A. MICROBIAL ASPECTS OF URINARY TRACT INFECTIONS

The following comments apply mainly to dogs, where cultured bacteria are most frequently correlated with urinary tract problems. UTI refers to the multiplication of bacteria at any site in the urinary tract proximal to the urethra. It is typically associated with $> 10^5$ bacteria/mL of voided urine.

1. Urinary tract as a microbial habitat

The kidneys, ureters, bladder and proximal urethra are normally free of microbes. Occasionally small numbers of bacteria enter the bladder but are rapidly removed by urination. The distal urethra and external genitalia have a resident and transient flora.

a. Resident flora - Generally non-pathogenic commensals (urinary microbiome) include Corynebacterium spp., CoNS, Lactobacillus spp., Streptococci, E. coli, Pseudomonas and Pasteurella spp. Mycoplasmas, etc.

b. Transient flora - These are typically agents of UTI and come from the intestine or skin. The most frequent pathogens in dogs include: Escherichia coli, Proteus mirabilis, Staph. intermedius group, Streptococcus and Enterococcus spp., Enterobacter spp., Pseudomonas aeruginosa, Streptococci (Lancefield group G), Mycoplasma’s such as M. canis.

In cats: E. coli, Enterococcus faecalis, Staphylococcus felis, Proteus spp., Enterobacter/Klebsiella spp., Pseudomonas aeruginosa etc.

In cattle: C. renale, C. cystitidis, C. pilosum, Arcanobacterium pyogenes.

In sows: Actinobaculum suis (previously Eubacterium suis).

In horses: Streptococci, E. coli, Staphylococcus aureus, Proteus spp., Klebsiella pneumoniae.
2. Routes of infection
   a. Hematogenous is less common. Notable exceptions include: *Actinobacillus equuli* in foals, *Leptospira* serovar infections.
   b. Extension from adjacent tissue
   c. **Ascending infection along urethra is the prime route**

3. Bacterial virulence factors:
   - colonization factors: adhesins (pili/fimbriae), flagella, biofilm formation prevent washout. Iron acquisition ensures growth.
   - host immune avoidance: capsule,
   - host tissue damage: cytotoxins, invasins, urease produces ammonia which irritates mucosal epithelium, increases urine pH and promotes crystalluria (struvite).

B. **HOST RISK FACTORS**

Clinical manifestation of uncomplicated UTI occurs more often in dogs than cats. Breeds including German Shepherd, Miniature Poodle, Retrievers, Dachshunds and Doberman pinschers have a greater tendency to develop UTIs. Risk factors in cats include genito-urinary tract surgical procedures, urinary incontinence. Additional risk factors not limited to dogs and cats include: urethral length, mating, obstruction (tumours, calculi), anatomical abnormalities, surgical procedures, catheterization, stasis (ie. pregnancy), diabetes (glucose), hyperadrenocorticism or chronic corticosteroid use and age.

In many cases there is no known predisposing factor. The consequences of infection in the bladder (cystitis) can include: bladder calculus formation (magnesium ammonium phosphate - struvite) and ascending infections (ureteritis, pyelonephritis).

C. **NORMAL DEFENSES OF URINARY TRACT**

1. Normal micturition (washout effect)
2. Normal anatomy
3. Intact mucosa - including normal epithelial exfoliation and glycosaminoglycan layer
4. Normal resident microbiome
5. Antibacterial compounds: antimicrobial peptides (small positively charged peptides produced by mucosal epithelium), IgA, Tamm-Horsfall mucoprotein (produced in kidney - prevents adhesion).
6. Antimicrobial properties of urine:
   - urea inhibits growth
   - hyperosmolality (Cat urine specific gravity is ~1.045, dog urine is ~1.028 )
   - pH < 6.5 inhibits bacteria; (cat pH: 6.4; dog pH: 6.8)

D. LABORATORY DIAGNOSIS
UTI refers to urethritis, cystitis, ureteritis, prostatitis, or pyelonephritis (kidney). Infections may be clinical (hematuria, pyuria, urination abnormalities) or subclinical (occult).

1. Urine collection
   a. Problem of urethral contamination.
   b. Mid-stream, free flow sample will always have contaminants (25-50% of animals will show > 10⁵/mL).
   c. Catheterized, often contaminated and may introduce infection.
   d. Cystocentesis (bladder tap) - preferred sample, safe procedure.
   e. Sample must be stored at 4 °C if not cultured within 1 hour of collection.

2. Gram or modified Wright-stain on urine sediment collected by cystocentesis.
   If done correctly this can be a particularly useful screen for dogs and cats and aid decisions to culture and initial antimicrobial therapy.
   Air dry one drop of urine sediment on microscope slide and stain.
   UTI if 1 bacteria per 10 x high-power oil fields (this is roughly equivalent to 10⁵ bacteria per mL of urine). The presence of WBC’s and/or RBC’s at > 5 per high power field (400x) is indicative of pyuria and/or hematuria, respectively.
3. **Culture** - requires specialized plating procedure.
   a. Quantitative urine culture: Inoculate BA and MacConkey agar with calibrated loops delivering 0.01 and 0.001 mL urine.
   b. Identification - use of these two media permits identification of common agents of UTI with little extra testing.

4. **pH of urine** - acidification suggestive of *E. coli* or *Enterococci/Streptococci*. Alkalinization suggestive of *Proteus spp.* or *Staphylococci*.

