Diagnosis of Urinary Tract Infections: Are the Old Methods Still Valid?

Bob Fader, Ph.D. D(ABMM)
Section Chief, Microbiology
Scott & White Memorial Hospital
BaylorScott&White HEALTH
Temple, TX
Objectives

• Discuss ways of improving pre-analytical variables in urine cultures
• Describe the changing concepts of the urinary tract microbiome
• Examine your local laboratory procedures for urine cultures and compare them to current guidelines
• Be willing to answer lots of questions!!
Urinary Tract Infection

- UTI in general
  - 1 in 3 females are treated for UTI by age 23
  - 50% of females have UTI during lifetime
  - Cost of community UTI > 1.5 billion $ / yr

- Nosocomial UTI
  - 30% of HAIs reported to NHSN
  - >560,000 nosocomial UTI/year with 13,000 deaths
  - Cost approx 0.5 billion/yr
  - 15-25% hospitalized patients catheterized
  - 75% HA UTI related to catheterization

Pre-analytical Variables

• How many attendees know the urine culture contamination rate (UCCR) in your facility?

• How many think that you can have an impact on the UCCR?
What is a “Random” Urine?

• A random urine is one that is unscheduled and is the usual term used to denote an unscheduled urine collection

• We noticed an increase in urines labelled as “random” from some OP clinics with high UCCR

• Found out that a “random” urine had morphed into something else again
  – Reserved for urine where the nursing staff knew the person was not going to be able to collect a proper specimen so they just have them urinate into a container and then poured it over to a transport tube!
Ways to Improve UCCR

• Work with nursing staff to standardize collection methods
  – Standardize urine collection materials
  – Training and competency
  – Posters on collection in ED bathrooms
  – Instructions sheets to review with patients in English and Spanish
• Establish (and adhere to) time limits and conditions of transport
  – Reject unpreserved urine with transport time > 2 h
  – Have urine split on floor/clinic to UA and boric acid transport tubes
• Get buy-in from TOP administrators!
PREVENTION OF CONTAMINATED URINE SPECIMENS

IMPACT:
- $900 per specimen (approx. $310,000./mo.)
- Unnecessary treatment
- Potential for false CAUTI
- Lack of confidence in healthcare team

GOAL:
Reduce specimen contamination rate to below 10%.

PLAN:  
- Improve collection procedures
- Reduce collection to transport time
- Monthly reports on unit level contamination rate

COLLECTING URINE FROM A CATHETER
- Swab catheter port with alcohol
- Connect Luer-Lok access device
- Swab tube top(s) with alcohol
- Center tube in holder & push in to fill
- Label at bedside & transport to lab within 1 hour

COLLECTING A CLEAN CATCH URINE SPECIMEN
- Instruct or assist patient to:
  1. Clean genito-urinary area with ALL towelettes
  2. Handle only outside of cup and place lid facing up
  3. Collect urine mid-stream (flow-collect-flow)
- Swab tube top(s) with alcohol
- Center tube in holder & push in to fill
- Label at bedside & transport to lab within 1 hour
Reflex urine cultures

• How many use “reflex” urine cultures?
• Cultures may be ordered only if certain parameters are met on the urinalysis
  – > 5 WBC (some use ≥ 10 WBC)
  – Positive leukocyte esterase
  – Positive Nitrate
  – Bacteria present
• ClinMicroNet/Division C survey
  – 52.9% of 128 respondents said yes
Reflex urine cultures

• Practice is fairly controversial
• Mostly limited to adult, outpatient urines
• Many that use report high percentage of “false positives”
• Some LIS/EMRs may have trouble handling request
Urine Screens: Microscopy

• Gram stain of non-centrifuged urine
  – 10 μL of urine on slide, air dry, methanol fix, stain
    • Quick, reliable, good correlations with > $10^5$ CFU/ml if 1 organism is seen in OIF
  – Alternatives
    • Centrifuged urine examinations
    • Unstained urine examination
• Automated Microscopy
  – DiaSys R/S 2000, Yellow IRIS, others
Urine Screens: Dipsticks

• Leucocyte esterase (LE)
  – Will be positive at 10 WBC/mm³
  – 90% UTI = pyuria
  – 75-96% sensitivity and 94-98% specificity

