

# Microbiology

— Lecture no. 2 —

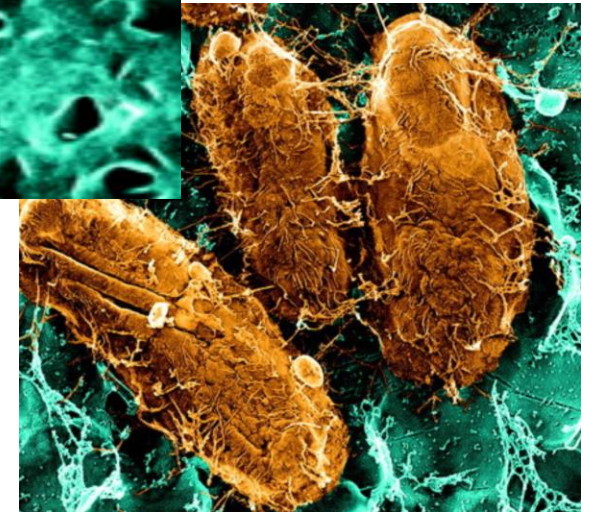
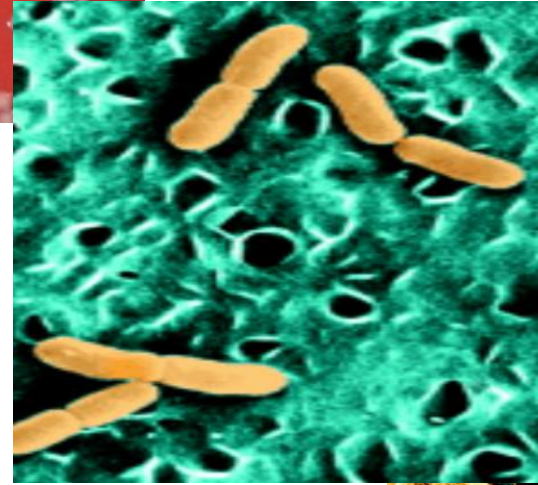
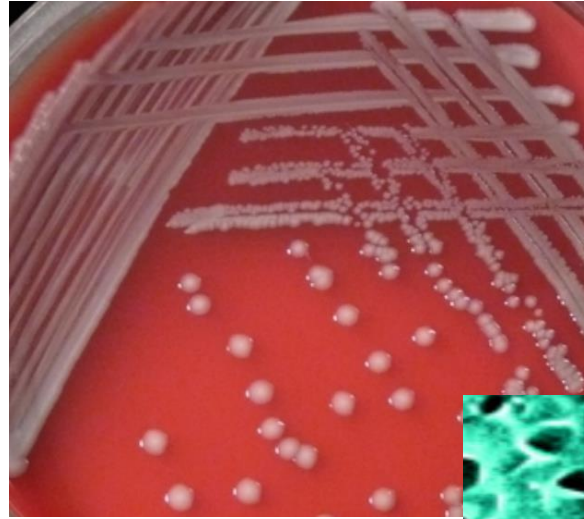
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# Microbiology of Urogenital system

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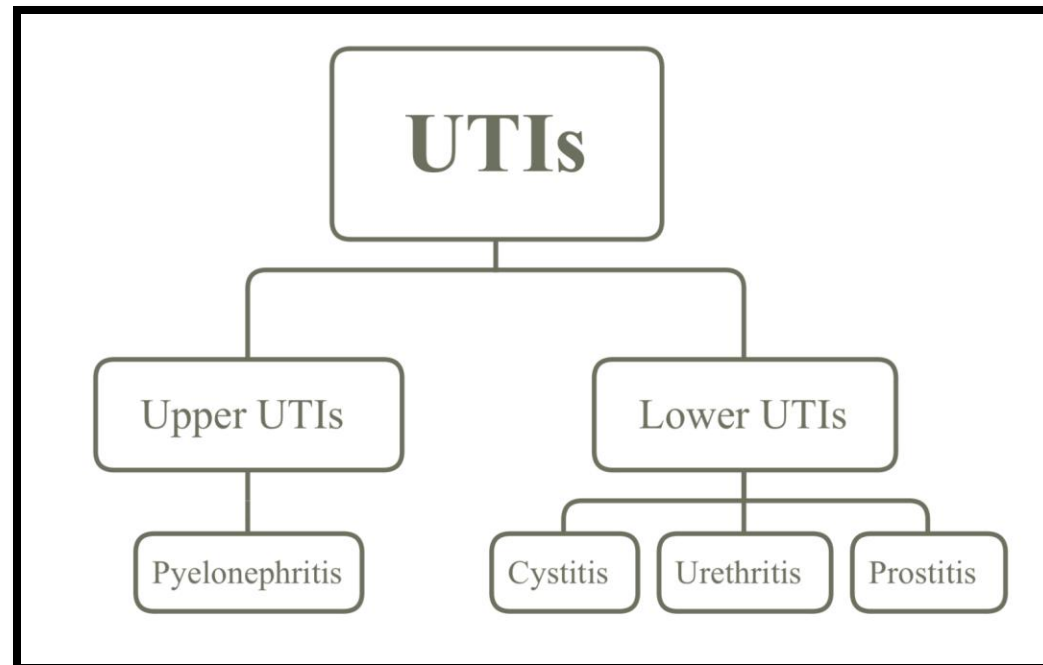
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How do UTI patients present?

