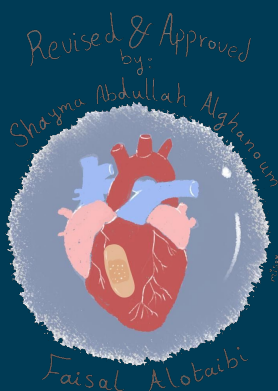


# OSPE

Renal- Practical

TEAM 439

**MICROBIOLOGY**



# Objectives

- ❖ Describe the different acceptable specimen types used for the diagnosis of cystitis.
- ❖ Discuss the laboratory work up of urine specimens for analysis and culture.
- ❖ Discuss important microbiological features of common causes of cystitis.
- ❖ Interpret urine analysis and culture results.
- ❖ List non bacterial causes of urinary tract infections

## Colour index:

PURPLE BORDERS : GIRLS

GREEN BORDERS : BOYS

Any future corrections will be in the editing file, so please check it

frequently.

Scan the code  
Or click [here](#)



**Important aspects of microbiological examination of UTI**

- Urine collection
- Urine analysis
- Interpretation of microbiology laboratory results

**Types of specimen**

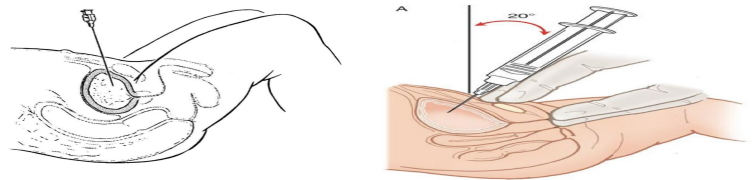
**Midstream urine (MSU)**

Should instruct the patient to collect the middle part of urine and discard the first 10ml of urine because it's contaminated by urine contaminants and the perineum colonizing flora.



**Suprapubic aspiration**

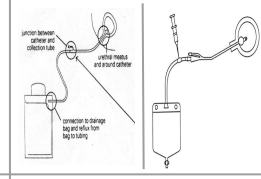
Usually done during the child/neonate period where they can't comply well in collection



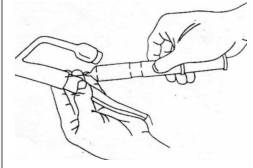
**Catheter sample**

Not recommended much but sometimes it's needed, for example if the patient has an obstructed neuropathy and can't pass urine normally.

-Urine specimens for laboratory investigations can be collected from catheterized patients



-The second port is for putting fluids into the bladder



-Urine from the drainage bag should not be tested because it may have been standing for several hours.

When collecting specimen from catheter it's better to collect urine from the tube rather than the bag itself because the bag usually contains colonizing flora or contamination.

**Sterile urine container**

Most commonly used which must be handled carefully and should be sent to the lab in a short time and processed by the technologist immediately because urine is a good media for bacteria to grow and we are also concerned from the contamination during collection and from the perineum even if it's very minimum it might give false result because we do quantitation of the urine specimen so the more time we leave the urine the more time we are giving the bacteria to grow and produce in significant numbers.



**Transport Media**

**Dipslide**

One slide is CLED media, the other can be MacConkey (MAC) agar or blood agar.  
2 different types of culture media

Not commonly used but it helps in avoiding contamination and it processes urine fast in the lab.



## Urine analysis

### One of the very important diagnostic tools used

How to differentiate between lower urinary tract infection (cystitis) and upper urinary tract infection (pyelonephritis) through urine analysis?  
By trying to find the cast

-Important function: is seeing some parasites like schistosoma egg which is diagnostic of schistosomiasis

-Schistosoma haematobium causes the bilharziasis, it's an organism that affect the bladder.

## Dip stick

-A strip which contains different type of analytical parameters.

-Most commonly used test to diagnose UTI

-Advantages: fast, reliable, used in emergency and sometimes in addition to clinical suspicion it's enough to diagnose and give proper medication and send the patient home without the need of a culture.

