Urinary Tract Infection
Investigations, Bacterial Virulence and Antibiotic Resistance

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Scope of Presentation

• Clinical investigations for UTI
  – Laboratory and non-laboratory
  – Rationale

• Bacterial virulence in UTI
  – Concepts and Definition
  – Virulence Factors

• Antimicrobial resistance in treating UTI
  – Pathogenesis
  – Implications
Urinary Tract Infection (UTI)

• Definition
  – Inflammatory response to infection of the urothelium

• Complicated vs uncomplicated
  – Anatomical and/or functional defects to urinary tract
  – Affects time to respond to antibiotic treatment

• Epidemiology
  – Common infection, GP antibiotic prescribing rate 57.1%¹
  – Sabah study²:
    • Female preponderance (F:M = 2.5:1)
    • Age > 60 years old
    • Organism: faecal origin: *E coli* (38.2%), *Klebseilla sp*(15.0%)

¹LT Cheong, et al. AustFamPhy 2011
²Mustafa M, et al. IOSRJPBS 2012
UTI: Clinical Investigations (1)

• Using symptomology to diagnose and treat UTI.
  – Dysuria, frequency (cystitis)
  – Adv: cost-saving, rapid treatment
  – Disadv: non-specific symptoms, under-diagnosis, over-treatment, promotes antimicrobial resistance, does not differentiate at-risk patients

• Evidence for treating UTI without Lab Test?
  – Patients from same locality known to a clinic
  – History alone: sensitivity 50% to 80%
  – Patient’s complaints clinically consistent with uncomplicated UTI with NO vaginal discharge or irritation (likelihood of uncomplicated UTI > 90% to 95%)

5Moore et al. Hosp Med Clin 2014
UTI: Clinical Investigations (2)

• Aims
  – Establish diagnosis
  – Confirm diagnosis
  – Select out patients who are more at-risk

• Types
  – Laboratory
    • Screening vs confirmatory
  – Non-laboratory
    • Imaging
UTI: Clinical Investigations (3)

• Laboratory
  – Urine studies: Urinalysis, Microscopy & Gram-staining, Culture
  – Diagnosis
    • Presumptive: direct/indirect analysis of urine
    • Confirmatory: urine culture
  – False results
    • False negative: early stage infection (↓WBC and ↓bacteria counts)
    • False positive: contamination

• Efforts taken to reduce contamination of collected urine to improve diagnostic accuracy
• How Urine specimens are collected:
  - **Suprapubic aspiration**
    • Unable to void on command, eg. newborns, neurology
    • Highly accurate (least contaminant)
    • But associated morbidity
  - **Voluntary voided specimen**
    • Mid-stream sample of urine (MSU)
    • Circumcised men: no preparation
    • Uncircumcised men: retracted prepuce, clean glans penis
    • Women: spread labia, cleanse periurethral area with moist gauze
  - **Per urinary (Foley’s) catheter: Catheter-sampled**
    • Uncooperative patient
    • Women: contaminated MSU – get catheterised specimen (mid-catheterised urine)

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• **Urinary Dipstick Test Parameters:**
  
  — Nitrites (Griess test)
    - Uropathogen reduces nitrate to nitrite
    - Positivity indicates bacteria in urine (bacteruria)
  
  — Leucocyte Esterase (LE) activity test
    - From neutrophils, LE catalyses formation of indoxyl which oxidises a diazonium salt on dipstick → colour change
    - Positivity indicates WBCs in urine (pyuria)
**UTI: Clinical Investigations (6)**

<table>
<thead>
<tr>
<th>Dipstick Test</th>
<th>Nitrite</th>
<th>Leucocyte Esterase (LE)</th>
<th>Nitrite + LE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>27%</td>
<td>57-96%</td>
<td>35-84%</td>
</tr>
<tr>
<td>Specificity</td>
<td>94%</td>
<td>94-98%</td>
<td>98-100%</td>
</tr>
<tr>
<td>False positive</td>
<td>Rare (contaminant)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>False negative</td>
<td>Low-grade bacteruria; <em>Staph Saprophyticus</em>, <em>Pseudomonas</em>, <em>Enterococci</em> do not reduce nitrate</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Positive Predictive Value 92%**
- Nitrite **AND** either
- Blood + or LE +
- IF none, Negative Predictive Value 76%

- Tests done in isolation is of limited value
- Nitrite positivity > specific than LE alone (85-88% vs 59-96%)

**Dipstick’s role: for screening esp asymptomatic patients**

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5 Moore et al. Hosp Med Clin 2014
6 Lane et al. Emerg Med Clin N Am 2011
• **Urinalysis by Microscopy (& Gram Staining):**
  
  – Indication: urinary symptoms present
  – **Adv:** rapid identification of bacteria and WBC
  – **Disadv:** presumptive UTI
  – Sediments from spun sample of 5-10 mL urine for microscopy. If \( \geq 10^5 \text{ CFU/mL} \), this is **highly specific** for UTI.
    
