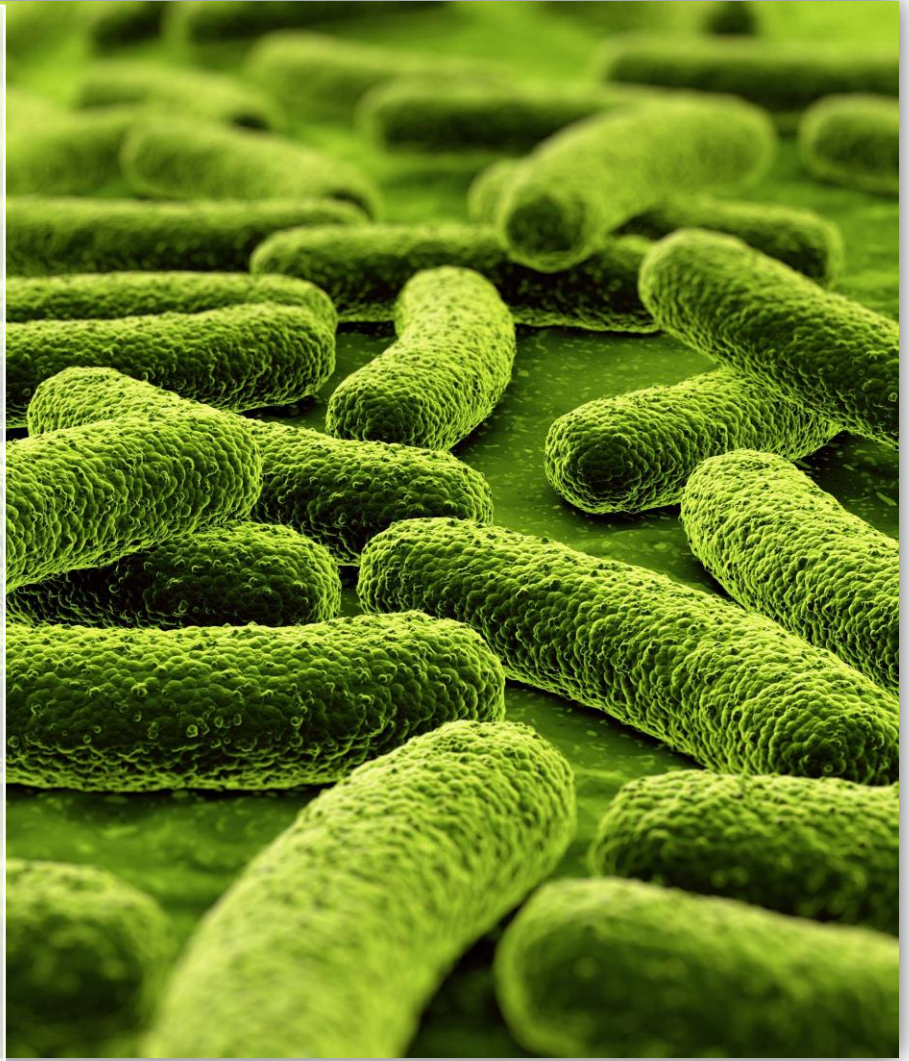


Microbiology Team

Organisms Causing UTI

PRACTICAL



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● **Important**

● **Additional Info**

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Microbiology – Renal Practical

● **Important**

● Additional Info

UTI: It is microbiologically defined as having significant bacteriuria + symptoms

* **Bacteriurea: $\geq 10^5$ colony forming unit \rightarrow UTI**
If less \rightarrow not UTI, maybe the reason is contamination

- Urine is the most common sample sent to the microbiology lab, because it's easy to collect.
- UTIs are very common
- Upper UTI symptoms: Flank pain, fever
- Lower UTI symptoms: Dysuria, frequency, suprapubic pain

EXAMINATION OF UTI:

- 1- Urine collection
- 2- Urine analysis
- 3- Interpretation of microbiology lab result