-Disadvantage: not very specific  
Ex: some bacteria don't produce positive nitrate test and some UTI are leukocyte esterase negative  
So you can't use it to rule out the infection

-**Leukocyte esterase** (WBC) the most important parameter which reflects the presence of pyuria and **indicates urinary tract infection**

-Pyuria is an important indicator of urinary tract infections.

-Pyuria is a urinary condition that is characterized by an elevated number of white blood cells in the urine.

-In addition we can find glucose, ketones, RBC and proteins which is important to tell us whether the patient has an **underlying urinary tract infection** or not.

-**Nitrate test** important **indication of presence of the bacteria** but it doesn't indicate it always, because some of the bacteria doesn't have the enzyme that converts nitrite into nitrate.

To know how to use a urine dski test please [click here](#)



## Microscopic

-Fast

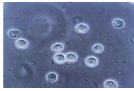
-Can see: WBC, RBC, cast, parasites (ex:schistosoma egg), bacteria without gram staining, candida

## Ex: cell-counting chamber

It is done in addition to the dip stick to look for the cellular elements including WBC and we start counting it. If we found more than 10 WBC per high power field then this is significant and we can say the patient has pyuria.

Might also help in looking for other parameters like RBC which signifies infection because hematuria is one of the diagnostic parameters that tells if the patient has UTI.

Bacteria can be seen using a microscope without gram staining.



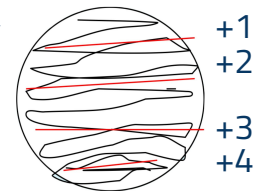
## Laboratory Examination of Urine:

**Culture is the gold standard** to diagnose urinary tract infection, identify the pathogen and to do sensitivity.

But it's not done for all specimens because it takes time (18-24 hours to grow the pathogen) which causes a delay in the diagnosis so we do it quantitatively

A urine sample is streaked on surface of Blood Agar plate and CLED agar / MacConkey agar with a special loop calibrated to deliver a known volume

For a video on the on how to streak please [click here](#)



Overnight incubation

Isolation of colonies, biochemical tests, **drug susceptibility test**

Drug susceptibility test is an Important test because:

-Not all cases of UTI is straightforward uncomplicated

-To put the patient on proper medication when it's complicated UTI

-Increasing prevalence of MRO (Multi-Resistant Organisms) many of the gram -ve including E.coli is resistant to almost most of the commonly used antibiotic and produce B-lactamase which are enzymes that destroys B-lactam agents from the penicillin or the cephalosporin group

Overnight incubation

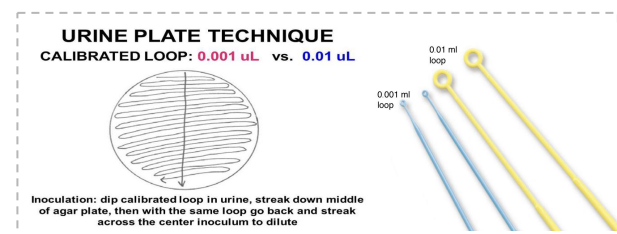
Result

**Quantitative urine culture**

Using 0.001/ml loop

1 colony = 1000 CFU/ml

100 colonies = 100,000 CFU/ml



-Quantitatively: use a specific loop which is calibrated (takes 0.001ml of the urine) and dip it in the urine specimen to collect a measured amount of the urine and then streak it on a specific culture media (usually it's differential or selective type of media) in different direction to get individual colonies that enables the technician to count the colonies if there is more than  $10^5$  signifies an infection and represent a significant growth then we identify it but low count might not signifies infection

-Selective: allows growth primarily of gram negative

-Agars used: CLED or MacConkey (mostly identifies gram -ve) in addition to blood agar (identifies gram +ve) because CLED doesn't grow all gram +ve

E.coli, enterococcus and group B are well known colonial normal flora which cause UTI through ascending infection.


