Viridans Group Strep****
- **Actinotignum schaallii**
- **Alloscardvia omnicolens**

✓ = Check marks indicate situations for which antibiotic use is either FDA-approved or off label use for antibiotics is illustrated in peer review literature. References available upon request.
Clinical Trials Results
# Current Clinical Studies

<table>
<thead>
<tr>
<th>Studies</th>
<th>Internal Validation</th>
<th>Retrospective UTI Urology Study (2018-RUUS)</th>
<th>Prospective UTI Urology Study (2018-PUUS)</th>
<th>Retrospective VPA Home Care UTI Study (2019 R-VPA-HUS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purpose</td>
<td>Technical Validity</td>
<td>Clinical Validity</td>
<td>Clinical Utility</td>
<td>Clinical Utility</td>
</tr>
<tr>
<td>Cohort Size</td>
<td>NA</td>
<td>500</td>
<td>2,518</td>
<td>110,000</td>
</tr>
<tr>
<td>Population</td>
<td>NA</td>
<td>60 and older</td>
<td>Mean age of 73</td>
<td>Mean age 70</td>
</tr>
<tr>
<td>Sites</td>
<td>Internal Test Validation</td>
<td>Urology Clinic (1)</td>
<td>37 Urology Offices in 7 states with 75 physicians</td>
<td>Home Care Patients</td>
</tr>
<tr>
<td>Study Design</td>
<td>NA</td>
<td>Retrospective analysis comparing Guidance UTI to culture</td>
<td>Compare outcomes of patients treated based upon Culture or Guidance UTI results</td>
<td>Comparison of culture to Guidance UTI in diagnosing and managing patients with UTI</td>
</tr>
<tr>
<td>Principal Investigator</td>
<td>Michael Opel, PhD</td>
<td>Kirk Wojno, MD</td>
<td>Kirk Wojno, MD</td>
<td>Kirk Wojno, MD</td>
</tr>
<tr>
<td>Status</td>
<td>Completed</td>
<td>Completed</td>
<td>Paper Submitted</td>
<td>Paper Submitted</td>
</tr>
</tbody>
</table>
## Bacterial Detection

<table>
<thead>
<tr>
<th></th>
<th>Guidance Positive n (%)</th>
<th>Guidance Negative n (%)</th>
<th>Total n (%)</th>
<th>Agreement n (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Culture Positive</strong></td>
<td>1,018 (40.5%)</td>
<td>80 (3.2%)</td>
<td>1,098 (43.7%)</td>
<td>92.7% (91.4,94.1%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Culture Negative</strong></td>
<td>557 (22.2%)</td>
<td>856 (34.1%)</td>
<td>1,413 (56.3%)</td>
<td>60.6% (58.0,63.1%)</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1,575 (62.7%)</td>
<td>936 (37.3%)</td>
<td>2,511 (100.0%)</td>
<td>74.6% (72.9,76.3%)</td>
<td></td>
</tr>
</tbody>
</table>

2018-PUUS Western IRB Number: 20181661 Not yet published.
## Compare Ability to Detect Polymicrobial Infections

<table>
<thead>
<tr>
<th></th>
<th>Guidance Polymicrobial (≥2 bacteria)</th>
<th>Guidance Positive (Monomicrobial)</th>
<th>Guidance Negative</th>
<th>Total</th>
<th>Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture Polymicrobial</td>
<td>141 (5.6%)</td>
<td>25 (1.0%)</td>
<td>2 (0.1%)</td>
<td>168 (6.7%)</td>
<td>83.9%</td>
</tr>
<tr>
<td>(≥2 bacteria)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture Positive</td>
<td>425 (16.9%)</td>
<td>427 (17.0%)</td>
<td>78 (3.1%)</td>
<td>930 (37.0%)</td>
<td>45.9%</td>
</tr>
<tr>
<td>(Monomicrobial)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture Negative</td>
<td>268 (10.7%)</td>
<td>289 (11.5%)</td>
<td>856 (34.1%)</td>
<td>1,413 (56.3%)</td>
<td>60.6%</td>
</tr>
<tr>
<td>Total</td>
<td>834 (33.2%)</td>
<td>741 (29.5%)</td>
<td>936 (37.3%)</td>
<td>2,511 (100.0%)</td>
<td>56.7%</td>
</tr>
</tbody>
</table>

Not yet published.
Bacterial Histogram - Prospective Study

Identification of Organisms

- Uses Real Time PCR to detect organisms associated with Urinary Tract Infections
- Quantity of Identified Organisms
  - Between 1,620 and 5,401 cells/mL (depending on organism) to 6,000,000 cells/mL or greater