* **E.COLI is the most common organism (pathogen) because it colonizes in the perineum, and it is the most likely organism to contaminate urine specimen**

TYPES OF SPECIMENS:

1. **Midstream urine (MSU)**** because it is the least contaminated sample
2. Adhesive bag
(for infants, but is easily contaminated because urine is stagnant in the bag)
3. Suprapubic aspiration (invasive)
(e.g.: Used when if there's Prostatic hypertrophy and MSU cannot be taken)
4. Catheter sample
(sample should be taken from the tube -where urine is flowing- not from the drained urine in the bag because bacteria might have colonized in it because it's stagnant)

INVESTIGATIONS:

1) URINE ANALYSIS

Quick investigations,
Less accurate

A. Dipstick: contains many biochemical tests

- **NITRATES test \rightarrow E.coli** and other enterobacteria
- **Leukocyte Estrase test:** to detect high WBC (pus)
- **Glucose:** to detect RBCs (Hematuria)

B. Microscopic: cell counting for **pyuria**

\rightarrow (high WBC >10 /hpf) hpf: high power field

What can we see under microscope?

RBCs (hematuria), WBC (pyuria), Casts

2) CULTURES:

Golden Standard,
Slow investigation,
More accurate

MEDIA (AGARS) USED TO CULTURE URINE:

1. MacConkey agar:

Differential media

Used more for gram - ve

It differentiates between lactose-fermenting and non-lactose fermenting bacteria:



- If the bacteria is **lactose fermenting**, the agar will turn **pink**. This indicates that the bacteria could be:

E.coli - Klebsella - Enterobacteriaceae (eg. as enterobacter)

→ WE USE **UREASE** TEST TO FURTHER DIFFERENTIATE BETWEEN THEM

[Lactose fermenting organism → acts on lactate in agar by producing acids → changes pH → Phenol which is the color indicator becomes red]

- If bacteria is **non-fermenting**, the **agar's color won't change**. This indicates the bacteria could be:

Pseudomonas - Proteus

→ WE USE **OXIDASE** TEST TO FURTHER DIFFERENTIATE BETWEEN THEM

2. Blood agar:

An enriched media for fastidious bacteria

Used more for **gram +ve** to show hemolysis (alpha, beta, gamma)

(E.g: staph saprophyticus, enterococcus feacalis, **group A beta hemolytic strept**)



