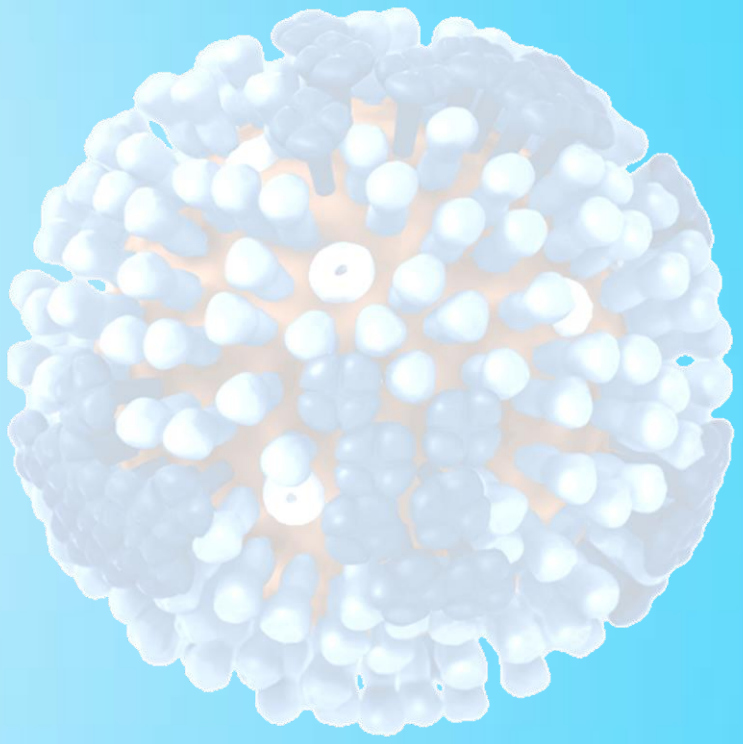
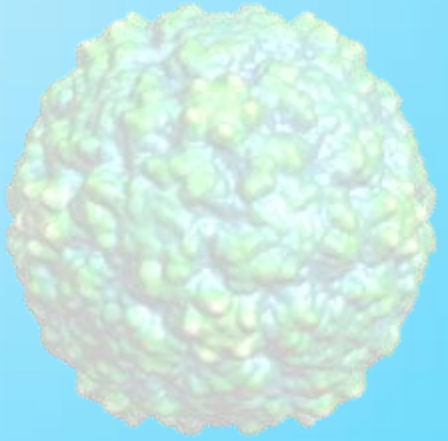


URINARY TRACT INFECTION

OSPE

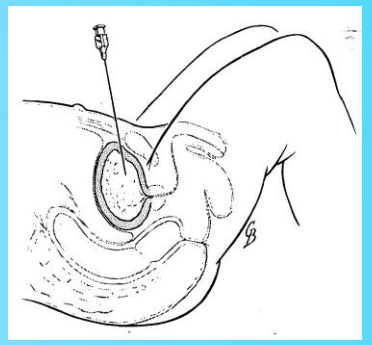


Examination of UTI:

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

Type of Specimens

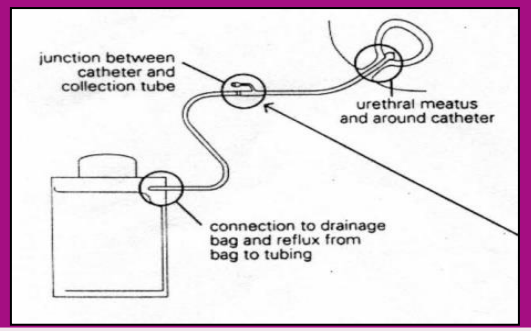
Midstream urine (MSU)	*To make sure that urine is not contaminated by bacteria from urethra. *Commonly used
Clean catch	
Adhesive bag	This is for newborn babies that are not able to collect midstream urine.
Suprapubic Aspiration	*Suprapubic aspiration if there is further difficulty in collecting urine. By using catheters. * Most specific
Catheter sample	*Should not be tested because it may have been standing for several hours.

