Urine: Significance of Reflexing, Counts and Important Organisms

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Wayne State University
Topics

- What is a UTI
- Microbiome of Urinary tract
- Misconceptions
- Unusual pathogens
- Reflex Testing
- New Methods
What is a urinary tract infection?

• An infection in any part of the urinary system — kidneys, ureters, bladder and urethra.

• Most infections involve the lower urinary tract — the bladder and the urethra.
Anatomy Of The Lower Urinary Tract
Routes of Infection:

- In healthy individuals
  - Uropathogens originate from rectal flora
  - Enter the urinary tract via the urethra into the bladder
  - Initially colonize urothelium of the distal urethra.
The logistics of infection:
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Uncomplicated cystitis</th>
<th>Complicated UTI’s</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. Coli</td>
<td>70-90%</td>
<td>40-50%</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>2-6%</td>
<td>10-17%</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>2-4%</td>
<td>5-10%</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>0-1%</td>
<td>5-10%</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>0-1%</td>
<td>2-10%</td>
</tr>
<tr>
<td>Staphylococcus saprophyticus</td>
<td>5-20%</td>
<td>0-4%</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>1-2%</td>
<td>1-20%</td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>0-1%</td>
<td>1-4%</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0-1%</td>
<td>1-2%</td>
</tr>
<tr>
<td>Candida spp.</td>
<td>0-1%</td>
<td>2-5%</td>
</tr>
</tbody>
</table>

Adherence

• Uroepithelial adherence is critical for establishment of UTI.
• Urinary tract: urethra, bladder, ureters and kidneys
• Mechanical barriers:
  • Epithelium lining the tract
  • Flushing action of urine
  • Aggregating actions of urinary mucins
Lines of Defense:

- First line of defense: prevention of adherence
  - Luminal surface of bladder is lined with highly sulfated and anionic glycosaminoglycans
  - Impermeability/antimicrobial anti-adherence properties
Umbrella cell exfoliation occurs by apoptosis like mechanism which removes adherent E. coli.

Tamm-Horsfall protein: high molecular weight protein present in human urine that binds E. coli fimbriae by mannose moieties, inhibiting fimbrial interaction with uroplakin receptors.
## Predisposing Factors For Adherence

<table>
<thead>
<tr>
<th>Genetic</th>
<th>Biological</th>
<th>Behavioral</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood group antigen</td>
<td>Congenital abnormalities</td>
<td>Intercourse</td>
<td>Decreased mental status</td>
</tr>
<tr>
<td>Non-secretor status</td>
<td>Urinary Obstruction</td>
<td>Some birth control systems</td>
<td></td>
</tr>
<tr>
<td>Increased density of adhesion</td>
<td>Calculi</td>
<td>Antimicrobial use</td>
<td></td>
</tr>
<tr>
<td>receptors</td>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anatomical</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Residual urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atrophic vaginitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urinary incontinence</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prior history of UTI</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maternal history of UTI</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Catheters and stents</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Immunologic suppression</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hormones and age</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Conventional Urine Culture and Urinalysis
The majority of specimen cultures are negative. Up to 70% are negative, up to 50% are contaminated, and most are not clinically significant. Processing varies from 10-30 minutes. Using 15 minutes and 50 urines/day results in 12.5 hours of 1.5 FTE/day.
Spinning our wheels on the road to nowhere.

- Lost and Tired
Consensus:

- Culture is not needed in most cases of initial uncomplicated cystitis
- Empirical treatment based on symptoms
- Save culture for upper and complicated UTI
Reporting by Culture Results and Numbers
What is significant count?

- Method of collection
  - Voided: \(> 10^4 - 10^5 \text{ cfu/ml}\)
  - Catheterized: \(> 10^3 - 10^4\)
  - Suprapubic: \(> 10^2 - 10^3\)
# Current Recommendations

<table>
<thead>
<tr>
<th>No. of Isolates</th>
<th>Colony Count</th>
<th>Definitive ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt;10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Yes, if PP</td>
</tr>
<tr>
<td></td>
<td>&lt; 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Both &gt; 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Yes, if PP</td>
</tr>
<tr>
<td></td>
<td>Both &lt; 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>1 &gt; 10&lt;sup&gt;4&lt;/sup&gt;; 1 &lt; 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Yes, if &gt; 10&lt;sup&gt;4&lt;/sup&gt; = PP</td>
</tr>
<tr>
<td>2,3</td>
<td>1 &gt; 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Yes, if PP</td>
</tr>
<tr>
<td></td>
<td>Any other</td>
<td>No</td>
</tr>
</tbody>
</table>