    • If \( < 10^2 \) to \( 10^4 \) CFU/mL, microscopic limitation to detect bacteria\(^4\)
    
    • **False negative (“no growth”) situation**
    
    • At least 30,000 CFU/mL in urine sediments to detect
  
  – **Bacteriuria**
    
    • Sensitivity to UTI 40-70%, specificity 85-95%
    
    • Without pyuria: contamination or bacterial colonisation
    
    • **False positive: cell shedding (squamous epithelial cells)**

\(^{4}\text{Schmiemann G, et al. DtschArzteblInt 2010}\)

\(^{6}\text{Campbell-Walsh Urology 2012}\)
• **Urinalysis by Microscopy**
  
  **Pyuria**
  - Sensitivity to UTI 80-95%, specificity 50-76%
  - If 1-2 WBCs per high power field (HPF) in sediment of centrifuged specimen = 10 WBCs/mL unspun specimen
  - Correlates with bacteruria if: >2 WBCs/HPF spun or >10 WBCs/mL unspun specimen
  - Abacterial pyuria: UTI, non-gonococcal urethritis, GU tuberculosis, stone disease, malignancy, staghorn (WBC clumping), trauma
  - Negative pyuria: questionable for UTI diagnosis

  **Haematuria**
  - Found in 40%-60% cystitis
  - Indicator of inflammatory response with pyuria

• **Gram-staining**
  
  - Sensitivity for UTI 90%, and specificity 88%
  - Un-centrifuged, MSU with 1 bacterium per oil-immersion field = $10^4$ bact/mL
  - A 5mL specimen centrifuged 5 minutes at 2000 rpm, allows lower number bacteria to be identified after Gram-staining
  - Not always positive with uncomplicated UTI

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Schmiemann G, *et al.* DtschArzteblInt 2010
Campbell-Walsh Urology 2012
UTI: Clinical Investigations (9)

- **Urine culture**
  - Criterion standard for diagnosis of UTI
  - Indications
    - Complicated UTI – including male UTI
    - Recurrent UTI – esp in female
    - Pyelonephritis – known or suspected, esp in female
    - Questionable UTI diagnosis in absent pyuria
  - Rationale: helps guide treatment in failed antibiotic therapy
  - Collected specimen sent for culture immediately, or else, refrigerate (4°C), and urine cultured within 24h
Culture techniques

- **Agar plating**
  - Quantitative
  - Urine dropped onto agar: more tedious
  - Urine specimens need to be refrigerated

- **Dip slide/Immersion culture media**
  - Difficult to identify bacteria species
  - Less tedious
  - Plastic rod coated with medium: soy agar//Eosin-methylene blue or MacConkey agar
  - Can be done at home: need not refrigerate
  - Sensitivity 73%, specificity 94%\(^4\)

\(^4\)Schmiemann G, *et al*. DtschArzteblInt 2010
• **What is a positive culture?**
  
  – Debatable, circumstantial
  
  – Cut-off point of number of colony forming units (CFU)
    
    • Traditionally, ≥ $10^5$ CFU/mL
    
    • In women, urine culture is positive if
      
      – 20%-40% symptomatic UTI have $10^2$ to $10^4$ CFU/mL
      
      – ≥ $10^2$ CFU/mL + clean catch urine + single bacterial isolate
    
  • Suprapubic aspirate: Positive if any bacteruria

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7Lane et al. Emerg Med Clin N Am 2011
# UTI: Clinical Investigations (12)

## 16.1 Criteria for the diagnosis of UTI, as modified according to IDSA/European Society of Clinical Microbiology and Infectious Diseases guidelines (1-3)