3. CLED agar: *CLED: cystine-lactose-electrolyte-deficient*

Differential media → has sugar

Selective for detection and isolation of E.coli and coliform bacteria

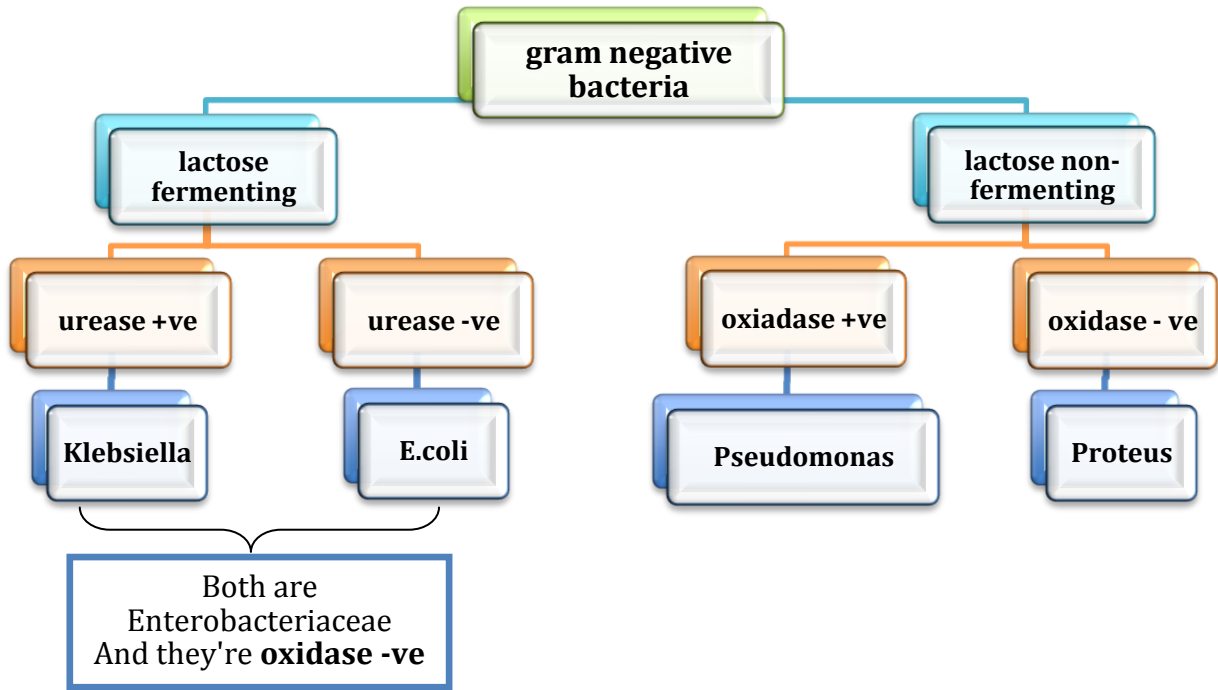
→ they show **yellow color**

It inhibits proteus swarming (proteus swarming will be mentioned later)



CLED agar has advantage over MacConkey agar because CLED allows growth of staph species.

Gram Negative Bacteria:



1. ESCHERICHIA COLI:

gram negative = red on gram stain

- Lactose fermenting (Pink on MacConkey, Yellow on CLED)
- Urease negative, Citrate negative
- Indole positive (gives red color)

Treatment:

- Nitrofurantoin
- Ciprofloxacin
- Co-Trimoxazole
- Ampicillin
- **1st generation cephalosporins**

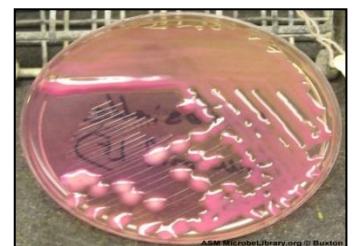


2. KLEBSIELLA PNEUMONIAE:

- Lactose fermenting
- Urease positive, Citrate positive
- Indole negative (color stays pale green)
- Mucoid appearance (encapsulated)

Treatment:

(Same as e.coli, except that it's resistant to ampicillin)



3. PROTEUS:

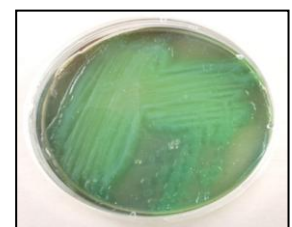
- a. Non-lactose fermenting
- b. Urease positive (splits urea into ammonia & CO₂)
- c. Oxidase positive
- d. **Shows SWARMING growth in blood agar because it has flagella (highly motile)**
→ but CLED & MacConkey agars inhibit swarming
(because they're low in electrolyte)
- e. Increase pH of urine (**alkaline urine**)
→ stone formation (by splitting urea to ammonia)



4. PSEUDOMONAS AEROGENOSA:

hospital acquired bacterial infection.
Multi-drug resistant

- a. Lactose non-fermenting
- b. Oxidase positive



Gram Positive Bacteria:

1. ENTEROCOCCUS (E.FAECALIS):

- a. Gram +ve cocci in chain (streptococcus)
- b. Catalase negative
- c. Positive bile esculin hydrolysis test (hydrolyzes esculin)
- d. gamma hemolytic = Non-hemolytic (black colonies)

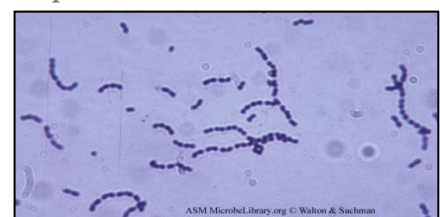


Complications: in heart valve the complication is: Infective Endocarditis.