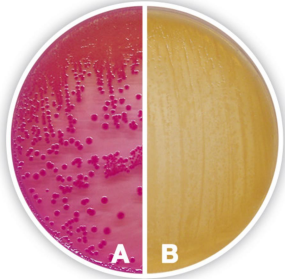
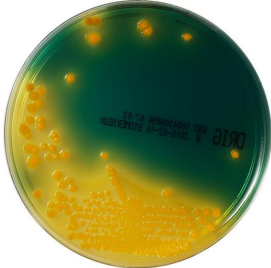
<p><b>Gram Negative</b></p> <p>Most common cause of urinary tract infection is gram negative particularly E.coli</p>	<p><b>Gram Positive</b></p>	<p><b>Other Organisms</b></p>
<p><b>Escherichia coli</b></p> <p>80-90% of the etiology of UTI</p> <p>-Most common cause of UTI and contamination which can give a false positive result</p> <p>-To differentiate between a contamination and a significant culture of E.coli we do quantitation</p> <p>-Exist in the colon as normal flora Goes outside the body colonizes the perineum then the urethra then ascends as ascending infection and causes UTI</p>	<p><b>Enterococcus</b></p> <p>Most common of gram +ve Exist in the colon as normal flora</p>	<p><b>Candida</b></p> <p>-Yeast fungal infection.</p> <p>-Very common in patients who are <b>catheterized</b>, have underlying chronic illnesses, on broad spectrum antibiotic, diabetic patients, pregnant woman.</p>
<p><b>Klebsiella</b></p>	<p><b>Staphylococcus saprophyticus</b></p> <p>We don't know but sometimes it's an animal origin</p>	
<p><b>Proteus</b></p>	<p><b>Streptococcus agalactiae (group B)</b></p> <p>-Normal flora in the colon</p> <p>-Cause of infection in pregnant women and diabetics</p>	<p><b>Schistosoma haematobium</b></p> <p>-A parasite</p> <p>-Causes schistosomiasis or bilharziasis of the bladder which is an endemic disease found in certain countries where people like to swim in contaminated water where this parasite invades the skin and passes to the bladder where it causes bladder infection.</p> <p>-It's problem that it causes chronic infection and might cause bladder cancer.</p> <p>- We diagnose it by seeing the schistosoma egg through microscope</p>
<p><b>Other Enterobacteriaceae (Enterobacter, Citrobacter...)</b></p> <p>Most are in the colon</p>	<p><b>Staphylococcus aureus (Associated with staphylococemia)</b></p> <p>Not a colonic flora and causes UTI through hematogenous spread (bacteremia)</p>	
<p><b>Pseudomonas aeruginosa</b></p> <p>-Hospital acquired UTI (Nosocomial infection)</p> <p>-Difficult pathogen most of the time, it's multi resistant to almost all antibiotic and very difficult to treat and it is treated by specific antipseudomonal agent.</p> <p>-If you found it in the urine think of complicated</p> <p>-Not a normal flora</p>		

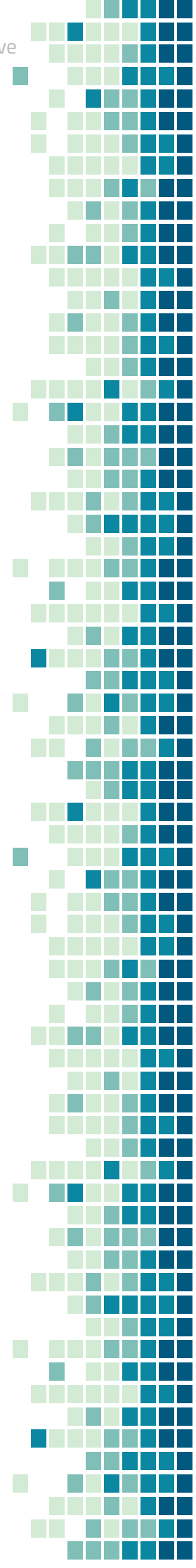
Numbers are not important just know what are the most common organisms in both gram +ve and gram -ve