- PP: potential pathogen
- .001 loop
- 16 h min incubation
## Current Recommendations

<table>
<thead>
<tr>
<th>No. of Isolates</th>
<th>Colony Count</th>
<th>Definitive ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$&gt;10^3$</td>
<td>Yes, if PP</td>
</tr>
<tr>
<td></td>
<td>$&lt;10^3$</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Both $&gt;10^3$</td>
<td>Yes, if PP</td>
</tr>
<tr>
<td></td>
<td>Both $&lt;10^3$</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>$1 &gt; 10^3; 1 &lt; 10^3$</td>
<td>Yes, if $&gt;10^3 = $ PP</td>
</tr>
<tr>
<td>2, 3</td>
<td>$1 &gt; 10^4$</td>
<td>Yes, if PP</td>
</tr>
<tr>
<td></td>
<td>Any other</td>
<td>No</td>
</tr>
</tbody>
</table>

Reporting:

• No growth: No growth of > 1,000 cfu/ml
• PP: Report organism ID, colony count and AST for 1 or 2 PP
• Culture with mixed species of various quantities
  • Report the presence of mixed flora, quantities and request a repeat specimen
• > 3 organism at $10^4$ cfu/ml (noninvasive) present in equal quantities

• > 3 organisms at $10^3$ cfu/ml (invasive collection) present in equal quantities

• Exception:
  • If > 3 organisms are present and 1 is predominant, the predominant organism should be identified if a PP.
## Treatment of UTI

<table>
<thead>
<tr>
<th>UTI</th>
<th>Pyelonephritis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uncomplicated</strong></td>
<td><strong>Acute</strong></td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>Fluoroquinolone</td>
</tr>
<tr>
<td>TMP-SMX</td>
<td>TMP-SMX</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>Ceftriaxone</td>
</tr>
<tr>
<td>Fluoroquinolone</td>
<td>Aminoglycoside</td>
</tr>
<tr>
<td>Oral B-lactam</td>
<td></td>
</tr>
</tbody>
</table>

Do not report, erythromycin, clindamycin, chloramphenicol, tigecycline
Resources for Routine Urine Culture Guidelines

- Cumitech 2C: Laboratory Diagnosis of Urinary Tract Infections
  - (McCarter, Burd, Hall and Zervos)
  - American Society of Microbiology

- Asymptomatic Bacteriuria
  - Published: Clinical Infectious Diseases; 2005; 40: 643-654
  - "Infectious Diseases Society of America Guidelines for the Diagnosis and Treatment of Asymptomatic Bacteriuria in Adults"
  - This guideline was last reviewed and deemed current as of 04/2013.

- Catheter-Associated Urinary Tract Infection
  - Published: Clinical Infectious Diseases; 2010; 50: 625-663
  - This guideline was last reviewed and deemed current as of 07/2013.

- Uncomplicated Cystitis and Pyelonephritis (UTI)
  - Published: Clinical Infectious Diseases; 2011; 52: e103-e120
  - "Guidelines for Antimicrobial Treatment of Acute Uncomplicated Cystitis and Pyelonephritis in Women"
  - This guideline was last reviewed and deemed current as of 07/2013.
NEW IDEAS
To Reflex/Not to Reflex

That is the question
Urinalysis

• Documentation of pyuria, hematuria, nitrates, leukocyte esterase, and bacteria.

• Pyuria present in almost all patients with urethritis, cystitis and pyelonephritis.