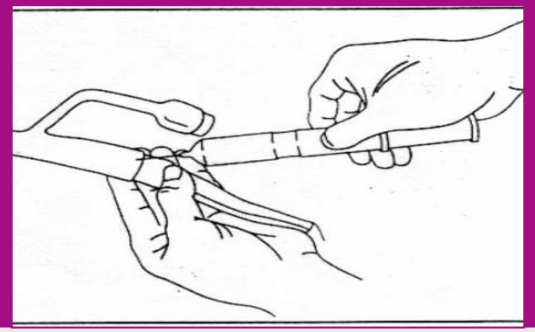


Suprapubic Aspiration

Adhesive bag

The urinary catheter





Urine specimens for laboratory investigations can be collected
from catheterized patients as shown (left). The second port is for
putting fluids into the bladder (right).

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

Urine in the bag should not be tested because it might be standing for several hours.

Transport media:

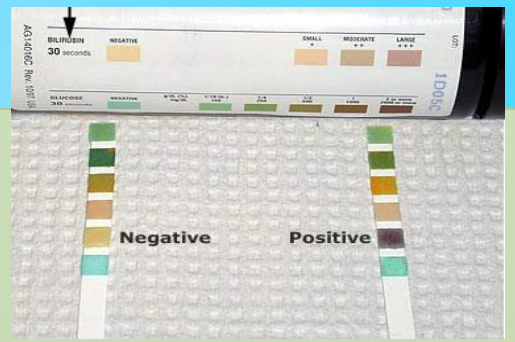
1- Sterile Urine

2- Dipslides

One side >> CLED media.

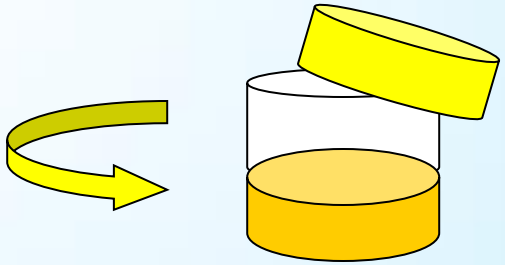
Other side >>MacConkey (MAC) agar or blood agar

Urine analysis

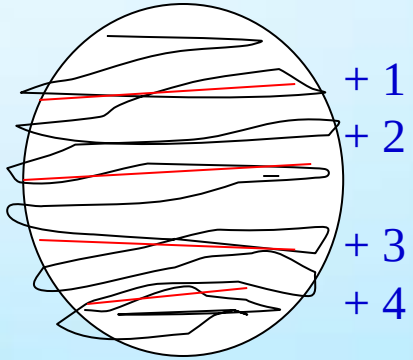


Biochemical Dip stick	(leukocyte esterase ,nitrate test) -Dip stick gives an indicator of presence of bacteria. Urine is a (وسط غذائي) bacteria can grow very quickly if we left the sample of urine for long time in the clinic before sending it to the lab which gives a confusing result because the number of bacteria will very much greater than the amount that is presented in the body. -Dip stick method is considered as a screening method that indicates presence or absence of bacteria.
Microscopic examination	cell-counting chamber (RBC,WBC..etc) - Cell counting chamber: to count the number of WBCs and to decide then if the number of WBCs is of great significance and if its number is related to a certain disease or not.

Laboratory examination of urine



Quantitative (Colony counts)



Note:
First streaking has the greatest number of bacteria . the following streaking contain less bacteria

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

Over night incubation

Isolation of colonies,
Biochemical tests,
Drug susceptibility test,

Over night incubation

RESULT

Causative organisms of UTI

Organism	Note
GRAM Negative Bacilli	
Escherichia coli	Most common in inpatients & outpatients
Klebsiella	Second most common in outpatients
Proteus	
Other Enterobacteriaceae (Enterobacter,Citrobacter….)	
Pseudomonas aeruginosa	
GRAM Positive Cocci	
Enterococcus	In chains
Staphylococcus saprophyticus	In clusters
Streptococcus agalactiae (group B)	Chains
Staphylococcus aureus (Associated with staphylococcemia)	Clusters
Other organisms	
Candida albicans	Second most common in inpatients (specially catheterized patients)
Schistosoma haematobium	

Culture media

blood agar

An enriched medium

MacConkey agar

A differential medium

CLED agar

Selective medium

Note:

Macconkey and CLED are differential media.