Causes of UTIs	Outpatients %	Inpatients %
<b>Escherichia coli</b> (Most common Gram -ve)	53-72	18-57
<b>Coagulase -ve Staphylococcus</b> (Gram +ve)	2-8	2-13
<b>Klebsiella</b> (Gram -ve)	6-12	6-15
<b>Proteus</b> (Gram -ve)	4-6	4-8
<b>Morganella</b> (Gram -ve)	3-4	5-6
<b>Enterococcus</b> (Gram +ve)	2-12	7-16
<b>Staphylococcus aureus</b> (Most common Gram +ve)	2	2-4
<b>Staphylococcus saprophyticus</b> (Gram +ve)	0-2	0-4
<b>Pseudomonas</b> (Gram -ve)	0-4	1-11
<b>Candida</b> (Fungi)	3-8	2-26

Note: both of E-coli & Enterococcus exist in colon as a normal flora, usually pseudomonas from outside of the body, the origin of staphylococcus saprophyticus is unknown, gp B streptococcus is a normal flora of the colon and usually infect the pregnant women.

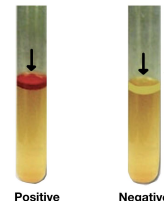
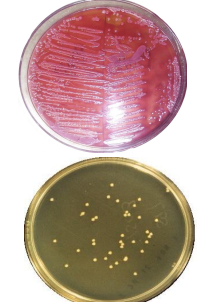

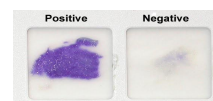

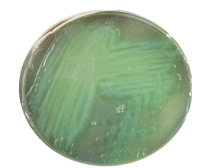


## Culture Media

<b>Blood agar</b>	<p><b>An enrichment medium</b></p> <p>An enriched medium, especially for culturing fastidious microorganism and observed the hemolytic reaction</p> <p>Only for fastidious and gram +ve bacteria</p>	
<b>MacConkey agar</b>	<p><b>Selective and differential medium</b></p> <p>MacConkey's agar showing both lactose and non-lactose fermenting colonies. Lactose fermenting colonies are pink whereas non-lactose fermenting ones are colourless or appear same as the medium.</p> <p>Selective = only for gram -ve</p> <p>Differential = differentiate between microorganisms</p>	
<b>CLED agar</b>	<p><b>Differential medium</b></p> <p>Differential = differentiate between microorganisms</p> <p>Differential culture medium for isolation and differentiation Of urinary pathogens</p> <p>If we use CLED no need to use macConkey and vice versa, because one of them substitutes the other.</p>	



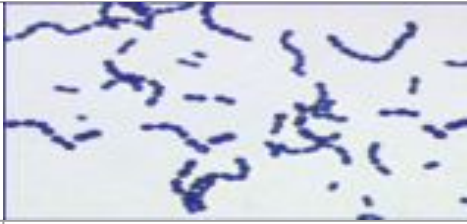
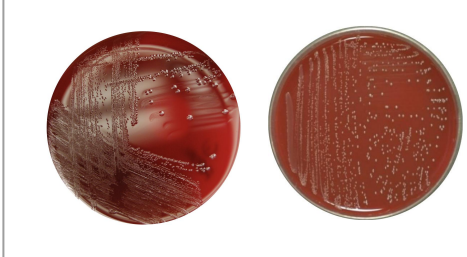
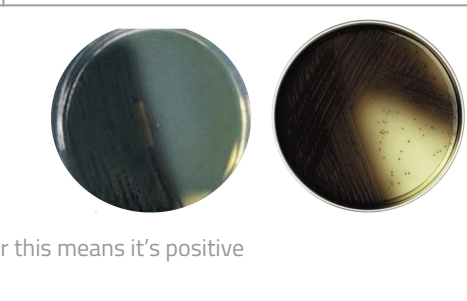
# Gram -ve bacilli