• Nitrite test
  – Detection of Enterics at >10⁵ CFU/ml
  – ~ 95% Specific, but only 35-85% sensitive
  – Some uropathogens are nitrate negative

• Combo LE + Nitrite
  – Rapid; may be negative with some bacteria like Enterococcus, S. saprophyticus
  – 79-93% sensitive and 82-98% specific
Urine Cultures: Indications

• Culture is **not** always necessary in women with dysuria, pyuria
• Uncomplicated UTI may be treated with empiric therapy for “common pathogens” – 75-90% are caused by E. coli
Urine Cultures: Indications

- Cultures are indicated in the following situations:
  - Complicated UTI
  - Suspicion of pyelonephritis
  - UTI in past 3 weeks indicating possible relapse or the presence of symptoms for > 7 days
  - Recent hospitalization or catheterization indicating possible healthcare-associated infection
  - Transplant patients
  - MS patients
  - Prostatitis patients
  - Pregnancy
  - Diabetes
  - Other
Collection: To clean or not to clean?

• Studies have demonstrated that if a proper mid-stream catch is collected, it is not necessary to clean the urethral meatus

• UpToDate supports this practice

• Problem with use of urine as source for GC/Chlamydia PCR
  – Requires unclean, first-catch urine
  – Recent JCM publication on over diagnosis of UTI and under diagnosis of STD on urine samples collected in ED

Urine: Collection

• Midstream urine is “usual” manner of collection
  – Patient instruction is crucial
    • No prior cleansing (UpToDate supports)

• Catheterized urine
  – Foley (big reduction in usage because of CMS refusal of CAUTI reimbursement)
    • Prone to contamination
    • Do not accept catheter tips for culture
  – Straight (in and out) cath (probably best specimen)
    • *Discard first 15-30 mls*

• Suprapubic aspirate:
  – Primarily on neonates
Urine Collection

• Cystostomy
  – Complicated procedure requiring bladder washout and collection of ureteral specimens before and after washout
  – 4 specimens labeled LK-1, RK-1, LK-2, RK-2)
• Nephrostomy
  – Usually collected from ureteral stents
• Prostatic massage (pre and post) (VB2 and VB3)
Urine Processing

• Process urines within 2 hrs of collection or refrigerate
• Reject > 2hr if no evidence of refrigeration
  – If delays in transport are expected or if unable to refrigerate, transfer to urine transport tube
    • Most types have boric acid; supposedly good for up to 96 h
    • Must fill to 3 ml mark or inhibition may occur
    • May change counts of some uropathogens (Enterococcus)
      • May inhibit small numbers of potential pathogens
• Reject urine samples collected with same collection method within 48 hrs of receipt of first specimen (duplicate)
Media for Culture

• Conventional: 5% sheep BAP and MAC (or EMB)
• CLED: Cysteine lactose electrolyte deficient medium
• Chromogenic media
  – BD CHROMAgar
  – bioMerieux chromID CPS
  – Remel Spectra UTI
  – Hardy BluEcoli
• Paddles or “Dip” type devices (often used in Dr offices)
  – SOLAR-CULT (Solar Biologicals)
  – OnSite™ (Trek Diagnostics)
  – DipStreak (Novamed)
CLED agar

- *Escherichia coli* ..... Yellow colonies, opaque, center slightly deeper yellow
- *Klebsiella* .... Yellow to whitish-blue colonies, extremely mucoid
- *Proteus*... Translucent blue colonies
- *Pseudomonas aeruginosa* .... Green colonies with typical matted surface and rough periphery
- Enterococci ..... Small yellow colonies, about 0.5 mm in diameter
- *Staphylococcus aureus* .......... Deep yellow colonies, uniform in color
- Coag Neg Staphylococci... Pale yellow colonies, more opaque than *E. faecalis*
EMB (Eosin Methylene Blue)

- *E. coli*: Large, blue-black, green metallic sheen
- *Enterobacter/Klebsiella*: Large mucoid, blue-black
- *Proteus*: Large, colorless
- *Salmonella*: Large, colorless
- *Shigella*: Large, colorless
- *Pseudomonas*: Irregular, colorless
- Gram-positive bacteria: No growth to slight growth
CHROMOGENIC MEDIA