CLED stop growth of gram positive and allows growth of gram negative bacteria and macconkey medium as well

Some media contain lactose sugar (macconkey) , so ,from this property we can differentiate between lactose fermenting and the non-lactose fermenting type of bacteria. (little types of bacteria that uses lactose for nutrition)

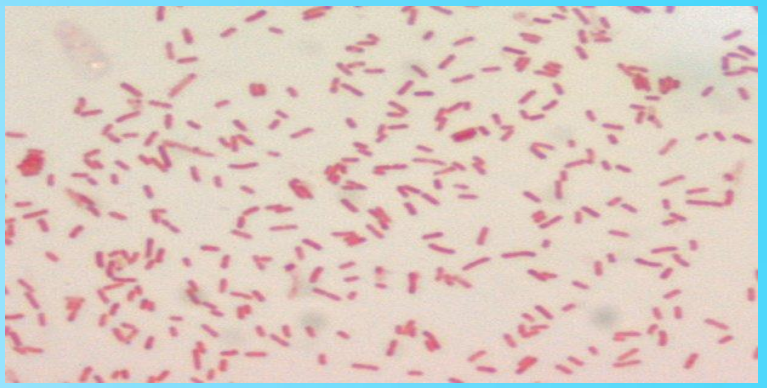
مهم نتعرف على انواع الاجار في العملي

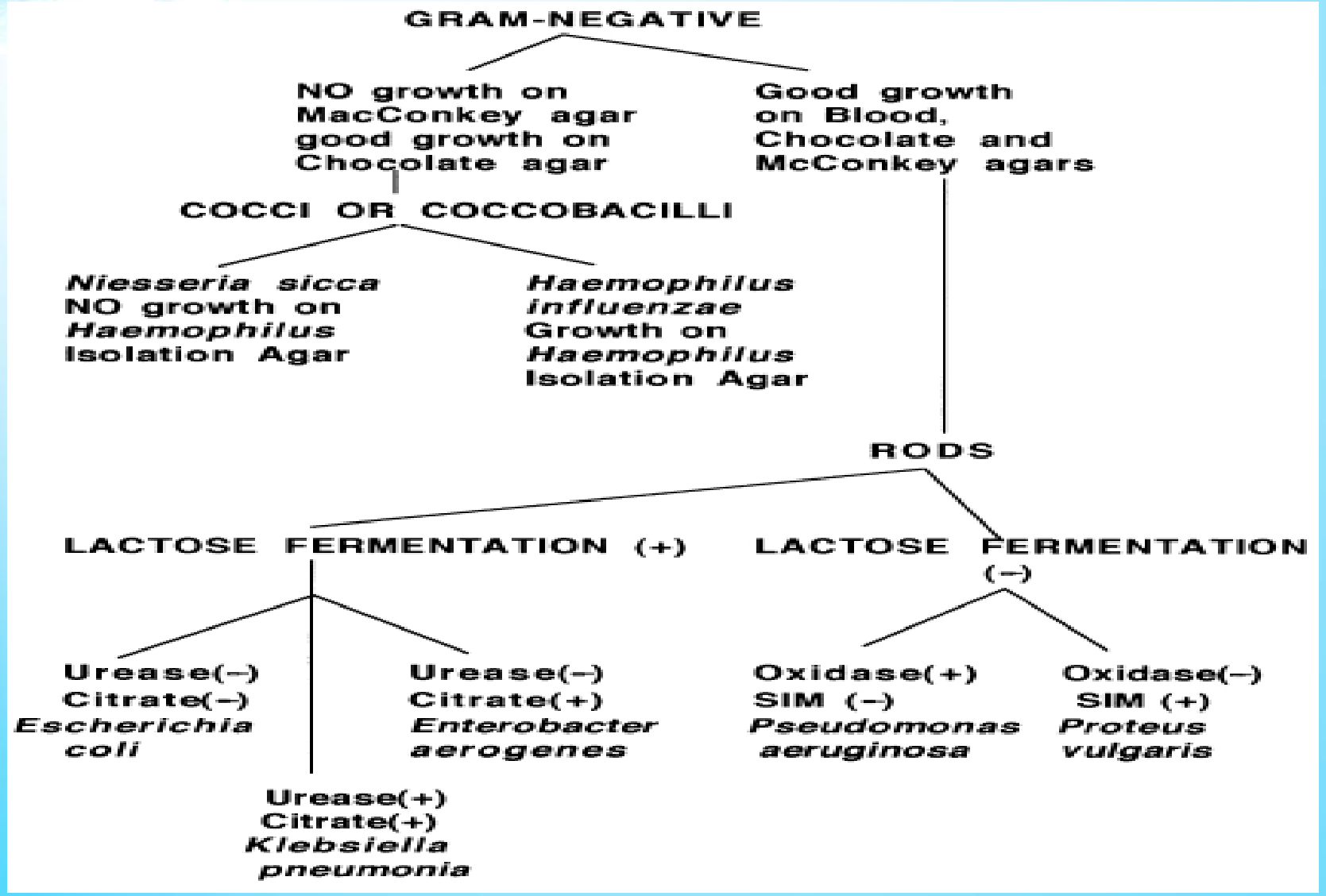
CLED is most commonly used in urine culture.