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
<th>Clinical features</th>
<th>Laboratory investigations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acute uncomplicated UTI in women; acute uncomplicated cystitis in women</td>
<td>Dysuria, urgency, frequency, suprapubic pain, no urinary symptoms in 4 weeks before this episode</td>
<td>$&gt; 10 \text{ WBC/mm}^3$</td>
</tr>
<tr>
<td>2</td>
<td>Acute uncomplicated pyelonephritis</td>
<td>Fever, chills, flank pain; other diagnoses excluded; no history or clinical evidence of urological abnormalities (ultrasonography, radiography)</td>
<td>$&gt; 10 \text{ WBC/mm}^3$</td>
</tr>
<tr>
<td>3</td>
<td>Complicated UTI</td>
<td>Any combination of symptoms from categories 1 and 2 above; one or more factors associated with a complicated UTI (see text)</td>
<td>$&gt; 10 \text{ WBC/mm}^3$</td>
</tr>
<tr>
<td>4</td>
<td>Asymptomatic bacteriuria</td>
<td>No urinary symptoms</td>
<td>$&gt; 10 \text{ WBC/mm}^3$</td>
</tr>
<tr>
<td>5</td>
<td>Recurrent UTI (antimicrobial prophylaxis)</td>
<td>At least three episodes of uncomplicated infection documented by culture in past 12 months: women only; no structural/functional abnormalities</td>
<td>$&lt; 10^3 \text{ cfu/mL}$</td>
</tr>
</tbody>
</table>
- **Asymptomatic Bacteriuria**
  - Bacteria in urine microscopy w/out s&s UTI
  - 2 x samples of urine with $\geq 10^5$ cfu/ml bacteria
  - Only 1 x sample needed for men or catheterised specimen
- Importance:
  - Determine risk for infection in pregnant women
  - For patients undergoing some urologic procedure
Rationale:

1. **Patient with UTI with risk factors that require intervention in addition to antimicrobial therapy**
   - Empirical UTI therapy relapses
   - Immuno-compromised host (e.g., DM/TB)
   - UTI with possible urinary tract obstruction
   - UTI in polycystic kidney
   - Men

2. **Radiologic evaluation to diagnose focus of bacterial persistence**
   - Failure to respond to treatment
     - Fever during treatment, consider abscess
   - Persistent or rapid UTI re-infections
• **Choice of imaging**
  
  – **Plain film**: localise stone density only not location
  
  – **IVP**: exact stone location and extent of ureteral obstruction
  
  – **US scan**: operator dependant. Useful for prostate abscess and testicular imaging
  
  – **CT scan**: choice investigation. Information on renal parenchyma and collecting system. Functional study: site and degree of obstruction
  
  – **MRI**: anatomic site, cause and extent of infection
Bacterial Virulence and UTI

• **Virulence – definition:**
  – The degree of pathogenicity of an organism, or the relative ability of a pathogen to cause disease

• **Bacterial virulence is effected by virulence factors (VFs).** These are:
  – Specific properties enabling bacteria to overcome host defences and to cause disease

• **When bacterial virulence ↑ or host defences ↓,**
  – Bacterial inoculation, colonisation and infection of urinary tract increases

• **But, ↑ bacterial virulence is needed to overcome strong host defences**
Bacterial Virulence: UTI and *E coli* (1)

- **Uropathogenic E coli (UPEC)**
  - Source: bowel flora. Most common and most studied.
  - Multiple genes code for VFs and function as adhesins, toxins, proteases, invasions, serum resistance factors, and cause severe histologic damage
  - *α-Hemolysin* (*HlyA*) *toxin*: porify host cell membrane
  - Iron acquisition system: *aerobactin*—acquire iron from host as nutrient to survive
  - Acid polysaccharide *capsule*: evade host phagocytosis
  - *Adhesion* molecules (pili): attach to urothelium
Bacterial Virulence: UTI and *E Coli* (2)

- **Adhesion molecules on bacteria**
  - Attaches to bacterial rigid fimbriae or pilus
  - Locks onto receptors urothelium

- **Types of Pili**
  - Type 1 (Mannose sensitive): most common pili, contains adhesin FimH to adhere to urothelium and colonise urinary tract
  - Type P (= pyelonephritis): confers tropism to kidney. Mediates haemagglutination of RBCs
  - Type S: binds to sialic acid residues via SfaS adhesin.

*Campbell-Walsh Urology, 2012*
Bacterial Virulence: UTI and *E coli* (3)

- **VF acquisition**
  - Gene coding for VF located on chromosome or plasmid.
  - Transmitted vertically or horizontally (via pathogenecity islands)

- **VF and phase variation**
  - Ensures bacterial survival at different sites of urinary tract
  - Type 1 in bladder mucosa, Type P in in kidney

- **VF and host urothelial cell**
  - Mildly virulent bacteria causes UTI when there is increased host cell receptivity to VF (genetic pre-disposition of the host)
  - Obstruction to urinary flow at all anatomic levels increases host susceptibility
  - Urine stasis: bacteria growth and increased adherence
Antimicrobial Resistance in UTI (1)

Definition

• When bacteria not eradicated after 7 to 14 days of treatment with antibiotics

• Use of antimicrobials is the main cause

• Resistance rates (in uncomplicated UTI)
  – At least 20%: amoxicillin, co-trimoxazole
  – Below 10%: fluoroquinolone, oral cephalosporin, co-amoxiclav
  – Very low: nitrofurantoin, fosfomycin