The term urinary tract infection (UTI) encompasses a variety of clinical entities, including :

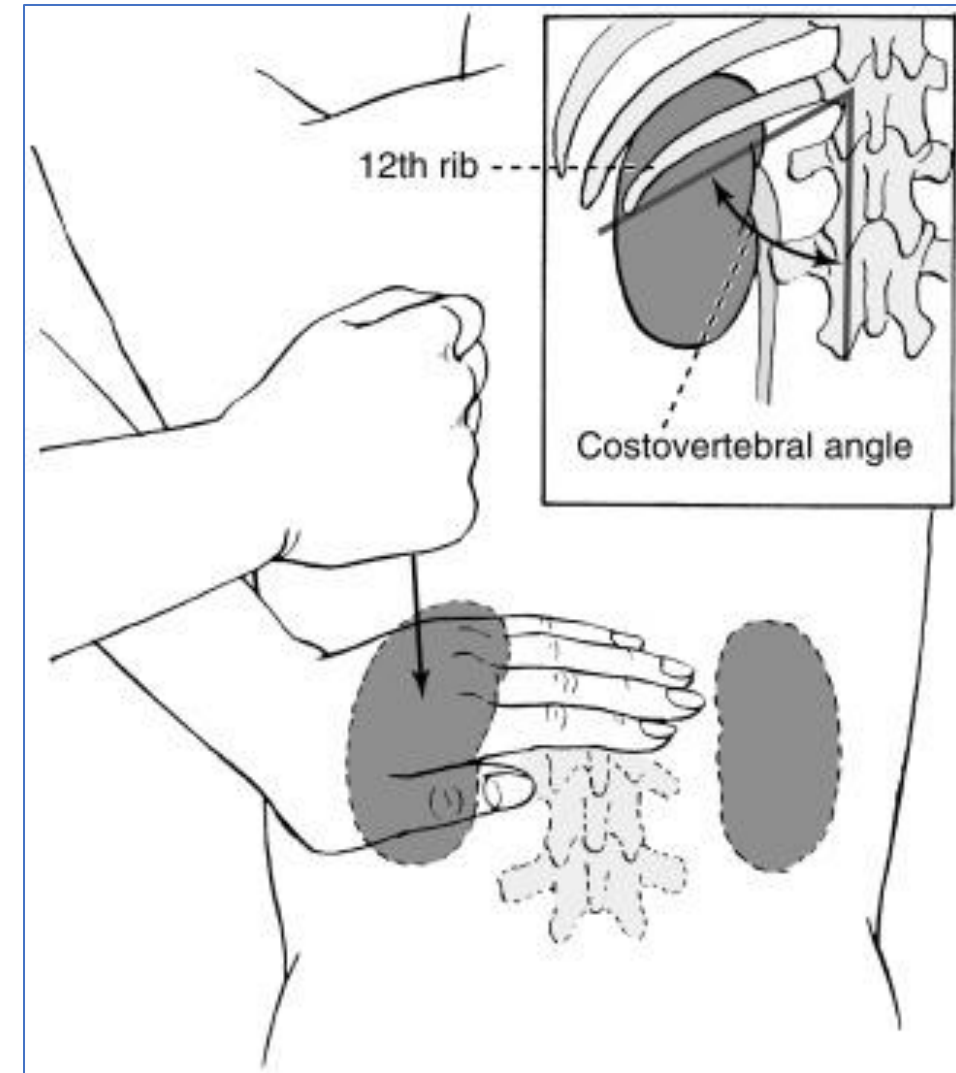
- **Asymptomatic bacteriuria (ASB)**
- **Cystitis**
- **Pyelonephritis.**
- **Prostatitis**

Before we start remember that UTI are more common in female.



## Cystitis and pyelonephritis

- The typical symptoms of **cystitis** are **dysuria**, urinary **frequency**, and **urgency**. **Nocturia**, **hesitancy**, suprapubic **discomfort**, and gross **hematuria** are often noted as well.
- **Mild pyelonephritis** can present as **low-grade fever** with or without lower-back or **costovertebral-angle pain**. Approximately where the kidney lies.
- **Severe pyelonephritis** can manifest as **high fever**, **rigors**, **nausea**, **vomiting**, and **flank and /or loin pain**.
- Constitutional symptoms, such as **fever**, are **mild or absent in cystitis**, but commonly **found in pyelonephritis**.



Murphy's percussion test is also known as costovertebral angle tenderness

# Explanation

Those typical symptoms are seen in all UTIs but mainly in cystitis:

- dysuria**: pain in urination.
- urinary frequency**: how many times you urinate.
- urgency**: feeling like you want to go to the bathroom.
- nocturnal**: waking up during night just to go to the bathroom.
- hesitancy**: the person feels that he would like to void but nothing goes out. (From the internet: Difficulty starting or maintaining a urine stream is called urinary hesitancy).

In severe cases hematuria may occur (which is blood in urine).

Sometimes pyelonephritis starts from cystitis then the bacteria ascends through the ureters to the kidney causing infection.

Pyelonephritis is a systemic inflammation with more inflammatory cytokines released causing systemic manifestations like fever, chills and rigors, in addition to the typical symptoms depending on the stage of the inflammation mild, moderate, or severe.

# Continuation

In mild cases:

-Low-grade fever.

-Tenderness during percussion of costovertebral angle shows inflammation of the kidney.

In severe cases:

Flank /Loin pain is pain in the costovertebral area.

Pyelonephritis can be a serious complication as the infection may progress to sepsis.

The constitutional symptoms are the main difference between upper and lower urinary tract infections as they **don't** appear in lower UTIs.

Remember that lower UTI are more common than upper.

# Cystitis and pyelonephritis

## Clinical Features and Virulence Mechanisms in Cystitis and Pyelonephritis

The causative organisms and the virulence factors are the same for both except for the presence of flagella that is important for the movement of the bacteria.