Both enterococci and group D streptococci produce positive bile esculin hydrolysis test.

Treatment:

- Ampicillin
(In old men with prostate enlargement)

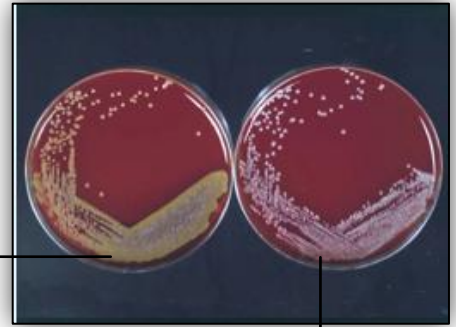


2. STAPHYLOCOCCUS SPECIES:

(Gram +ve cocci in clusters)

A. STAPH AUREUS: (hematogenous)

- a. Catalase positive (bubble reaction)
- b. Coagulase positive (forms a clot by converting fibrinogen into fibrin)
- c. Color: Golden colonies



B. STAPH SAPROPHYTICUS:

(Causes honeymoon cystitis, by sexual intercourse mechanism)

- a. Catalase positive (Bubbles)
- b. Coagulase negative (no clotting)
- c. Color: Whitish colonies
- d. **Novobiocin resistant**

How does catalase reaction produce bubbles?

By releasing O₂ from oxygen peroxidase

Both staph saprophyticus & staph epidermidis are coagulase negative.

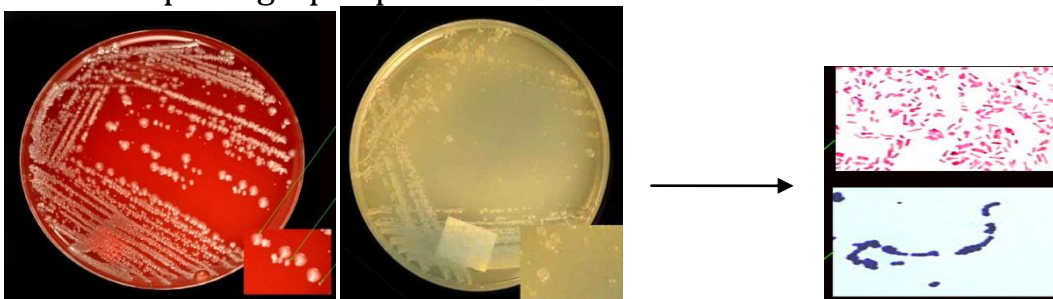


FURTHER INFORMATION:

- Urine analysis is done quickly after collecting the sample in order to prevent colonization of bacteria in urine which will contaminate the sample.
- When taking MSU, the first millimeters of urine are discarded because they're most likely to be contaminated.
- Where does contamination of a urine sample come from?
It comes from the bacteria in the perineal area
(from anus because of stool, vagina & urethra)
- How to protect a urine sample from contamination?
1- Fridge urine 2- Put in dip slide 3- ---- acid??
- If the patient has high bacteriuria without any symptoms and no WBC elevation this doesn't mean UTI because urine is susceptible to contamination.
- **Pyuria is highly indicative** when associated with symptoms
- Sensitivity test is important for recurrent infections and nosocomial infections
- **Causes of hematuria:**
Tumors Stones Infections Schistosomiasis Cystitis
- Pyelonephritis treatment requires 10-14 days
- Cystitis treatment requires 3-7 days

CASE 1:

The blood agar plate and CLED plate provided were inoculated with a sample of urine from a patient with a suspected urinary tract infection. Examine the plates and photographs provided.