Either on MacConkey or CLED

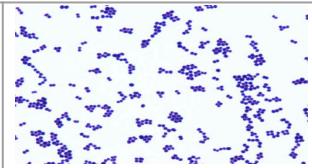

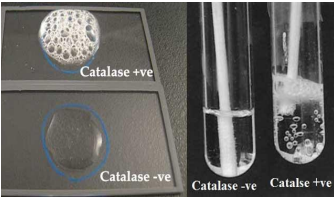

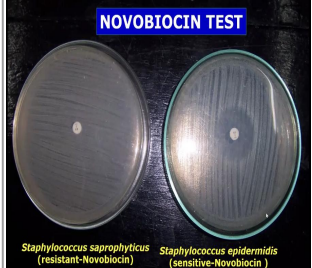
<p><b>Lactose Fermentation (+)</b></p> <p><b>Lactose Fermenter = Pink colonies</b> Due to lactose sugar that present in the agar, when the bacteria utilize it the color changes to pink.</p>	<p><b>Escherichia Coli</b> -Most common organism in UTIs -Dry colonies</p>	<p><b>Urease (-)</b> <b>Citrate (-)</b></p>	<p><b>Indole reaction</b></p>  <p>E-coli is characterized by <b>Positive indole</b> test. You can differentiate E-coli from other organisms by this test.</p>	
	<p><b>Klebsiella pneumonia</b></p>	<p><b>Urease (+)</b> <b>Citrate (+)</b></p>	<p>Notes: -The main difference between E-coli and klebsiella colonies is the mucoid appearance " due to production of capsules " in klebsiella unlike E-coli which is characterized by the dry colonies. -capsulated microorganism means it's more virulent than other non-capsulated organisms  -Klebsiella is <b>indole negative</b>. -More resistant than E-coli due to production of <math>\beta</math>-lactamase and presence of plasmid in the cytoplasm of the bacteria which code genes and become resistant.</p>	
	<p>Enterobacter aerogenes</p>	<p><b>Urease (-)</b> <b>Citrate (+)</b></p>		
<p><b>Lactose Fermentation (-)</b></p> <p><b>Non-lactose Fermenter = yellow or pale colonies</b></p>	<p><b>Pseudomonas aeruginosa</b>  If we culture it on CLED media it will give us greenish color pigment called <b>pyocyanin pigment</b> and its very unique and special for pseudomonas it's the only organism in the world that produces this pigment.</p>	<p><b>Oxidase (+)</b> <b>SIM (-)</b> (Sulfide, Indole, Motility)</p>	<p>Notes: It causes <b>complicated UTI</b>, hospital acquired infection, very difficult to treat, resistant to almost all of the antibiotics because it has a special pseudomonas agent. If it's <b>oxidase Positive</b> then we Automatically say it's Pseudomonas. It has a mucoid appearance on MacConkey agar</p> <p>Risk factors: -Hospitalized patients -catheterized patients -neutropenic patients.</p> 	<p>MacConkey  CLED  Pyocyanin pigment</p>
	<p><b>Proteus vulgaris</b></p>	<p><b>Oxidase (-)</b> <b>SIM (+)</b> (Sulfide, Indole, Motility)</p>	<p>CLED (Cystine-Lactose-Electrolyte-Deficient) inhibits the proteus swarm.</p> <p>Notes: It's a highly motile organism. <b>It's characterized by the swarming phenomena</b> only on blood agar because it's non-inhibitory movement medium unlike other media.</p>	
			<p>proteus is <b>Urease positive</b> Urease splits urea into ammonia; and alkalinizes the urine with production of crystals.</p> <p>Notes: The broth contains two pH buffers, urea, a very small amount of nutrients for the bacteria, and the pH indicator phenol red. Phenol red turns yellow in an acidic environment and fuchsia in an alkaline environment. If the urea in the broth is degraded and ammonia is produced, an alkaline environment is created, and the media turns pink.</p> <p>Broth: liquid medium containing proteins and other nutrients for the culture of bacteria.</p>	 <p>Urease producing bacteria cause <b>stone formation</b> in the urine.</p>

# Gram Positive Bacteria :- (only blood agar)

## 1-Enterococcus species catalase-,non hemolytic

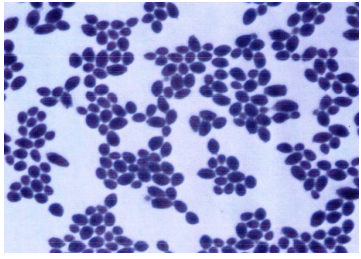
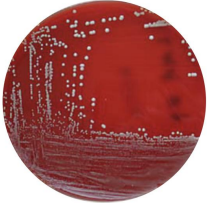
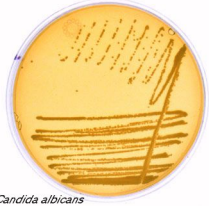
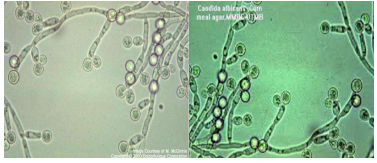