• Hematuria: cystitis and pyelonephritis (Sensitivity-44%)

• Leukocyte esterase: detection of WBC (Sensitivity 74-96%)

• Nitrite test: (Sensitivity-39%)
**Rapid Screens:**

<table>
<thead>
<tr>
<th>Method</th>
<th>10⁵ cfu/ml</th>
<th>10⁴ cfu/ml</th>
<th>10³ cfu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopic</td>
<td>95</td>
<td>90</td>
<td>78</td>
</tr>
<tr>
<td>Enzymatic</td>
<td>85</td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>Filtration</td>
<td>94</td>
<td>85</td>
<td>76</td>
</tr>
<tr>
<td>Bioluminescence</td>
<td>98</td>
<td>&gt;85</td>
<td></td>
</tr>
<tr>
<td>Photometry</td>
<td>97</td>
<td>&gt;87</td>
<td></td>
</tr>
</tbody>
</table>

Screening for Pyuria to reflex to culture

- Pyuria is present in cystitis but also in other processes.

- Leukocyte esterase
  - Sensitivity of 74-96%
  - False (+): vaginal secretions
  - False (-): glycosuria, urobilinogen, and high specific gravity.
    - A negative result does not exclude infection
    - Symptomatic patients with a negative leukocyte esterase should receive empiric therapy or further testing.
Sterile Pyuria

- 5-8 WBC/HPF in a urinalysis in the absence of bacteria in culture.

- Causes:
  - Injury
  - Catheterization
  - Diseases
  - Gram stain important to rule out presence of organisms.
Screening for Nitrate to reflex to culture

- Presence of nitrites is highly specific for bacteriuria
- Sensitivity is low
- Detects between $10^3$ and $10^5$ cfu/ml
- Staphylococcus, Enterococcus and Pseudomonas are nitrite negative.
Reflex urine culture cancellation in the ED

- 1546 ED patients met study criteria.
- 314 (20%) had positive urine cultures.
- Restriction of culture to samples with WBC > 10/HPF, positive nitrites, positive LE or positive bacteria = sensitivity of 96.% and specificity of 48.1%
- 604/1546 (39%) culture eliminated
- 11/314 (3.5%) positive cultures missed

Role of Urinalysis in the Diagnosis of UTI:

- The presence of both pyuria and bacteriuria microscopically increases the probability of accurate detection of a UTI

There is always light at the end of every dark tunnel...

You simply have to find courage to go wherever the road leads you.
New Rapid Screens:
New Rapid Screens:

- IQ 200
- UF1000/ Sysmex Analyzer
- Maldi-TOF MS
- Pocared P-1000
- Automated Microscopy
  - Diasys
  - Yellow IRIS
UF-1000i

- Automated urine sediment analysis
- Flow cytometry platform
- Particles stained by fluorescent dyes
- Identification by impedance, scattering and fluorescence.
• Image-based Automated Particle Recognition (APR)
• Digitally documented, auto-identification of 12 particle classes, with 26 additional particle sub-classifications
• Captures the emitted light from the interaction between photons and molecules to detect the pathogens' optical properties
• Subsequently an algorithm determines results.
- 4 ml urine centrifuged to remove WBC, then bacteria for testing.
- 260 urines designated positive by UF-1000i were processed by conventional culture and MALDI-TOF.

<table>
<thead>
<tr>
<th>Conventional ID (No. of Cases)</th>
<th>MALDI-TOF (No. of Cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (20)</td>
<td>Negative (20)</td>
</tr>
<tr>
<td>Positive, 2 morphology colony types (5)</td>
<td>Not reliable ID (2) Microorganisms ID (3)</td>
</tr>
<tr>
<td>Positive, 1 morphology colony type (235)</td>
<td>Positive with same ID (205) Positive with different ID (2) Negative or not reliable ID (28)*</td>
</tr>
</tbody>
</table>

*14/28 were culture with 10% cfu/ml

Direct ID Of Bacteria Causing UTI By Combining Maldi-TOF With UF-1000i Urine Flow Cytometry.

- Method: UFC to eliminate negatives and MALDI-TOF to ID positives.
- 1456 urine samples/932 negative urines
- Combined automation matched conventional methods 94.8% of time.
- An adequate bacterial count was essential for obtaining good results with MALDI-TOF directly from clinical samples (> $10^5$ cfu/ml)

Chromagar
Is $10^5$ cfu/ml still relevant?
Redefining Urinary Tract Infections by Bacterial Colony Counts

• Kass 1956 criteria:
  • UTI=pure urinary culture of $> 10^5$ cfu/ml of a uropathogen