### Organisms

- **Escherichia coli**
- **Enterococcus faecalis**
- **Klebsiella pneumoniae**
- **Coagulase Negative Staph**
- **Streptococcus agalactiae**
- **Viridans group Strep**
- **Pseudomonas aeruginosa**
- **Proteus mirabilis**
- **Other species**
- **Enterobacter species**
- **Aerococcus urinae**
- **Klebsiella oxytoca**
- **Staphylococcus aureus**
- **Enterobacter aerogenes**
- **Enterococcus species**
- **Citrobacter freundii**
- **Morganella morganii**
- **Citrobacter koseri**
- **Serratia marcescens**
- **Acinetobacter baumannii**
- **Providencia stuartii**
- **Ureaplasma urealyticum**
- **Mycoplasma hominis**
- **Corynebacterium riegelii**
- **Alloscardovia omnicolens**
- **Actinobaculum schaalii**

Number of Prospective Patients Testing Positive
Multiplex PCR Based Urinary Tract Infection (UTI) Analysis Compared to Traditional Urine Culture in Identifying Significant Pathogens in Symptomatic Patients


OBJECTIVE
To evaluate whether multiplex PCR-based molecular testing is equivalent to urine culture for detection of bacterial infections in symptomatic patients.

METHODS
Retrospective analysis of 592 consecutive patients presenting to the emergency department with symptoms of lower urinary tract infection (671) were included. All patients had multiple lower cultures and NGS molecular testing.

RESULTS
A total of 592 patients (mean age 73 years, range 40–95) with symptoms of lower UTI had both urine culture and diagnostic NGS between March and July 2016. PCR detected uropathogens in 571 patients (95.3%). Urine cultures were positive in 571 patients (95.3%). PCR and urine culture agreed in 398 of these patients (68.4%). Negative results were seen in 11 (1.9%) patients, with PCR reporting 16 and culture reporting 17. Positive results were seen in 153 patients (26.2%), with PCR reporting 144 and culture reporting 138. Agreement between PCR and culture for positive cultures was 98.6%.

CONCLUSION
Multiplex PCR is useful in the detection and identification of bacteria. Further investigation is needed to evaluate molecular testing for detection and identification of bacteria. Further investigation is needed to evaluate molecular testing for detection and identification of bacteria.

Traditional urine culture is currently regarded as the gold standard for detection and identification of pathogens. However, this method has been associated with cumbersome and time-consuming procedures such as NGS. With the emergence of multiplex PCR, both ease and accuracy of detection can be improved.

Traditional urine culture is currently regarded as the gold standard for detection and identification of pathogens. However, this method has been associated with cumbersome and time-consuming procedures such as NGS. With the emergence of multiplex PCR, both ease and accuracy of detection can be improved.

Traditional urine culture is currently regarded as the gold standard for detection and identification of pathogens. However, this method has been associated with cumbersome and time-consuming procedures such as NGS. With the emergence of multiplex PCR, both ease and accuracy of detection can be improved.

Traditional urine culture is currently regarded as the gold standard for detection and identification of pathogens. However, this method has been associated with cumbersome and time-consuming procedures such as NGS. With the emergence of multiplex PCR, both ease and accuracy of detection can be improved.

Traditional urine culture is currently regarded as the gold standard for detection and identification of pathogens. However, this method has been associated with cumbersome and time-consuming procedures such as NGS. With the emergence of multiplex PCR, both ease and accuracy of detection can be improved.

Traditional urine culture is currently regarded as the gold standard for detection and identification of pathogens. However, this method has been associated with cumbersome and time-consuming procedures such as NGS. With the emergence of multiplex PCR, both ease and accuracy of detection can be improved.

Traditional urine culture is currently regarded as the gold standard for detection and identification of pathogens. However, this method has been associated with cumbersome and time-consuming procedures such as NGS. With the emergence of multiplex PCR, both ease and accuracy of detection can be improved.

Traditional urine culture is currently regarded as the gold standard for detection and identification of pathogens. However, this method has been associated with cumbersome and time-consuming procedures such as NGS. With the emergence of multiplex PCR, both ease and accuracy of detection can be improved.

Traditional urine culture is currently regarded as the gold standard for detection and identification of pathogens. However, this method has been associated with cumbersome and time-consuming procedures such as NGS. With the emergence of multiplex PCR, both ease and accuracy of detection can be improved.