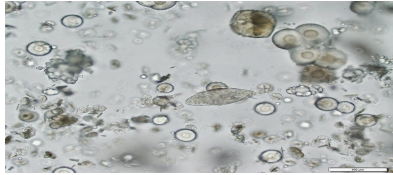

Morphology	Microscopic appearance: Gram positive cocci in chains	
Culture	<b>Blood Agar:-</b> Blood culture shows growth of $\gamma$ -hemolytic colonies	
Identification	1- Bile Esculin hydrolysis test*:  Both Group D streptococci and enterococci produce a <b>positive</b> bile Esculin hydrolysis test. <small>*Put bile in the media and it will give black color this means it's positive</small>	

## 2-Staphylococcus saprophyticus catalase +,coagulase-

Morphology	Microscopic appearance: Gram positive cocci in cluster		
Culture	<b>Blood Agar:-</b> Blood culture shows growth of white non-hemolytic Dry colonies.		
Identification	<p>1-Catalase test=<b>Positive</b> with bubbles (to differentiate between staph or strept bacteria)</p>  <p>Catalysed to  <math>2H_2O_2 \longrightarrow O_2 + 2H_2O</math>                  Streptococci vs. Staphylococci</p>	<p>2-Coagulase test=<b>Negative</b>                  Staphylococcus aureus is the only one that has positive coagulase test</p> 	<p>3-Novobiocin Test:  <b>Resistant</b> ( to differentiate between S.saprophyticus and S.epidermidis) it's an antibiotic</p>  <p><b>NOVOBIOCIN TEST</b>                  Staphylococcus saprophyticus (resistant-Novobiocin)      Staphylococcus epidermidis (sensitive-Novobiocin)</p>



# Other Organisms that might Cause URI:

<p><b>1-Fungi: Candida albicans</b></p> <p>Most common cause of UTI and difficult to treat.</p> <p>Risk Factors: catheters/DM/pregnancy /immune suppression</p>	Morphology	<p>Microscopic appearance: <b>Yeast with Budding pseudohyphae</b></p> 	
	Culture	<p>1- On blood agar</p> 	<p>2-Dextrose Media</p>  <p><i>Candida albicans</i></p>
	Identification	<p>1- Chlamyospore test <b>Positive</b></p> 	<p>2- <b>Germ tube test Positive</b></p> <p>(Test to differentiate between Candida albicans &amp; other types of candida, in albicans you will see elongation of yeast/ pseudohyphae after incubation in other candida will see only yeast)</p> 
<p><b>2-Parasites : Schistosoma haematobium</b></p> <p>cause chronic infection in bladder&gt;fibrosis &gt;scars&gt; cancer</p>	<p>(urine; eggs 115-170 x 45-65 micrometers) (primates)</p> <p>*<b>May cause Urinary Bladder cancer</b></p>  		

## ● Antibiotic sensitivity test: Agar diffusion method

What is Antibiotic sensitivity test ?

It is a test we do to see if the microorganism is sensitive to the drug or not , and we use with Staphylococcus saprophyticus with Novobiocin Test .

\*it is possible to use multiple drugs on the same agar  
it is called : Agar diffusion method

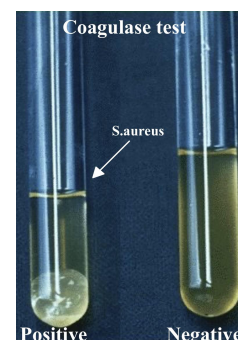


## ● Staphylococcus aureus could be differentiated from other Staphylococcus organisms through the following method :-

**Coagulase :-** is an enzyme produced by *S. aureus* that converts (soluble) fibrinogen in plasma to (insoluble) fibrin .

