Reducing Time to Result for Urinary Tract Pathogen Detection Utilizing Real-Time PCR Technology

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Pathnostics
Evolving Picture of Urinary Tract Infections

- The Scope of the Problem
- Evaluating the Tools Available
- Refining the Definition
- Moving from a Monocentric Thought Process
- Understanding the Prevalence and Nature of Polymicrobial Infections
- Development of New Tools for the Diagnosis and Management of Urinary Tract Infections
Objectives

• At the conclusion of the presentations, participants will be able to:
  – Understand the Impact of UTI’s in Patient Care
  – Evaluate the Technical Challenges Associated with Urine Culture
  – Identify the Different Classes of Uropathogens
  – Explain the Role of the Urinary Microbiome and Polymicrobial Infections in the Management of Urinary Tract Infections.
  – Assess the Use of qPCR in the Diagnosis of Urinary Tract Infections
  – Compare the Clinical Utility of Genotypic and Phenotypic Methods in Treatment of Urinary Tract Infections
THE SCOPE OF THE PROBLEM
MARKET SIZE AND INCIDENCE
Symptoms of Urinary Tract Infections

- Pressure in Lower Pelvis
- Dysuria
- Frequency
- Urgency
- Nocturia
- Abnormal Color or Odor
- Hematuria
- Flank Pain
- Fever/Chills
- Mental Changes/Confusion
The Impact

- Cost to System

  Responsible for
  \[\approx 10.5 \text{ MILLION office visits/year}\]  
  UTI complications result in
  \[9-11 \text{ DAYS longer for each hospital stay}\]  
  Health care cost exceed
  \[\approx 13 \text{ BILLION in the US}\]

- Cost to Humanity

  Up to \(\frac{1}{3}\) of infections illustrate resistance to an antibiotic
  \[2 \text{ MILLION INFECTIONS} \quad \text{and} \quad 23,000 \text{ DEATHS/YEAR}\]

According to the CDC, antibiotic resistance gives rise to at least
UTIs Segmentation (US)

**Urinary Tract Infections (UTIs)**

- **Symptomatic**
  - Cystitis
    - Majority are uncomplicated
  - Pyelonephritis
    - Majority are uncomplicated
  - Recurrent UTI
    - 25% of UTIs in women will recur within 6 months of initial infection
  - Catheter-associated UTI
    - Incidence: 449,334 CAUTI events (2002); 35,600 CAUTI events (2011) in acute care hospitals
  - UTI in men
    - Incidence: 0.1% in 30-65 years old; 5% in 65-85 years old
  - Urosepsis
    - Incidence: 0.1% in 30-65 years old; 5% in 65-85 years old

- **Complicated UTIs**
  - UTI in men
  - Urosepsis

- **Uncomplicated UTIs**
  - Low risk
  - High risk

- **Asymptomatic**
  - Pregnant women
    - Asymptomatic bacteriuria (ABU): 2-10% of pregnant women
  - Patient who will undergo invasive urologic procedure (eg, prostate biopsy)
  - Renal transplant recipient
  - Patient with urinary catheter

**Incidence**:
- UTIs in men: ~10-15% of adult women/year; 2-3M ER visits (2013)
- UTI in men: ~1.1M
- UTI in pregnant women: 25% of UTIs in women will recur within 6 months of initial infection
- UTI in men: Incidence: 0.1% in 30-65 years old; 5% in 65-85 years old
- Urosepsis: Incidence: 0.1% in 30-65 years old; 5% in 65-85 years old

**Majority are uncomplicated**

**High risk**

**Low risk**
US Market

- **Long Term Care Facilities**
  - 1.5M under care in 16,700 Nursing Homes with a total of 5.3M forecasted for 2030
    - Up 1.2 Million infections per year with patients averaging 3-4 courses of antibiotics annually
    - Up to 120,000 Hospital admissions annually which make up 30-50% of all Medicare Hospital admissions annually
- **Average Cost of Treating UTI in Nursing Home of $150 Per Patient**
  - Total Cost of $180M
- **Up to 120,000 Hospitalization**
  - from Average Hospital stay per admission costs an average of $1947 per night
- **Total Cost of Hospital Admission from Long Term Care Facilities - $467M**
US Market

• Hospital Acquired Urinary Tract Infections
  - 25% of patients in the US have catheters.
    • Catheter-acquired urinary infection is the source for about 20% of episodes of health-care acquired bacteremia in acute care facilities, and over 50% in long-term care facilities
  - 561,667 infections per year with a patient
  - Average 2 additional nights based on UTI

• Average Hospital stay per admission costs an average of $1947 per night

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<tr>
<th>Type</th>
<th>Number</th>
<th>Cost per Night</th>
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<td>State/Local</td>
<td>1053</td>
<td>$1,974.00</td>
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<tr>
<td>Non-Profit</td>
<td>1003</td>
<td>$2,346.00</td>
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<td>For Profit</td>
<td>2870</td>
<td>$1,798.00</td>
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<tr>
<td></td>
<td>4926</td>
<td>$1,947</td>
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</table>