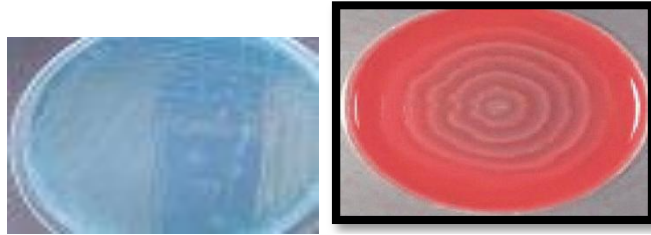
• **Identify the colonies on the blood agar plates and photographs.**

The photographs show the results of the Gram stain of each colony type.

• **Large colonies** are **Gram NEGATIVE** and **small colonies** are **Gram POSITIVE**

CASE 2:

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 micturation. Urine examination showed :
Moderate number of WBC and a PH of 8



A) What is the likely this pathogen?

Proteus bacteria

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

Urease test

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

.....Alkalize urine by breaking down urea into ammonia leading to formation of stones.

Mention one organism from each of the following which may cause urinary tract infection

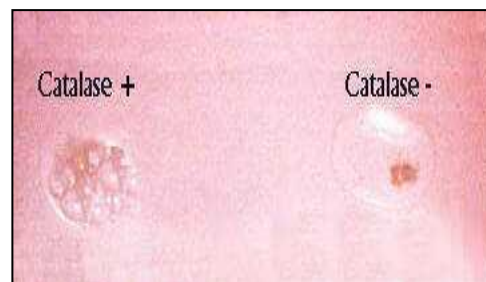
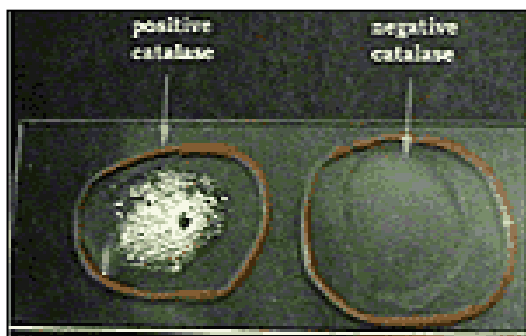
A) Bacteria: E.coli

B) Parasites: Schistosoma

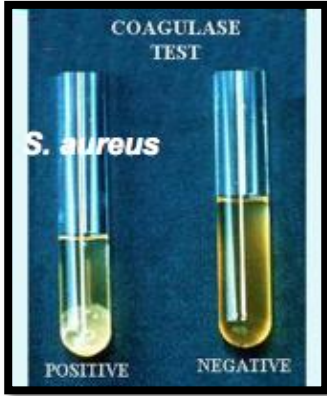
C) Fungi: Candida

PICTURES AND CHARTS

Catalase Test: (BUBBLES)



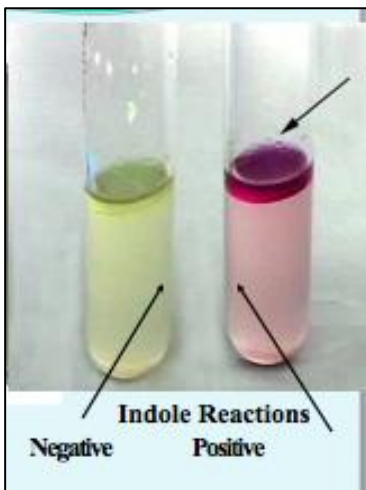
Coagulase Test: (CLOT)



Urease Test:



Indole test:

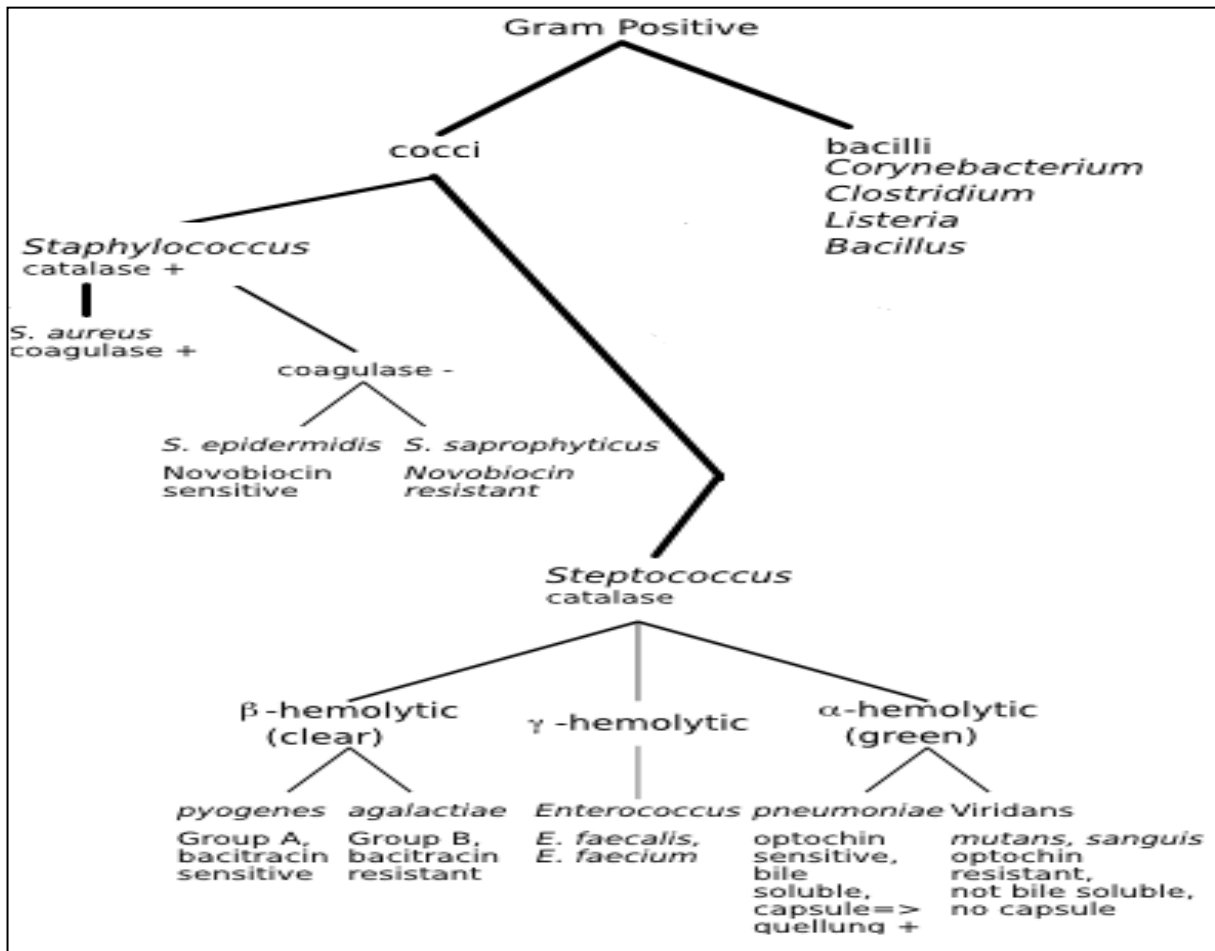


API: machine for culturing

Three API 20E strips :

- Immediately after inoculation
- After 24 hours incubation
- That in (b) after addition of reagents to certain wells.

The organisms here is *Escherichia coli*. Here the first carbohydrate well (glucose) is also used for the nitrate reduction test



QUICK SUMMARY OF ORGANISMS

	E.Coli	Klebsiella Pneum.	Proteus	P. Aerogenosa	Entero cocci	Staph Aureus	Staph Saprophyt.
Gram	-ve	-ve	-ve	-ve	+ve	+ve	+ve
Lactose fermentation	✓	✓	X	X			
Oxidase	-ve	-ve	-ve	+ve			
Urease	-ve	+ve	+ve	---			
Indole	+ve	-ve					
Catalase					-ve	+ve	+ve
Coagulase						+ve	-ve
Novobiocin						Sensitive	resistant

Good Luck!