Cost versus convenience?
Dip Slides

NovaMed Dip Slides

- **Routine or non-invasive:**
  - Clean-Catch mid-stream
  - Indwelling catheter
  - Pediatric “bagged” urine
- **0.001 ml (1 μL) calibrated loop onto primary media (BAP and MAC or others)**
  - Streak down center; spread out from there
    - 4 quadrant streak also works
- **>16 hr incubation, 35°C in an O₂ incubator before reading plates initially; many laboratories discard mixed culture or no growth at ~ 24 hrs.**
- **Catheterized** – hold additional overnight to r/o yeast
Inoculation of Conventional Media:
Cumitech 2C/Clin Micro Proc Handbook

• **Invasive collection methods:**
  – Straight catheter (in and out)
  – Suprapubic aspirate
  – Cystoscopy
  – Nephrostomy

• **0.01ml (10 µL) calibrated loop onto BAP and MAC**
  – May want to add chocolate agar to cystoscopy, nephrostomy or surgically-obtained specimens
  – Consider > 24 - 48 hr incubation, 35°C in a O₂ incubator
  – May want to include a 0.001 ml inoculum on BAP for easier CFU determination

• **We need to accurately know what type of urine it is!**
NOW FOR THE REAL QUESTIONS

• What to work-up?
• How to Report & Interpret
Reporting Culture Results - By the Numbers?

- **Pyleonephritis**
  - > $10^5$ CFU/ml

- **Asymptomatic Bacteriuria**
  - Kass. 1956. Trans Assoc Am Physicians 69:56-64
  - > $10^5$ CFU/ml x 2

- **Women Acute Cystitis**
  - Stamm et al. 1982. NEJM 307:463-8
  - > $10^2$ CFU/ml

- **Men Acute Cystitis**
  - Lipsky et al. 1987. JID 155:847-54
  - > $10^3$ CFU/ml

- **UTI in Catheterized Patient ??**
  - Stark and Maki. 1984. NEJM 311:560-4
  - $10^2$->$10^5$ CFU/ml

- **UTI in the Infant 2-24 mo**
  - > $5x10^4$ CFU/ml-cath

Slide courtesy of Dr Paul Schreckenberger
HOW TO REPORT URINE CULTURES: Cumitech Recommendations

• Negative urines (no growth)
  – 0.01 ml inoculum
    • Sterile or < 100 CFU/ml or
    • No growth of ≥ 100 CFU/ml
    • No organisms isolated???
  – 0.001 ml inoculum
    • Sterile or < 1000 CFU/ml or
    • No growth of ≥ 1000 CFU/ml
    • No organisms isolated???

• Positive cultures: colony count reported along with ID (with or without AST)

• Mixed cultures: reported as such with “canned comment”

• Unusual pathogens/isolates
  – Bring it to attention of supervisor
  – Call clinician or other health care provider
What to do with Routine CC-MS or Non-Invasive Urine

• Work up 1 or 2 pathogens each at ≥ 10,000 CFU/ml with ID and AST* (some use >50,000 CFU)

• If 1 or 2 pathogens at < 10,000 CFU/ml
  – simplified ID including gram stain, spot tests, hemolysis, and rapid biochemical tests for both
  – full ID probably not necessary
  – Check urinalysis result (if available)

• If 1 pathogen is ≥ 10,000 CFU/ml and another is <10,000 CFU/ml
  – Perform ID and AST on predominant isolate and give simplified ID for isolate at < 10,000 CFU/ml
What to do with Routine CC-MS or Non-Invasive Urine

- If there are ≥ 3 organisms
  - If one organism is predominant, is uropathogen and ≥ 100,000 CFU/ml even with mixed flora, perform ID and AST
  - If there are 2 isolates in this mixed flora that have counts 10,000 – 100,000 CFU/ml each, and others < 10,000, use the simplified identification only for both > 10,000 CFU/ml
  - If the 3 or more are in roughly same numbers report as mixed flora and/or give morphologic ID of components only.
    - Suggest recollection??
    - Sign out as mixed skin flora or mixed urogenital flora (microbiota?)
Contamination comments

• MULTIPLE ORGANISMS CONSISTENT WITH URETHRAL FLORA PRESENT AT <10,000 CFU/mL.