### Cystitis

**Risk factors**

- Female sex, history of UTI
- Sexual activity
- Vaginal infection
- Diabetes, obesity, genetic susceptibility

**Clinical symptoms**

- Frequent and urgent urination
- Dysuria, suprapubic pain
- Nocturia, hematuria, malaise

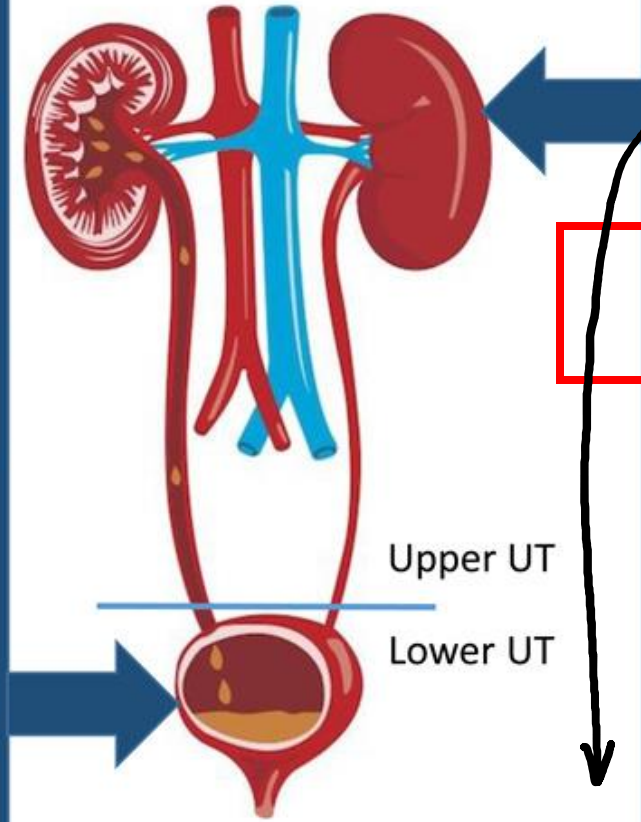
**Causative organisms**

- UPEC
- Klebsiella pneumoniae*
- Staphylococcus saprophyticus*
- Enterococcus faecalis*
- Others

**Selected UPEC virulence factors**

- Adhesins (Type 1 & other chaperone-usher pili)
- Toxins (HlyA, CNF1)
- Siderophores (aerobactin, enterobactin, yersiniabactin)
- Capsule

Pyelonephritis has more severe risk factors.



Vesico-uretral reflux (found only in males) in which the urine goes back to from the bladder to the kidney through the ureters so increases the chance for the pathogen to reach the kidney.

### Pyelonephritis

**Risk factors**

- Diabetes
- HIV/AIDS
- Iatrogenic immunosuppression,
- Congenital or acquired urodynamic abnormalities

**Clinical symptoms**

- Back and/or flank pain
- Fever, chills, malaise
- Nausea, vomiting, anorexia

**Causative organisms**

- UPEC
- Klebsiella pneumoniae*
- Staphylococcus aureus*
- Enterococcus faecalis*
- Proteus* spp
- Others

**Selected UPEC virulence factors**

- Adhesins (Type 1 & P pili)
- Toxins (HlyA, CNF1)
- Siderophores (aerobactin, Iha, TonB siderophore receptor)
- Flagella

## UTI diagnosis – History and self diagnosis

- The diagnosis of any of the UTI syndromes or ASB begins with a **detailed history**. (E.g. in women presenting with at least one symptom of UTI ( dysuria, frequency, hematuria, or back pain) and without complicating factors, the probability of acute cystitis or pyelonephritis **is 50%** ).
- If vaginal discharge and complicating factors are absent and risk factors for UTI are present, then the probability of UTI is close to **90%**, and no laboratory evaluation is needed before initiation of therapy. (We prescribe the appropriate antibiotics)
- One significant concern is that **sexually transmitted disease**—that caused by Chlamydia trachomatis in particular—may be inappropriately treated as UTI.

Complicating factors: immunosuppression, antibiotic exposure, catheters, congenital abnormalities, even DM.



## UTI diagnosis – dipstick and urinalysis

If there were complicating factors or you aren't sure then further work up is done for the patient.

- **dipstick and urinalysis** provide point-of-care information. point of care: no need for specialized lab and easy to perform

Some points to consider:

- Only members of the **family Enterobacteriaceae** convert nitrate to nitrite.
- When **voiding frequently**, the dipstick test or nitrite is less likely to be positive.
- The **leukocyte esterase test** detects this enzyme in the host's polymorphonuclear leukocytes in the urine.
- A **negative dipstick test** is not sufficiently sensitive to rule out bacteriuria in pregnant women, in whom it is important to detect all episodes of bacteriuria.

**Extra:** Point of care test for covid is antigen test not the PCR.

كل الجدول مهم Table 1. Urinalysis Results

Test	Usual Range	Indicators of Infection	Accuracy
Bacteria	Absent	Any amount	Low sensitivity, <sup>a</sup> high specificity <sup>b</sup>
Leukocyte esterase	Absent	Positive = pyuria, presence of WBCs in urine	High sensitivity, low specificity
WBC	<5	Pyuria: WBC >10	High sensitivity, low specificity
Nitrite	Absent	Positive = presence of bacteria that reduce nitrate	Low sensitivity, high specificity
RBC	<5	Hematuria common in infection	Low sensitivity, high specificity
Epithelial cells	<5	<5 = good urine sample	High epithelial cells indicate contamination with skin flora
pH	4.5-8	pH ↑ if urea-splitting organism (e.g., <i>Proteus mirabilis</i> ) is present	Low specificity (there are many other causes of alkaline urine)

<sup>a</sup> Sensitivity = likelihood of positive test when disease is present.  
<sup>b</sup> Specificity = likelihood of negative test when disease is not present.  
Source: Reference 1.