• Types of resistance:
  – Inherited chromosomal-mediated
  – Acquired chromosomal- or extrachromosomal-mediated

Campbell-Walsh Urology 2012
Antimicrobial Resistance in UTI (2)

- **Inherited Resistance (Vertically transmitted)**
  - Chromosomal
    - Due to an absence of proper mechanism on which antimicrobial agents can act
    - Eg. *Proteus* sp and *Pseudomonas* sp: NITROFURANTOIN

- **Acquired Resistance**
  - Chromosomal
    - Due to treatment initiated for UTI
    - Selection of resistant clones (5%-10%)
  - Non-chromosomal (**Horizontally transmitted**)
    - Acquired or transferred via plasmids
    - Called R-factor resistance
    - Occurs in bowel flora
    - Due to selection of pre-existing mutants in urinary tract
    - All antimicrobial classes capable of plasmid-based resistance
      - Rare: FLUOROQUINOLONE
      - Never: NITROFURANTOIN
Antimicrobial Resistance in UTI (3)

• R-factor against current and other antimicrobial agents:
  – β-lactams, aminoglycosides, sulfonamide, trimethoprim, tetracycline

• Multi-drug resistance organisms
  – Plasmid-mediated resistance

• Other factors for resistance development
  – Previous use of fluoroquinolones and presence of urologic disease
Antimicrobial Resistance (4)

• **Enzyme inactivation**
  – β-lactamase: S.aureus, N.gonorrhoea, enterobacteriae: penicillins, cephalosporins, carbapenems

• **Altered permeability**
  – Access of antibiotic to bacteria prevented
  – Bacteria receptor alterations or transport mechanisms

• **Alteration of binding site**
  – Genetic variations alter antibiotic target
### Mechanism of Action of Common Antimicrobials Used in the Treatment of UTIs

<table>
<thead>
<tr>
<th>DRUG OR DRUG CLASS</th>
<th>MECHANISM OF ACTION</th>
<th>MECHANISMS OF DRUG RESISTANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Lactams (penicillins, cephalosporins, aztreonam)</td>
<td>Inhibition of bacterial cell wall synthesis</td>
<td>Production of β-lactamase Alteration in binding site of penicillin-binding protein Changes in cell wall porin size (decreased penetration)</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Inhibition of ribosomal protein synthesis</td>
<td>Downregulation of drug uptake into bacteria Bacterial production of aminoglycoside-modifying enzymes Mutation in DNA gyrase-binding site Changes in cell wall porin size (decreased penetration)</td>
</tr>
<tr>
<td>Quinolones</td>
<td>Inhibition of bacterial DNA gyrase</td>
<td>Active efflux Not fully elucidated—develops slowly With prolonged exposure</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>Inhibition of several bacterial enzyme systems</td>
<td>Draws folate from environment (enterococci)</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>Antagonism of bacterial folate metabolism</td>
<td>Enzymatic alteration of peptidoglycan different point than target</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Inhibition of bacterial cell wall synthesis (at β-lactams)</td>
<td></td>
</tr>
</tbody>
</table>

*Campbell-Walsh Urology, 2012*
Antimicrobial Resistance (6)

• Implications – compromised treatment in...
  – Uncomplicated UTI:
    • Eg. CTXM-15 ESBL producing *E. coli* (ST-131 strain)
    • Predictable
    • Resistance ≠ complications
    • Risk factor: previous antimicrobials
  – Complicated UTI:
    • Eg. ESBL *E. coli*, NDM-1 *E. coli*, *Klebsiella pneumoniae*
    • Less predictable
    • Multidrug resistance

*Nicole L. Curr Infect Dis Rep 2011*
Antimicrobial Resistance (7): Prevention

• Prophylactic antibiotics
  – Long term, use low dose
• Non-antimicrobial
  – Cranberry juice
• Vaccine
  – Not successful
• Threshold of prevalence of resistance
  – IDSA: threshold of prevailing resistance (community), above which, a drug is NOT recommended
    – 20% for trimethoprim-sulfamethoxazole (TMP-STX)
    – 10% for fluoroquinolones
• Study your hospital’s antibiogram
  – May overestimate resistance pattern in uncomplicated UTI
• Establish UTI diagnosis from clinical history and lab screening investigations +/- imaging
• Culture urine for at-risk patients or in complicated UTI
• Avoid obtaining contaminated urine samples
• Screen for asymptomatic bacteriuria in pregnant women and patients undergoing interventional urology
• Interpret urinary culture results based on patient circumstances
• Understanding of bacterial virulence vital to target antimicrobial therapy
• Antimicrobial resistance pattern in UTI can be problematic