Total Cost of Hospital Acquired UTI $2.2 Billion
• 3% of children per year develop a urinary tract infection accounting for 1 million officer visits per year
• Recurrent infections occur in up to 50% of patients
• Permanent renal cortical scarring may occur in up to 65% of affected children, especially in recurrent UTI and its long-term complications include hypertension and chronic renal failure which may result in end stage renal disease
• 1.5 million office visits annually
  – $150M in annual costs
• >50,000 hospital admissions
  – Average cost of hospitalization is $10,489 per patient
  – Annual cost of $520 Million
• Total cost >$670 Million
  – Does not include costs associated with treatment of patients for consequences of renal/cortical scarring

Pediatric Urinary Tract Infections
Sepsis

- 30 million sepsis cases worldwide annually
- 1.1 million cases in US annually
- Urosepsis comprises 25% of that total
- Total US cost for sepsis treatment is $24B annually
- Total cost for sepsis due to urinary tract infection is >$6B annually

Fig 1. hospital admissions for sepsis have overtaken those for stroke or myocardial infarction. Adapted from Seymour et al. [16]
# Total Cost of UTI Treatment in US Healthcare System

- **Physician Office Based**: $3.9B
- **LTC Facility Based**: $647M
- **HAI Based**: $2.2B
- **Pediatric UTI**: $.67B
- **Sepsis (UTI Based)**: $6B

**Total**: $13 Billion Dollars Annually
The Situation is Complicated by Lack of New Antibiotics and Increasing Rates of Antibiotic Resistance
Limited Efficacy of Current Testing Methodologies
Limit Treatment Options

Due to Prolonged Turn around Times (48-72 Hours) and Limited Sensitivity Associated with Urine Culture Clinicians Frequently Treat Patients Empirically Resulting in Poor Antibiotic Stewardship and Increased Rate of Antibiotic Resistance.
EVALUATING THE TOOLS
The Method – 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.
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*. The biased results developed using this methodology often creates findings that are not consistent with the clinical symptoms.

- Is unable to detect slow growing organisms including fastidious and non aerobic 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.

What Is the Fundamental Problem With Culture as a Detection Method?
• The Loyola Study followed 150 patients who were split into two groups based on whether they believed they were symptomatic for UTI
  
  – They 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%.

Expanding the Number and Types of Uropathogens
qPCR Assay Results

Cases with Organism Detection

- Escherichia coli: 24 cases
- Klebsiella pneumoniae: 8 cases
- Aerococcus urinaria: 3 cases
- Enterococcus faecalis: 11 cases
- Pseudomonas aeruginosa: 12 cases
- Staphylococcus aureus: 5 cases
- Streptococcus agalactiae: 10 cases
- Actinobaculum schaali: 1 case
- Allo lovedonia omnicolens: 5 cases
- Candida albicans: 6 cases
- Candida parapsilosis: 2 cases
- Citrobacter Koseri: 4 cases
- Corynebacterium urealyticum: 3 cases
- Proteus mirabilis: 0 cases
- Staphylococcus marcessans: 1 case

This urine is not sterile
Enhanced quantitative urine culture

Blood agar, 1 ml, 24 hours, aerobic
Blood agar, 100 ml, 48 hours, 9% CO2

Adapted from June 3, 2016 ASM Microbe presentation by Alan J. Wolfe, PhD. "Urine is Not Sterile: Why We Should Care."
### Frequency of Uropathogens - Type

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of Positives</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecalis</td>
<td>252</td>
<td>50.4%</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>246</td>
<td>49.2%</td>
</tr>
<tr>
<td>Actinobaculum schaali</td>
<td>139</td>
<td>27.8%</td>
</tr>
<tr>
<td>Streptococcus anginosus</td>
<td>133</td>
<td>26.6%</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>121</td>
<td>24.2%</td>
</tr>
<tr>
<td>Aerococcus urinae</td>
<td>114</td>
<td>22.8%</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>92</td>
<td>18.4%</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>89</td>
<td>17.8%</td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>53</td>
<td>10.6%</td>
</tr>
<tr>
<td>Alloscardovia omnicolens</td>
<td>43</td>
<td>8.6%</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>35</td>
<td>7.0%</td>
</tr>
<tr>
<td>Corynebacterium riegelii</td>
<td>30</td>
<td>6.0%</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>28</td>
<td>5.6%</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>26</td>
<td>5.2%</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>25</td>
<td>5.0%</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>25</td>
<td>5.0%</td>
</tr>
</tbody>
</table>