The dipstick test procedure:

You dip a stick in a tube with urine and the color of the brackets in of the stick will change according to the constitution of the urine, some are for pH, nitrites and so on.

**-what can we find in urine?**

1- Polymorphonuclear cells.

2- **bacteria**, so we can do gram stain looking for gram positive or gram negative organisms.

3- **leukocyte esterase enzyme** —> high sensitivity, low specificity, indicating for inflammation.

4- **number of WBCs** should be <5 but in pyuria (presence of WBCs in urine) more than 10 —> high sensitivity for inflammation, low specificity for bacterial infection.

5- **nitrite** is presented because Enterobacteriaceae produce enzyme that convert nitrate to nitrite —> low sensitivity, high specificity for infection.

6- **RBCs** may be in the form of gross hematuria or microscopic hematuria.

7- **PH** increases because bacteria breakdown urea into ammonia that increases the pH. (Normally the urine is acidic).

In more than 90% of UTI cases we find positive leukocyte esterase and nitrite test.

Helpful video (start from 1:45 min) [https://youtu.be/udqv-3og9\\_k](https://youtu.be/udqv-3og9_k)

## UTI diagnosis – Urine culture

After confirming an organism using the previous tests we do culture to know the pathogen and antibiotic susceptibility.

- The detection of bacteria in a **urine culture** is the diagnostic “**gold standard**” for UTI.
- Colony formation needs around 24h in E.coli, but to know the antibiotic susceptibility we need to grow the colonies with antibiotic susceptibility test for another 24h.
- Studies on women with symptoms of cystitis have found that a **colony count threshold of  $>10^2$  bacteria (colony forming unit CFU more specifically) /mL** is more sensitive (95%) and specific (85%) than a **threshold of  $10^5$ /mL** for the diagnosis of acute cystitis in women.
- Urine specimens frequently become **contaminated** with the normal microbial flora of the distal urethra, vagina, or skin. Thus using Midstream Clean Catch Specimen or suprapubic aspiration might be needed.

Helpful video by the doc

<https://youtu.be/KR9yyaZSp-c>

culture **results** do not become available **until 24 h** after the patient’s presentation. Identifying specific organism(s) can require an **additional 24 h**



# Question

- **How can we decrease contamination while collecting urine????**
  1. we can ask the patient to **clean the tip of the urethra or the vagina.**
  2. **midstream clean catch specimen:** don't start filling the urine cup at the beginning of urination to avoid contamination with skin microbiota found on the tip of the urethra as *S.saprophyticus* or *S.epidermidis*. So patients start collecting the urine sample after a couple of seconds.
  3. if contamination still occurs we do **suprapubic aspiration:** by inserting a needle suprapublically into the bladder and aspirate urine directly from the bladder.
  4. in hospitalized patients and after surgeries we can collect urine through the **catheters** (but with high chance of contamination).

## UTI treatment

- Antimicrobial therapy is warranted for **any symptomatic UTI**.
- The choice of antimicrobial agent and the onset and duration of therapy depend on **the site of infection** **due to antimicrobial resistance** and the presence or absence of **complicating conditions**.
- Antimicrobial resistance among uropathogens **varies from region to region** and impacts the approach to empirical treatment of UTI.

**Table 1. Antibiotics for non-pregnant women aged 16 years and over**

Antibiotic <sup>1</sup>	Dosage and course length <sup>2</sup>
<b>First choice<sup>3</sup></b>	
Nitrofurantoin – if eGFR ≥45 ml/minute	50 mg four times a day or 100 mg modified-release twice a day for 3 days
Trimethoprim – if low risk of resistance and not used in the past 3 months	200 mg twice a day for 3 days
<b>Second choice (no improvement in lower UTI symptoms on first choice taken for at least 48 hours, or when first choice not suitable)<sup>3,4</sup></b>	
Nitrofurantoin – if eGFR ≥45 ml/minute and not used as first choice	50 mg four times as day or 100 mg modified-release twice a day for 3 days
Pivmecillinam	400 mg initial dose, then 200 mg three times a day for a total of 3 days
Fosfomycin	3 g single dose sachet

## UTI treatment

- Antimicrobial therapy is warranted for **any symptomatic UTI**.
- The choice of antimicrobial agent and the onset and duration of therapy depend on **the site of infection** and the presence or absence of **complicating conditions**.
- Antimicrobial resistance among uropathogens **varies from region to region** and impacts the approach to empirical treatment of UTI.