**How to know if the test is positive or negative ?**

A: if the plasma coagulated it means the bacteria has released it is enzymes in the plasma which is why it coagulated, We know that staphylococcus aureus is the the only one of it family that could cause the plasma to coagulate .



# Case1

These Blood agar and CLED agar plates were inoculated with MSU from a 45 years old man suspected of having bladder stone and complaining of burning micturition. Urine examination showed :

Moderate number of WBC and a PH of 8



CLED Plate



Blood Agar\*

A) What is the likely this pathogen?

Answer : Proteus.

B) How would you confirm the identity of this pathogen?

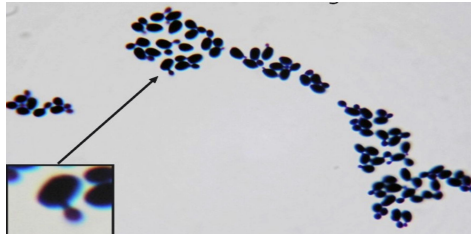
Answer : CLED plate , swarming and urease test.

C) What is the role of this organism in forming stones?

Answer : The bacteria will release Urease, which is an enzyme that splits urea into ammonia; and alkalinizes the urine with production of crystals which causes the kidney stone .

# Case2

64-year old male with history of chronic obstructive pulmonary disease and esophageal stricture. He developed septic shock following dilation of an esophageal stricture. He developed symptoms of urinary tract infection following urinary catheterization. An initial urine culture on hospital day 19 revealed 10<sup>4</sup> CFU/L of budding yeast following a change of his catheter.



A)What is your diagnosis?

Answer : Candida albicans.

B)What is the risk factor in this case?

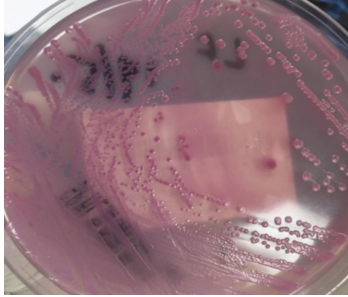
Answer : Urinary catheterization.

## Case3

3 year old woman presents to her doctor complaining of 1 day of increased urinary frequency, dysuria and sensation of incomplete voiding. She is otherwise healthy, takes no medications, and does not have fever, chills, vaginal discharge, or flank pain.

Urinalysis: pyuria (WBC 300/hpf), RBC and bacteria present.

Urine dipstick: positive leukocyte esterase and nitrite. Urine culture grew LF colonies that are indole positive. The organism was resistant to trimethoprim-sulfamethoxazole, cefalotin and amoxicillin-clavulanic acid but sensitive to ciprofloxacin.



A)What is the diagnosis?

Answer : Cystitis.

B)What is the most likely organism?

Answer : E.coli (Gram-, indol+,most common cause of cystitis).

## Case4

woman with DM presents to the ER complaining of chills, nausea and low back pain for the past 2 days. Earlier in the week she developed increased urinary frequency and dysuria. Recognizing the symptoms of UTI she took two days of TMP/SMX but was unable to finish treatment because of nausea and vomiting. Past medical history is notable for frequent UTIs treated with TMP/SMX and a history of Diabetes Mellitus. Blood culture grew Gram-Negative Bacilli. Mucoid LF and indole negative.