Traditional urine culture is currently regarded as the gold standard for detection and identification of pathogens. However, this method has been associated with cumbersome and time-consuming procedures such as NGS. With the emergence of multiplex PCR, both ease and accuracy of detection can be improved.

Traditional urine culture is currently regarded as the gold standard for detection and identification of pathogens. However, this method has been associated with cumbersome and time-consuming procedures such as NGS. With the emergence of multiplex PCR, both ease and accuracy of detection can be improved.
Genotype v Phenotype
# Resistance Gene and Susceptibility Agreement

<table>
<thead>
<tr>
<th>ABR and Genotype Agree</th>
<th>ABR and Genotype Disagree</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td><strong>Bacteria</strong></td>
</tr>
<tr>
<td><strong>42%</strong></td>
<td><strong>16%</strong></td>
</tr>
<tr>
<td><strong>58%</strong></td>
<td></td>
</tr>
</tbody>
</table>

*There are either more genes to detect or organism interactions impacting resistance patterns*

**Genes may be present in low quantity, inactive, or nonfunctional**
Bacteria Interactions
Figure 5: Correlations between organisms, excluding E. coli. The strength of the correlation is represented by the width of the edge connecting the genes. Only correlations greater than 0.1 shown.
Organism Interactions Impact Susceptibility Results

Pair increases resistance.

Would expect the antibiotic resistance levels to equal *E. coli*. *K. pneumonia* causes resistance to drop.
## Organism Interactions: Cephalosporin

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Bacteria that increase odds of resistance (odds-ratio)</th>
<th>Bacteria that decrease odds of resistance (odds-ratio)</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefaclor</td>
<td>E. faecalis ▲ (2.3)</td>
<td>Klebsiella pneumonia (0.69)</td>
<td>E. coli &amp; E. faecalis and Klebsiella &amp; E. faecalis together <strong>decrease</strong> the odds of resistance</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas aeruginosa (8.6)</td>
<td></td>
<td>E. coli &amp; Klebsiella together <strong>increase</strong> the odds of resistance</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>Actinobaculum schaalli (1.3)</td>
<td></td>
<td>Pseudomonas &amp; E. faecalis together <strong>decrease</strong> the odds of resistance</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas aeruginosa (2.2)</td>
<td></td>
<td>E. coli &amp; Klebsiella together <strong>increase</strong> the odds of resistance</td>
</tr>
<tr>
<td>Cefepime</td>
<td>E. faecalis ▲ (9.4)</td>
<td></td>
<td>E. coli &amp; Klebsiella and E. coli &amp; Proteus together <strong>increase</strong> the odds of resistance</td>
</tr>
<tr>
<td></td>
<td>E. coli (1.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefoxitin</td>
<td></td>
<td></td>
<td>Pseudomonas &amp; E. faecalis together <strong>decrease</strong> the odds of resistance</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E. coli &amp; Klebsiella together <strong>increase</strong> the odds of resistance</td>
</tr>
<tr>
<td>Ceftazidine</td>
<td>E. faecalis ▲ (7.3)</td>
<td>Klebsiella pneumonia (0.53)</td>
<td>CNS &amp; Klebsiella together <strong>decrease</strong> the odds of resistance</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E. coli &amp; Klebsiella and E. coli &amp; Pseudomonas together <strong>increase</strong> the odds of resistance</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>E. faecalis ▲ (6.9)</td>
<td>Klebsiella pneumonia (0.65)</td>
<td>Pseudomonas &amp; CNS together <strong>decrease</strong> the odds of resistance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proteus mirabilis (0.41)</td>
<td>E. coli &amp; Klebsiella together <strong>increase</strong> the odds of resistance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VGS (0.73)</td>
<td></td>
</tr>
</tbody>
</table>

▲ Even though agent is found susceptible in vitro, it is ineffective clinically.
Conclusions

- Multiplex PCR for UTI demonstrates
  - improved detection of organisms in urine
  - improved detection of polymicrobial urinary tract infections
- Pooled Antibiotic Sensitivity and identification of Antibiotic Resistance Genes may better clinical utility than culture
  - Reduces the need for empiric therapy with quicker turn around time
  - Identifies effective antibiotics for the patient’s specific group of organisms causing the UTI which improves time to symptom resolution
Disclaimer

Thermo Fisher Scientific and its affiliates are not endorsing, recommending, or promoting any use or application of Thermo Fisher Scientific products presented by third parties during this seminar. Information and materials presented or provided by third parties are provided as-is and without warranty of any kind, including regarding intellectual property rights and reported results. Parties presenting images, text and material represent they have the rights to do so.