TABLE 33-1

### TREATMENT STRATEGIES FOR ACUTE UNCOMPLICATED CYSTITIS

DRUG AND DOSE	ESTIMATED CLINICAL EFFICACY, %	ESTIMATED BACTERIAL EFFICACY, <sup>a</sup> %	COMMON SIDE EFFECTS
Nitrofurantoin, 100 mg bid × 5–7 d	84–95	86–92	Nausea, headache
TMP-SMX, 1 DS tablet bid × 3 d	90–100	91–100	Rash, urticaria, nausea, vomiting, hematologic abnormalities
Fosfomicin, 3-g single-dose sachet	70–91	78–83	Diarrhea, nausea, headache
Pivmecillinam, 400 mg bid × 3–7 d	55–82	74–84	Nausea, vomiting, diarrhea
Fluoroquinolones, dose varies by agent; 3-d regimen	85–95	81–98	Nausea, vomiting, diarrhea, headache, drowsiness, insomnia
β-Lactams, dose varies by agent; 5- to 7-d regimen	79–98	74–98	Diarrhea, nausea, vomiting, rash, urticaria

# Urinary Tract Infection in South Jordanian Population

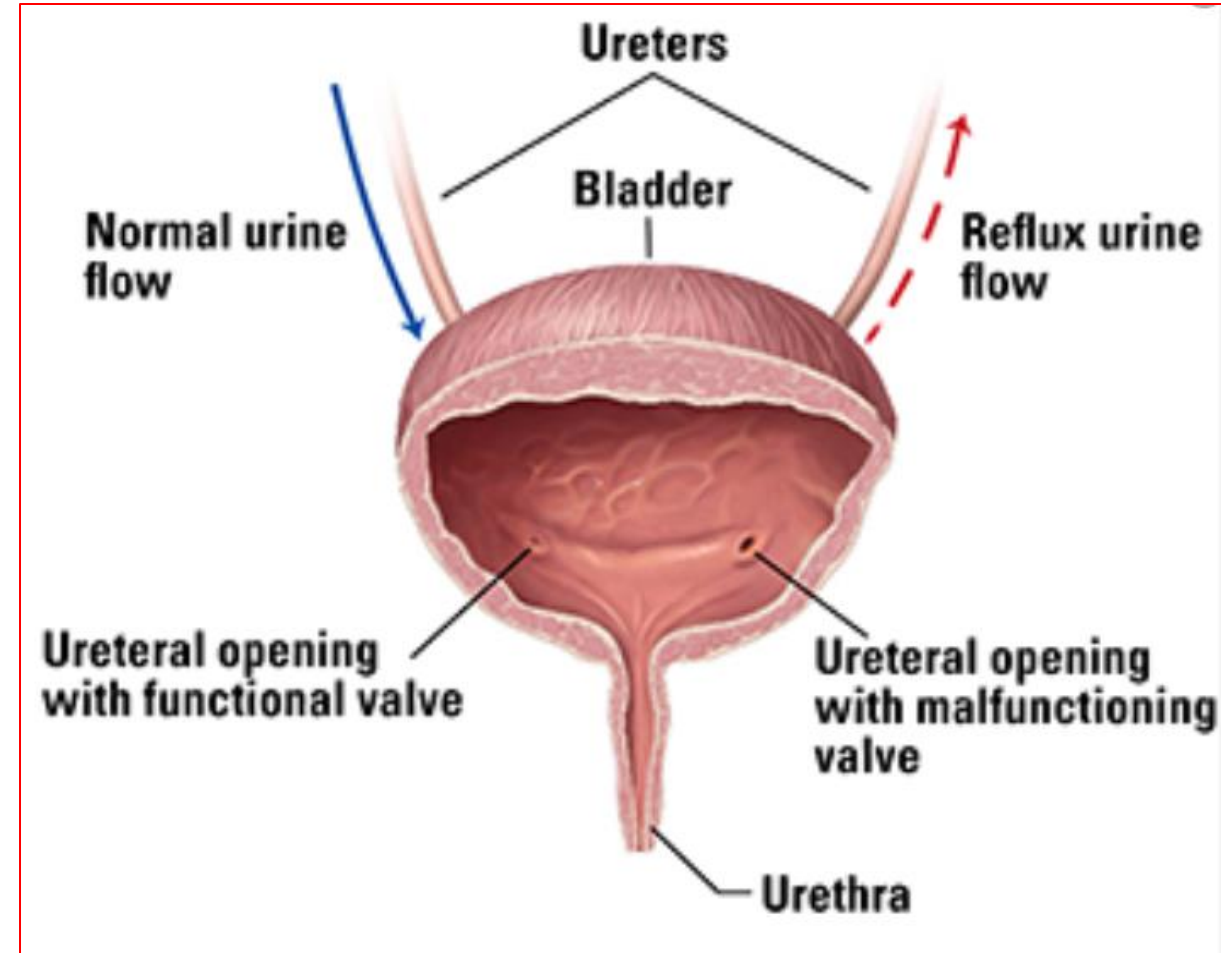
Khaled M. Khleifat , Muayad M. Abboud , Sharaf S. Omar and Jafar H. Al-Kurishy

The result of different examination test suggest that the *Escherichia coli* is responsible for the large proportion of infection (53.24%), followed by other strains like *Enterococcus faecalis* (24.05%), *Proteus* sp. (19.537%), *Staphylococcus aureus* (19.206%), *Staphylococcus epidermidis* (7.8%), *Staphylococcus saprophyticus* (13.2%), *Klebsiella* sp. (11.96%), *Enterobacter* sp. (5.128%), *Pseudomonas aeruginosa* (3.4%), *Citrobacter* sp. 1.92% and *Serratia marcescens* (0.8%).

For the treatment of urinary tract infection the antibiotic Ciprofloxacin (5 µg disk-1) was found to be most effective antimicrobial agents against all isolated bacteria strains, while Oxacillin (1 µg disk-1) was found to be the least effective.

The UTIs have good prognosis and the symptoms start to disappear after 48 hours of using antibiotics and the whole course is only 3 days.

- A single episode of acute pyelonephritis in an adult woman can lead to **renal scarring** that will affect renal function and may end with renal failure.
- Pyelonephritis becomes potentially fatal when secondary conditions develop such as **emphysematous pyelonephritis** (20– 80% mortality rate), **perinephric abscess** (20– 50% mortality rate), or **sepsis**.
- **Chronic pyelonephritis** might develop following acute pyelonephritis in childhood in the context of (**vesico- ureteric reflux**) VUR. They are more susceptible for recurrence of the infection.