• MULTIPLE ORGANISMS CONSISTENT WITH URETHRAL FLORA PRESENT AT >10,000 CFU/mL; NO PREDOMINANT ORGANISM PRESENT.
What about the low colony count urine (0.01 mL inoculum)?

• Similar to “routine” urine culture, but with ID of lesser CFU/ml since inocula is 10-fold less

• If both 1 µL and 10 µL inocula are planted, may use the 1 µL inoculum plates for quantitation if counts are > 10,000 CFU/ml

• Mixed cultures are probably still “mixed” and may represent
  – Contamination or improperly transported urine specimens
  – Need to take patient population into account
  – May consider need to treat suprapubic aspirates differently since any growth of potential pathogen is probably significant.
### What’s Important and What’s Not

<table>
<thead>
<tr>
<th>Uropathogens</th>
<th>Commensals (Urogenital/skin flora)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteric GNRs</td>
<td>Lactobacilli</td>
</tr>
<tr>
<td>Pseudomonas &amp; other NF GNRs</td>
<td>Alpha-Strep</td>
</tr>
<tr>
<td>Enterococcus (predominant?)</td>
<td>Diphtheroids</td>
</tr>
<tr>
<td>Staphylococcus saprophyticus</td>
<td>Coag Neg Staph (non-catheterized)</td>
</tr>
<tr>
<td>Gp B Streptococci</td>
<td></td>
</tr>
</tbody>
</table>
What about Group B Strep?

• GBS can cause a “true” urinary tract infection
  – Symptomatic and no other uropathogens isolated
  – AST probably not needed (canned comment)
  – May need AST if penicillin allergic patient

• Urine can also be a ‘surrogate” for the vaginal/rectal specimens when screening for GBS in the pregnant female
  – Asymptomatic carriage without UTI symptoms
    • Most often in mixed urogenital flora
  – Any quantity was considered significant
    – Newer recommendations, report if \( \geq 10,000 \text{ CFU/ml} \)
      (Report any???)
Other Infrequent Pathogens

• *Corynebacterium urealyticum*
  – Associated with alkaline-encrusted cystitis and pyelitis in adults and children and urinary tract struvite calculi
  – Rapid urease production

• *Gardnerella vaginalis*??
  • Normal vaginal flora; increased numbers in bacterial vaginosis;
  • unclear as to UTI significance

• **Anaerobes**
  – very low incidence
  – Consider only in suprapubic aspirate
What about *Aerococcus* sp.?

- GPC in *clusters*; catalase negative; α-hemolytic; may resemble enterococcus or other viridans streptococci
  - *A. urinae*: PYR – and LAP +
    - Most commonly isolated from UTI; probable pathogen
  - *A. sanguinicola*: PYR + and LAP +
    - Not commonly isolated, but can be a UTI Pathogen
  - *A. viridans* = PYR +/- LAP –
    - Can be isolated from urine; significance unknown
- Don’t chase it in a mixed urine!

Aerococcus sp.

A. urinae: PYR – and LAP +
Actinobaculum spp.

- Fastidious Gram positive bacillus
- Facultative anaerobe, CO$_2$-requiring
- Slow growth; need 2-5 days in culture
- Weak beta-hemolysis in 3-5 days
- Noted to cause UTI in elderly
- Most resistant to fluoroquinolones and trimeth/sulfa

Le Brun, C. et al. 2015. Urinary tract infection caused by *Actinobaculum schaalii*: a urosepsis pathogen that should not be underestimated. JMM Case Reports. DOI 10.1099/jmmcr.0.000030
Actinobaculum schaalii

Chocolate agar
This begs the question of what are we missing with our current techniques?
Is there a urinary microbiome?

- Study from Loyola Univ. Medical Center in Chicago suggests so
- Consenting participants who were free of known UTI provided urine samples by voided, transurethral, and/or suprapubic collection
- Presence of bacteria assessed by extended culture, microscopy and 16S rRNA gene sequencing

Is there a urinary microbiome?