• Current Study
  • UTIs defined by a culture of $> 10^5$ cfu/ml produces a 7.2% false-positive rate.
  • Increasing cutoff to $> 10^6$ cfu/ml halves the false-positive rate
  • Culturing 2 urine samples reduces the false positive rate to just 0.6%

Coulthard et al. Pediatrics, 2010; 125;335
**FIGURE 2**
Frequency distribution curves of concentrations of bacteria cultured from the first urine samples of a pair collected from 205 children.
Redefining urinary tract infections by bacterial colony counts

- Compared with patients with counts <10^5 cfu/ml, patients with counts ≥10^5 were 74% more likely to have a clinically significant UTI.
- Reporting positive results only for patient with >10^5 cfu/ml would have reduce the number of positive culture by 38%
- Reporting counts < 10^5 cfu/ml encourages treatment of clinically insignificant UTIs in hospitalized patients
- Result: inappropriate antibiotic use.
% Of Patients W/Wo UTI And Colony Counts

Kwon et al. Am J Clin Pathol 2102; 137: 778-784
Microbiome
Urinary Microbiome

Culture Negative ≠ Sterile
Evidence supports that the lower urinary tract can have a microbiota.

Evidence of diverse bacteria not routinely cultivated by the lab in the urine of both men and women.

Studies performed using 16S rRNA gene sequencing, MALDI-TOF, and more sensitive culture techniques of “sterile urine”.
Viable but nonculturable bacteria are alive but do not give rise to visible growth under nonselective growth conditions.

Urine from female volunteers w/wo UTI were found to contain significantly more viable than culturable forms of bacteria.

Members of the urinary microbiota are not reported in routine culture because:

- The quantity is $< 10^3$ cfu/ml
- Growth of microbes require special culture conditions
• The most prevalent genera isolated:
  • Lactobacillus
  • Corynebacterium
  • Streptococcus
  • Actinomyces
  • Staphyloccoccus
Bacterial composition in the urine collected within studies is highly variable regardless of sex or age. Studies indicate the existence of a core bladder microbiome with variability in the amount of the core bacteria and flux of other bacteria with ageing.
Core Urinary Microbiome in Women

- Corynebacterium
- Dialister
- Neterobacter
- Enterococcus
- Facklamia
- Finegoldia
- Fusobacterium
- Lactobacillus
- Mobiluncus
- Murdochella
- Negativicoccus
- Peptoniphilus
- Porphyromononas
- Provetella
- Propionimicrobium
- Sporanaerobacter
- Streptococcus
- Varibaculum
OverActive Bladder:
- Increased Gardnerella and decreased Lactobacillus in microbiome

Other genera
- Actinobaculum, Actinomyces, Aerococcus, Arthrobacter, Corynebacterium, Garnerella, Oligella, Staphylococcus, Streptococcus and
- Lactobacillus gasseri.
Persistence of Intracellular Bacteria in the Urinary Bladder

- Persistent bacterial populations within the bladder are often intracellular.
- Intracellular bacteria can reemerge sporadically and grow to high titers within the bladder lumen.
- May be one aspect of recurrent UTIs in women
Unusual Isolates
Aerococcus Urinae

• Risk Factors:
  • Elderly patients with urologic conditions

• Identification
  • GPC in tetrads and clusters
  • Catalase neg, PYR neg, LAP-pos
  • No standardized method for AST
Corynebacterium urealyticum

- Alkaline urine pH, struvite crystals, leukocytes and erythrocytes
- Important in renal transplant patients with post transplant UTI
- Pleomorphic diphteroid-like small gram-positive rod
- Strict aerobe requiring incubation beyond 24 hours
- Multi-drug resistant
Actinobaculum

- First described as cause of infection 1997
- Closely related to genus actinomyces
- 4 species: suis, schaalii, massiliae and urinale
- Isolated from urine after prolonged incubation in 5% CO₂
- Patients reported to have underlying urological conditions.
• A 24-year-old female presented 5 weeks after an uncomplicated vaginal delivery with fever, rigors, and malaise of 7 days duration.

• Prior to admission, she was treated with oral penicillin for 5 days for a possible mastitis but was without any clinical improvement.

• Her pregnancy was normal apart from a urinary tract infection (UTI) during the last trimester.

• She had a past medical history of UTIs, for which she had not been investigated.