UTI patients usually don't need to be admitted to the hospital except for pyelonephritis patients with a lot of systemic manifestations.

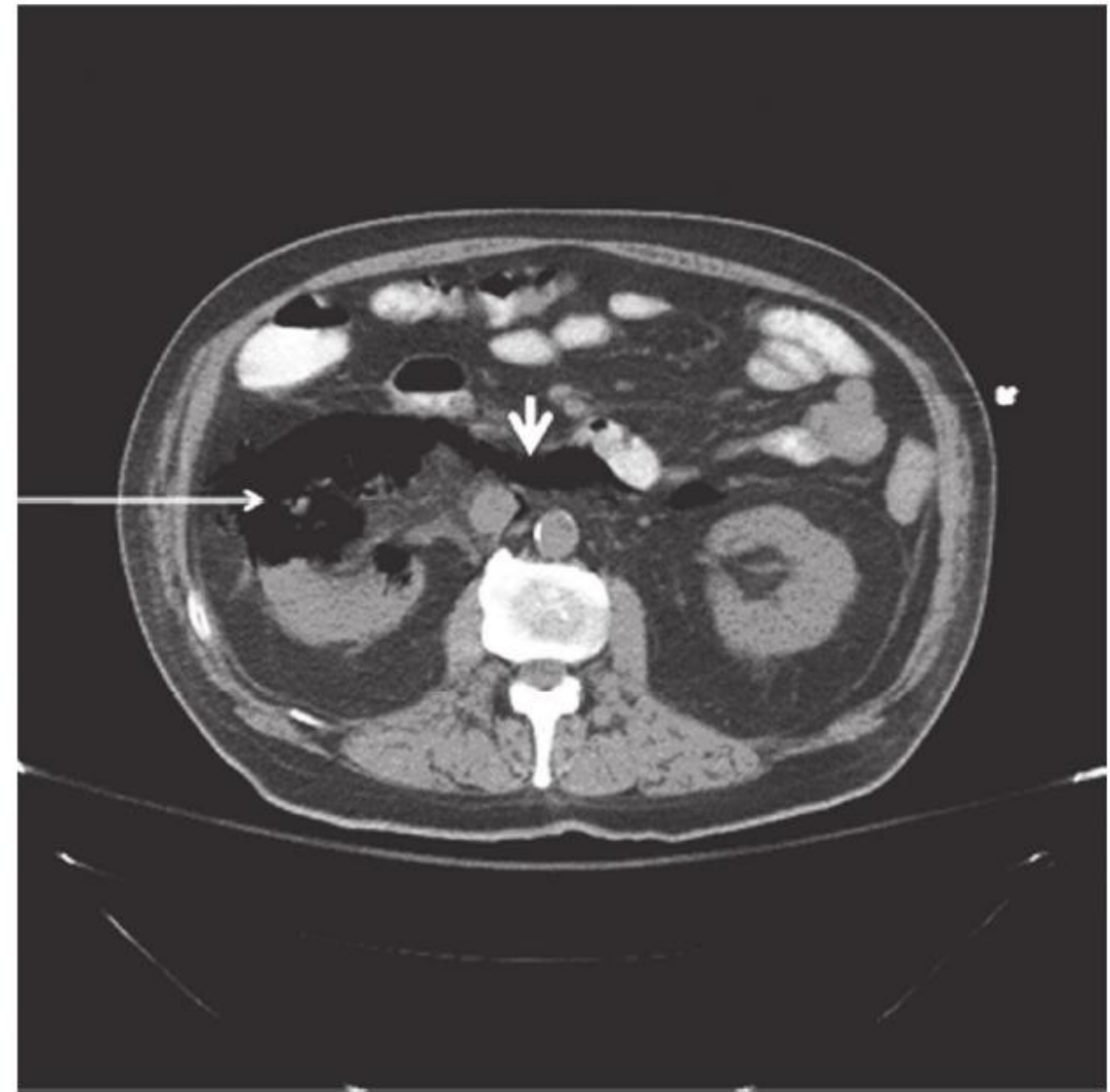


## Complications of pyelonephritis

- **Emphysematous pyelonephritis:** A severe, necrotizing, acute, multifocal bacterial nephritis, with extension of the infection through the renal capsule. Gas is found in the renal substance and perinephric space. Often happens in diabetics.

Remember: The patients will have a UTI symptoms in addition to systemic signs and symptoms such as fever, chills and rigors.

- Diagnosis is confirmed by **CT scan**.
- Treatment involves **antibiotics, drainage, nephrectomy**.
- Mortality is high (around 60%)