- Bacteria that are not or cannot be routinely cultivated were common in all three specimen types regardless of whether the subjects had urinary symptoms
- Transurethral and suprapubic aspirates contained similar communities of bacteria
- Voided urine contained mixtures of urinary and genital tract bacteria

Most abundant genera

Bladders of culture-negative women contain bacteria

Slide courtesy of Dr. Paul Schrekenberger
Which organism(s) is responsible for urinary symptoms sequenced samples from single culture-positive participant (>10^5 cfu/ml *E. coli*)?
What is the significance of a urinary microbiome?

• It is safe to say we don’t know at this point!
• There is plenty more work to be done
• E. coli is still the primary pathogen
Antimicrobial Susceptibility

• How many know the percent resistance of the common UTI pathogens to routine oral agents at your institution?
• How many provide a urine antibiogram?
UTI Treatment

• UpToDate recommends trimethoprim/sulfa (Bactrim) as first line empirical agent unless the percent susceptible is <80%

• Other agents include fluoroquinolones (Cipro, Levo) and nitrofurantoin (Macrobid)
What about fosfomycin?

• How many are receiving requests for fosfomycin susceptibility testing?

• Fosfomycin (Monuril) is a broad spectrum antibiotic that was developed in the late 1960’s

• Mechanism of action is to block the cross linking of the peptidoglycan portion of the cell wall

• Has wide range of activity but FDA approved only for treatment of uncomplicated UTI caused by *E. coli* and *Enterococcus faecalis*
Fosfomycin

- Taken as a single dose for UTI
- Prostatitis: 3 doses
- Has fairly good activity against ESBLs and CREs
- Can be formulated as a parental for treatment of CRE infections
- CLSI guidelines have breakpoints only for E. coli and E. faecalis
- Requires glucose-6-phosphate for activity
  - Disk and E test strip have it incorporated
- Breakpoints are for Urine only!!!
## CLSI Fosfomycin breakpoints

<table>
<thead>
<tr>
<th>Zone diameter</th>
<th>MIC interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S</strong></td>
<td><strong>S</strong></td>
</tr>
<tr>
<td><strong>I</strong></td>
<td><strong>I</strong></td>
</tr>
<tr>
<td><strong>R</strong></td>
<td><strong>R</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>E. coli</strong></th>
<th>≥ 16</th>
<th>13-15</th>
<th>≤ 12</th>
<th>≤ 64</th>
<th>128</th>
<th>≥ 256</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. faecalis</strong></td>
<td>≥ 16</td>
<td>13-15</td>
<td>≤ 12</td>
<td>≤ 64</td>
<td>128</td>
<td>≥ 256</td>
</tr>
</tbody>
</table>

**If you are asked to test a MDRO for parenteral therapy, use E test (do QC!) and report only MICs. Document that susceptibility testing was requested by Dr. XXXX**
What about cefazolin?

• M100 S24 made a change that cefazolin could be used as the surrogate oral cephalosporin in addition to cephalothin

• However, different breakpoints for cefazolin are in effect depending on site of infection

<table>
<thead>
<tr>
<th>Zone diameter</th>
<th>MIC interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S</strong></td>
<td><strong>I</strong></td>
</tr>
<tr>
<td>Cefazolin parenteral</td>
<td>≥ 23</td>
</tr>
<tr>
<td>Cefazolin oral (UTI)</td>
<td>≥ 15</td>
</tr>
</tbody>
</table>
M100 comment

- Cefazolin results predict results for the oral agents cefaclor, cefdinir, cefpodoxime, cefprozil, cefuroxime, cephalexin and loracarbef when used for therapy of uncomplicated UTIs due to E. coli, K. pneumoniae and P. mirabilis. Cefpodoxime, cefdinir and cefuroxime may be tested individually because some isolates may be susceptible to these agents while testing resistant to cefazolin.
Summary

• You can have an impact on the quality of the urine specimen at your facility but proper collection and transport is still essential to quality performance of the urine culture

• Communication with clinicians concerning ordering, processing and AST is crucial for proper diagnosis and treatment of urinary tract infections

• Don’t be shy about using urinalysis results to determine specimen work-up

• At this point, the old rules still apply for urine specimens - but stay tuned!
Questions?