**Common Organisms Culture Identified Positive**

- **Gram+**: Escherichia coli, Klebsiella
- **Gram-**: Enterococcus, Proteus
Fungal Infections most often due to Candida species including
- Candida albicans
- Candida glabrata
- Candida parapsilosis

Can cause both UTI’s and Prostatitis

Most patients asymptomatic but symptomatic patients are indistinguishable from those with bacterial UTI.
Virus in the Urinary Tract

- Virus typically difficult to detect in the bladder or prostate.
- Most common viral causes of urinary tract infections include BK virus, JC virus, Adenovirus, CMV and HSV
- Impacts those with low immunity, for example:
  - Bone marrow or organ transplantation
  - Blood cancers/malignancies (e.g. leukemia)
  - HIV infection
  - Pregnancy
  - Diabetes, alcoholism, malnutrition, liver cirrhosis
- In UTI, high viral load is associated with high mortality in patients with low immunity
UNDERSTANDING THE IMPACT OF THE MICROBIOME AND POLYMICROBIAL INFECTIONS

REFINING THE DEFINITION – MOVING FROM A MONO-CENTRIC THOUGHT PROCESS
Evolving Picture of the Microbiome of the Bladder and Urethra

- Urine is not sterile
- The bladder contains a microbiome that has been overlooked primarily because of our limited capacity to culture microorganisms
- The net result has been an understatement of the frequency and scope of bacterial infections
The Female and Male Microbiome

- The characterization of the male and female urinary microbiome are in their infancy but recent studies have begun to define the basic parameters associated with them.
  - In Females, the FUM
    - Tend to be at lower colony counts as compared to other human microbiomes with counts in the $10^3$ to $10^5$ range.
    - They are dominated by Lactobacillus, Gardnerella, Sneathia, Staphylococcus and Enterbacteriaceae as well as other diverse species.
    - They consist of genital and urinary tract organisms.
The Female and Male Microbiome

• In Males, the MUM
  • They are dominated by Lactobacillus, Sneathia, Veillonella, Corynebacterium Prevotelloa, Streptococcus, and Ureaplasma.
  • They consist of genital and urinary tract organisms.
Asymptomatic Bacteriuria and Dysbiosis

• Asymptomatic bacteriuria is the presence of a high number of bacteria >100,000/ml without symptoms.
• Not treated unless patient is has renal disease, is immunocompromised or pregnant (to prevent pyelonephritis)
• May represent an ecological balance between pathogenic bacteria and the urinary microbiome.
Dysbiosis in the Urinary Tract

- Antibiotic Use
- Immune Suppression
- Diet
- Stress
- Lack of Exercise
Evolving From an Monocentric View of Urinary Tract Infections

• Wolfe and Brubaker have proposed moving from a E.coli-centric view of urinary tract infections -
  – As importantly with an increasing number of studies demonstrating that the majority of 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.
  • A simple truth – Polymicrobial Infections may be the norm rather then the exception.
• The sharing of metabolic products provides polymicrobial infections an advantage
  – In the presence of antibiotics the sharing of metabolic products plays a protective role increasing resistance and virulence
• Brings into question the current practice of isolating organisms prior to determining the antibiotic resistance
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.
Polymicrobial Interactions Change MIC Levels
Prevalence of Polymicrobial Infections Require the Development of New Methodologies

- Using current culture guidelines polymicrobial infections would most often be classed as mixed flora—probable contamination and not be worked up…
  - In that there are a number of studies showing polymicrobial infections in the blood with corresponding UTI findings – this supports the clinical importance of proper characterization of samples

- This lack of sensitivity seen with traditional culture coupled with this guidance underlies the growing incidence of patients presenting with symptoms of UTI and no diagnosis—resulting in ineffective treatment
DEVELOPING NEXT GENERATION TOOLS FOR THE EVALUATION OF URINARY TRACT INFECTIONS
Development of a Next Generation Assay for the Identification of Urinary Tract Infections

• Guidance is a quantitative PCR based assay that identifies organisms associated with UTI’s without the need of culture.
Open Array Format with 56 Assays and 48 Samples
- TaqMan assays are spotted inside each through-hole.
- Hydrophilic through-holes are surrounded by hydrophobic surfaces that keep the reaction contained; 48 subarrays & 64 through-holes per subarray = 3072
**Sample Type**
- Urine
  - Voided, Catheter, or Suprapubic Aspiration
- Quantity of Identified Organisms
  - Between 500 cells/mL (depending on organism) to 6,000,000 cells/mL or greater
High Sensitivity and Specificity with ATCC Inclusivity Panel

The UTM assays demonstrate high sensitivity and specificity with gDNA controls.
Assays Demonstrate 5 Logs of Dynamic Range and Strong Linearity
Serial Dilution of Pooled ATCC gDNA Inclusivity Panel

UTM assays demonstrate 100 copies/ul sensitivity of sample input with gDNAs
Detection Objectives