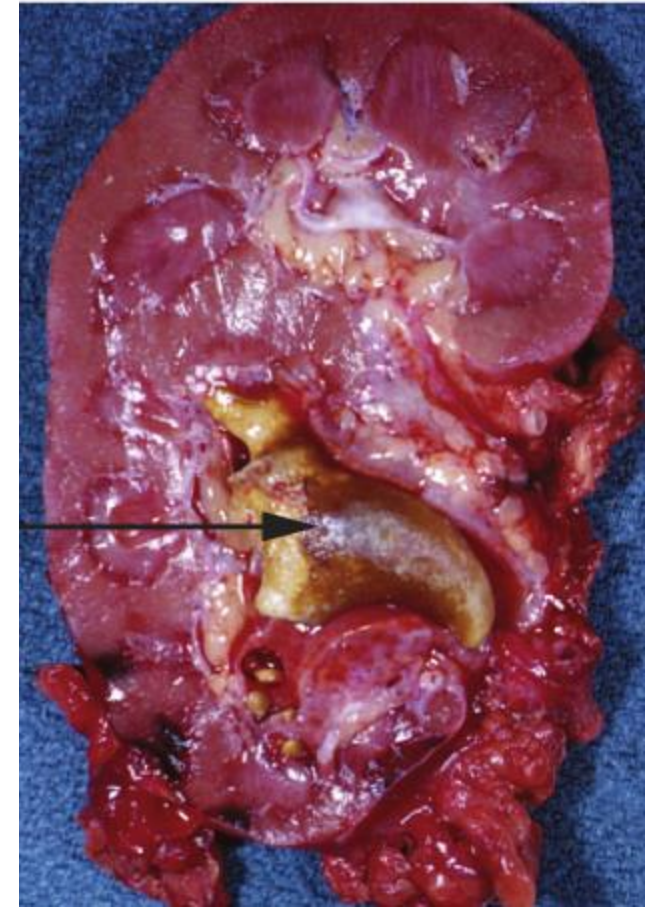
**FIGURE 33-2**

Emphysematous pyelonephritis. Infection of the right kidney of a diabetic man by *Escherichia coli*, a gas-forming, facultative anaerobic uropathogen, has led to destruction of the renal parenchyma (arrow) and tracking of gas through the retroperitoneal space (arrowhead).

## Complications of pyelonephritis

- **Xanthogranulomatous pyelonephritis**, A rare, serious, debilitating illness characterized by a chronic inflammatory mass originating in the renal parenchyma.
- Gross appearance: mass of yellow tissue composed of **lipid-laden macrophages** and inflammatory cells regional **necrosis**, and **haemorrhage**.
- Often associated with infection by *Proteus* [mainly] ,
- *E. coli*, or *Pseudomonas* spp. in the context of chronic obstruction.

Some pathogens change the pH leading to formation of stones and these stones may be large enough to obstruct the renal pelvis → this will exert pressure on the nearby blood vessels → necrosis and death of the nearby structures.



A large staghorn calculus (arrow) is seen obstructing the renal pelvis and calyceal system. The lower pole of the kidney shows areas of hemorrhage and necrosis with collapse of cortical areas.

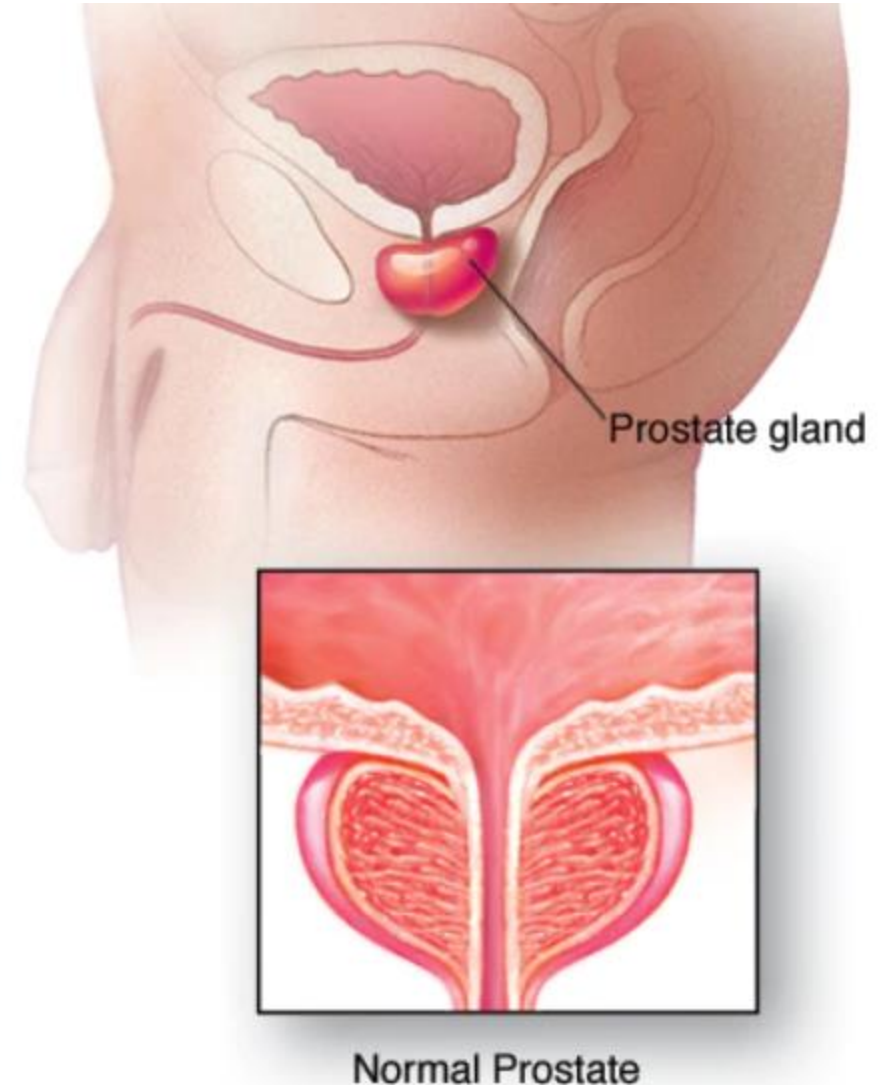
Need surgery to be removed

## Prostatitis (the underlined sentence is newly added to the file)

- Up to 50% of men will experience symptoms of prostatitis at some time in their lives. However, actual bacterial infections of the people account for a minority of these cases.