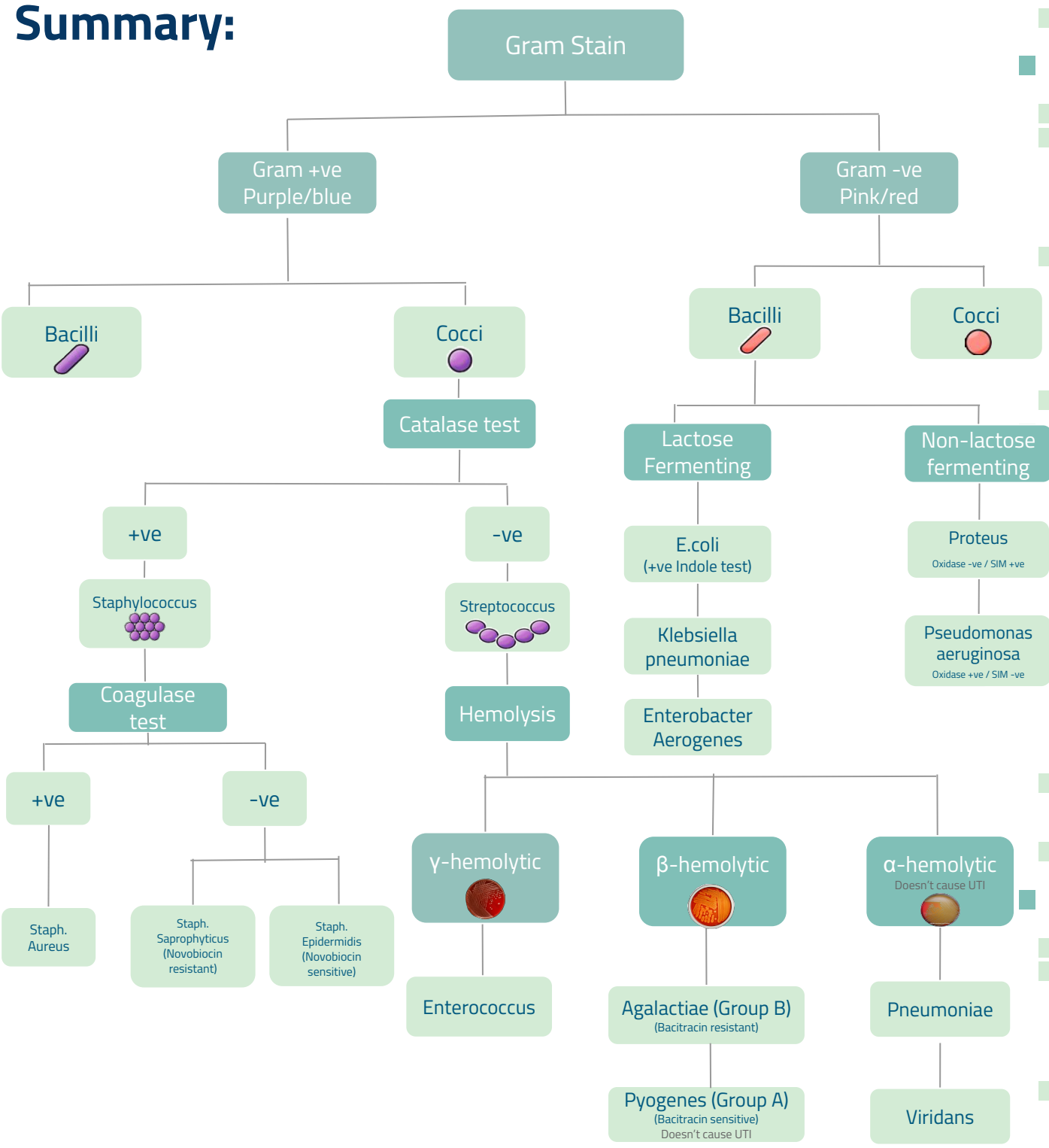
A)What is the most likely organism?

Answer : Klebsiella pneumoniae.( indol- )

B)The most likely diagnosis?

Answer : Pyelonephritis.

# Summary:



 Special thanks to Muneerah Al Sadhan

# Team Leaders

- Yara Alasmari
- Manee Alkhalifah

## Sub Leader

Mohammed Beyari

## Team Members

- Shahad Almezel
- Noura Alsalem
- Ghadah Alsuwailem
- Noura Alshathri
- Rand AlRefaei
- Muneerah Alsadhan
- Sarah Alaidaroos
- Sara AlQuwayz
- Sadeem Alhazmi
- Abdulaziz Alderaywsh
- Faisal Alomri
- Abdulaziz Alomar
- Meshal Alhamed
- Homoud Algadheb
- Abdulaziz Alsuham