• **Primary Objective**
  – Compare the ability of Guidance and traditional urine culture in detecting organisms causing a UTI

• **Secondary Objective**
  – Identify the frequency of observed polymicrobial infections and compare the ability of Guidance and traditional urine culture in detecting polymicrobial infections
### Comparing Detection Levels

<table>
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<tr>
<th>Guidance</th>
<th>Generation 3</th>
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<tbody>
<tr>
<td>Number of Patient Samples</td>
<td>196</td>
</tr>
<tr>
<td>Number Bacterial Organisms in Panel</td>
<td>25</td>
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<tr>
<td>Inclusion Criteria</td>
<td>DX Code for UTI from Urology Office</td>
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<tr>
<td>Exclusion Criteria</td>
<td>DX Code Not UTI</td>
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Comparison Study

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Total Number of Cases</td>
<td>196</td>
</tr>
<tr>
<td>Total Number of Cases - Male</td>
<td>96</td>
</tr>
<tr>
<td>Total Number of Cases - Female</td>
<td>100</td>
</tr>
<tr>
<td>Total Number of Negative Cases</td>
<td>42</td>
</tr>
<tr>
<td>Total Number of Positive Cases</td>
<td>154</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Culture</th>
<th>Guidance</th>
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<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
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<tr>
<td>Positive</td>
<td>101</td>
<td>3</td>
</tr>
<tr>
<td>Negative</td>
<td>50</td>
<td>42</td>
</tr>
<tr>
<td>Total</td>
<td>151</td>
<td>45</td>
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</table>

**At Case Level Agree**

**Missed by Guidance**

**Missed by Culture**
Organisms Detected

Organisms Detected by Method

- **Molecular**
- **Culture**
Developing Methodologies for Managing Antibiotic Resistance Testing in a Polymicrobial Environment
**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>
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<tr>
<td>Macrolide resistance</td>
<td>ermA + Erm B</td>
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<tr>
<td></td>
<td>ermC</td>
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<tr>
<td></td>
<td>mefA</td>
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<tr>
<td>Extended-Spectrum-Betalactamase</td>
<td>TEM</td>
</tr>
<tr>
<td></td>
<td>CTX-M group 1</td>
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<tr>
<td></td>
<td>SHV</td>
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<td>VEB</td>
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<td>OXA-1</td>
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<td>PER-1</td>
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<td>GES</td>
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<td>blaNDM-1</td>
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<td>Quinolone and fluoroquinolone resistance</td>
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<tr>
<td>Aminoglycoside</td>
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<td>anti-la-aph2</td>
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<tr>
<td>Trimethoprim/Sulfamethoxazole</td>
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<tr>
<td>AmpC resistance</td>
<td>ampC, FOX, ACC</td>
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<tr>
<td></td>
<td>DHA, MOX/CMY, BIL/LAT/CMY</td>
</tr>
</tbody>
</table>
Phenotypic Testing

1. Media is plated into a 96-well plate, each with a different antibiotic.
2. Urine sample (1mL minimum) added to each of the 96 wells and incubated.
3. Urine in each well read by spectrophotometer for optical density (OD), which measures cell density.
4. Determine potential antibiotic resistance or sensitivity based on OD (cell density).

Each well has different antibiotic
All of the bugs (polymicrobial)

Sample

Above threshold = Resistant
Below threshold = Susceptible
Antibiotic Resistance

Average Resistance Detected

- ISOLATE ABR TESTING: 22%
- "SOUP" METHOD: 51%
THE GUIDANCE THERAPEUTIC MANAGEMENT SOLUTION

Identify symptomatic patients that require treatment

Identification

What is Causing the Symptoms?
Bacterial / Viral / Fungal

Therapeutic Solution

Genotypic ABR Testing
The Genetic Markers Are Found in the Urine That Indicate Resistance

Phenotypic ABR Testing
What Antibiotics Kill the Organisms in the Urine?
Conclusion

• UTI’s Constitute 13B Impact to US Economy with Significant Morbidity and Mortality
• Urine is Not Sterile
• The Urinary Tract Contains a Microbiome That Plays an Important Role in Maintaining Health
• Polymicrobial Infections are Common and Result in Increased Rates of Virulence and Antibiotic Resistance
• Routine Urine Culture Has a High False Negative Rate and Miss the Majority of Uropathogens
• qPCR is a Powerful Tool for Identifying both the Identity of the Infectious Agent as well as the Presence of Antibiotic Resistance Genes
  – Presence of the ABR Genes Does Not Necessarily Correlate with Actual Resistance
• Phenotypic Assays Evaluating Pooled Resistance Allows for the Assessment of the Antibiotic Resistance of the Pooled Sample
• Combining Genotypic and Phenotypic Data Provides a Functional Answer with Respect to Both Fast and Slow Growing Organisms.
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