Most of the time it is not infectious. Mostly it is caused by other factors such as drugs, autoimmune diseases or malignancies.

- Prostatitis includes both infectious and non-infectious abnormalities of the prostate gland.
- **Acute bacterial prostatitis** presents as **dysuria, frequency, and pain** in the prostatic pelvic or perineal area. Fever and chills are usually present, and symptoms of bladder outlet obstruction are common. **Usually they are superimposed on an infection in the bladder or the urethra, so there is a predisposing condition.**
- Chronic/ recurrent bacterial prostatitis occurs in young and middle-aged men. Risk factors include **previous acute prostatitis**, history of prior **manipulation** of the urinary tract, diabetes, smoking.

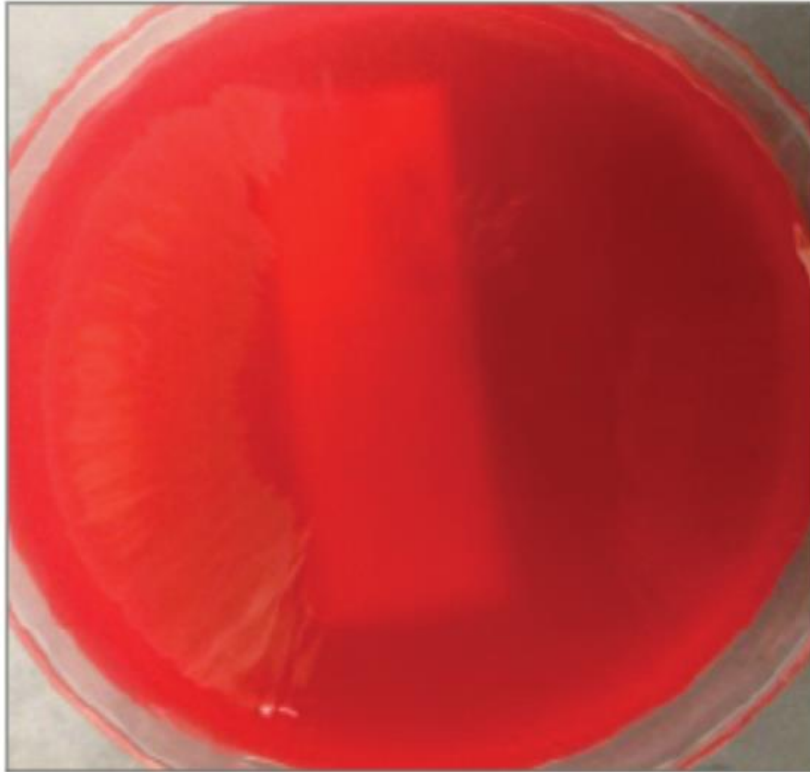


## Bacterial prostatitis/management **(this slide is newly added to the file)**

- The pathogens associated with acute prostatitis **reflect the spectrum of organisms causing cystitis**, urethritis, and deeper genital tract infections (such as epididymitis). Thus, gram-negative infections, especially with **Enterobacterales**, are the most common.
- The presence of typical symptoms of prostatitis should prompt digital rectal exam, and the finding of an **edematous and tender prostate on physical exam** in this setting usually establishes the diagnosis of acute bacterial prostatitis.
- In order to establish the microbial etiology, a urine Gram stain and culture should be obtained in all men suspected of having acute prostatitis. Gram stain of the urine, if positive, can be used as a guide to initial therapy.
- Treatment of acute prostatitis includes antimicrobial therapy and supportive measures to reduce symptoms (**same as UTIs treatment**). Rarely, more invasive intervention is indicated to manage complications.

## This urine is not sterile

### Standard urine culture



**Blood agar, 1  $\mu$ L, 24 hours, aerobic**

### Enhanced quantitative urine culture



**Blood agar, 100  $\mu$ L, 48 hours, 5% CO<sub>2</sub>**

Adapted from June 9, 2016 ASM Microbe presentation by Alan J. Wolfe, PhD: "Urine Is Not Sterile: Why We Should Care."

"I thought, like everybody thought, that urine was sterile except when there's an infection," he says. The Loyola team tried more than two dozen different combinations of atmospheric conditions, media, incubation times, and urine volumes before arriving at the streamlined EQUC. The process of studying each of these and seeing under which conditions the uropathogens grew best took about a year.

**The Clinical Urine Culture: Enhanced Techniques Improve Detection of Clinically Relevant Microorganisms**

Journal of clinical microbiology 2016

## Is urine sterile?

- Recent data suggest that the normal, healthy bladder is not always sterile, and a picture of the urinary microbiome is emerging.
- This urine sample was obtained by transurethral catheter from a woman:

**a** | 1  $\mu$ l urine was spread on a blood agar plate and incubated for 24 h at 35°C. This technique is part of the standard urine culture protocol.

**b** | 100  $\mu$ l urine from the same patient was spread on a blood agar plate and incubated at 35 °C for 48h with 5% CO<sub>2</sub>.

Increased growth of bacterial colonies can be seen, demonstrating that the urine sample was not sterile, despite being obtained directly via a transurethral catheter.

- The previous results suggested that maybe we don't have the proper techniques to retrieve normal flora of the urine. Further investigation is required to establish a full profile of the urinary microbiota.

## Further reading:

- Oxford handbook of infectious diseases and microbiology-  
Part4: Clinical syndromes  
Chapter 17 Urinary tract infections
- Harrison's Infectious Diseases 3rd Edition  
SECTION III Infections in organ systems  
Chapter 33