5. **Localization** - It is sometimes important to determine whether the kidney is involved. Methods are:
   a. Radiography
   b. Ultrasonography
   c. Bladder mucosal biopsy/microscopy/culture may be indicated in urine culture- negative, recurring cases and/or urolithiasis.

6. **Antibiotic susceptibility testing** - Results from disc diffusion tests (Kirby-Bauer) may be misleading when applied to UTI because of the capacity of the kidney to concentrate a significant number of antimicrobials.

   **Generally speaking, antibiotic choice requires a capacity to achieve 4-5 x the minimum inhibitory concentration (MIC) value in urine.**
<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Dose (mg/kg) / route / frequency</th>
<th>Mean Urine Concentration (µg/mL)</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td>Trimethoprim- Sulphonamide</td>
<td>30/PO/24</td>
<td>25/75</td>
<td>0.5</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>12.5/PO/12</td>
<td>300</td>
<td>8</td>
</tr>
<tr>
<td>Amoxicillin- Clavulanic acid</td>
<td>12.5/PO/12</td>
<td>200</td>
<td>8</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>30-40/PO/12</td>
<td>225</td>
<td>&gt;64 (R)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>16/PO/8</td>
<td>150</td>
<td>&gt;64 (R)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2-4/SC/24</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>25-50/PO/8</td>
<td>125</td>
<td>16</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>2.5-5.0/PO/12</td>
<td>40</td>
<td>0.25</td>
</tr>
</tbody>
</table>

1. Generally, an antibiotic can be considered if 4-5 x the MIC value is less than the Mean Urine Concentration Value. For example- Ampicillin would not be a good choice for Proteus.


E. LABORATORY EXERCISES

1. You are provided with a canine urine sample collected using cystocentesis. Inoculate BA plates using the quantitative plating technique (this will be demonstrated). One student will plate 10.0 µLs and the other 1.0 µL’s - label your plates. Do a Gram stain and examine under oil. Record your results.
2. You are provided with urine samples collected by cystocentesis from cats with a recurring history of inappropriate urination. Samples are labelled A, B or C. Using the quantitative plating technique plate 10.0 uL’s of urine on BA and MAC plates - label properly. Do a Gram stain and examine under oil. Record your results.

F. DEMONSTRATIONS

1. BA and MAC cultures from the kidney of a 4 day-old foal which died suddenly are provided. A Gram stain prepared from colony is provided. On postmortem examination the most striking finding was multiple microabscesses in the kidneys. Your presumptive identification:

2. Urine sample from a cow with frequent urination, pyuria and hematuria and decreased milk production. A Gram stain prepared from urine (Slide set 9-1) and a 48 hour BA culture and a biochemical test are provided. Your presumptive identification:

3. A silver-stained section of a kidney from a sow with leptospirosis. Examine for the presence of *Leptospira* and note their morphology and location.
A. LABORATORY EXERCISES

1. This is the canine UTI case you set up yesterday. Consult the Table "Interpretation of Quantitative Urine Culture Results in Dogs" below and determine if you have an infection. Does the colony appear to be primarily one type? Assess colony, microscopic morphology and Gram stain reaction. If this a Gram-positive cocci what test can you do to confirm genus? Demonstration 3 provides Tube Coagulase test results.

### Table: Interpretation of Quantitative Urine Culture Results in Dogs

<table>
<thead>
<tr>
<th>Method of Collection</th>
<th>Results indicative of infection*</th>
<th>Equivocal results</th>
<th>** Results indicate not infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystocentesis</td>
<td>&gt; 1,000</td>
<td>100 to 1,000</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>Catheterization</td>
<td>&gt; 10,000</td>
<td>1,000 to 10,000</td>
<td>&lt; 1,000</td>
</tr>
<tr>
<td>Voided</td>
<td>&gt; 100,000</td>
<td>10,000 to 100,000</td>
<td>&lt; 10,000</td>
</tr>
</tbody>
</table>

* Number of bacteria (CFU)/ml of urine - CFU = Colony Forming Units
** Normal flora or contaminants

BA
Counts
Microscope
Presumptive identification:
2. This is the cat case set up yesterday. Evaluate your plates as you did for Exercise 1. Did you have growth? If not why? Demonstrations 1 and 3 may help you with your identification.

BA  
MAC

Counts

Microscope

Presumptive identification:

B. DEMONSTRATIONS

1. Tentatively identify the 5 bacterial cultures on BA and MAC recovered from cases of UTI in dogs last week.

   (i)  
   (ii)  
   (iii)  
   (iv)  
   (v)  

2. Quantitative Urine Plate + Calculations.

3. Tube Coagulase tests for canine case (Exercise 1) and Gram-positive cocci from feline case (Exercise 2).
C. **QUESTIONS**

1. What are the common serotypes of *Leptospira* in this country? How is the infection transmitted? How is it commonly prevented? How are these organisms isolated?

2. Name an anaerobic urinary pathogen that specifically causes infection in pigs.

3. What is MIC?

   The MIC of tetracycline against *Staphylococcus aureus* (see Table in section D) is > 64 µg/mL.

   Is it a good drug to use for an *S. aureus* infection?

   What about ampicillin and *Proteus*?

4. Convenia™ (Cefovecin) is a newer injectable cephalosporin with a relatively broad spectrum of activity. It is unique in that a single dose can provide therapeutic levels in tissue or urine for 10-14 days (for susceptible organisms).

   Would this be a good “broad-spectrum” choice prior to information on pathogen and MIC?