In blood agar any bacteria can grow because it is an enriched medium.

Blood agar 	An enriched medium, especially for culturing fastidious microorganism and observed the hemolytic reaction	Advantages of blood agar: Blood agar can grow all types of bacteria as it is an enriching media even Fastidious bacteria that is very hard to be grown. In blood agar from the type of hemolysis we can differentiate between types of bacteria.
MacConkey agar 	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.	Macconkey's agar is a selective medium.We differentiate between types of bacteria based on its ability to ferment lactose (bacteria that is able to ferment lactose has lactase enzyme to break lactose) Indicator of fermentation is pink color and non fermenting indication is pale color.
CLED agar 	**Selective culture medium for detection and isolation Of *Escherichia coli* and *coliform* bacteria in urine**	Allow growth of some types of bacteria only and stop growth of others. Enhances growth of gram negative enterobactericia We see single colonies

Gram negative bacilli





First : GRAM NEGATIVE

Lactose fermenting

1- E.coli

Indole Reactions	Positive
Urease	Negative

2- Klebsiella pneumoniae

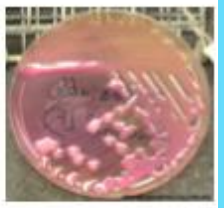
Indole Reactions	Negative

3-Enterobacteriaceae

Nitrate	Positive

Negative Positive
Indole Reactions

MacConkey agar



MacConkey agar
Klebsiella

Non-Lactose fermenting

4-Proteus

Urease	Positive → act on urea →splits ammonia → alkalinizes the urine →production of crystals
Swarming (motility)	Positive (seen in a blood agar)
NOTE:	✓Commonly infect: People with kidney stone ✓CLED inhibits the proteus swarm

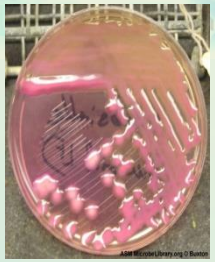
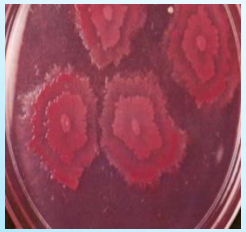
Positive Negative
Urease test

Swarming

5-Pseudomonas aeruginosa

Oxidase	Positive → purple
Note	Produce special greenish pigment

agar
Pseudomonas

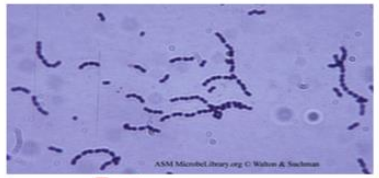
Notes: 	**E coli**	**Usually 80% of UTI is caused by E.coli** **E.coli is the most common cause of UTI in females.** **It is the most common cause of UTI because it is colon normal flora.** **Indole is a biochemical test to differentiate**
	***Klebsiella pneumoniae***	Klebsiella pneumonia has a mucoid appearance because it has a capsule. Bacteria that has capsule is more virulent. E.Coli forms single colonies that are not shinny. Both klebsiella and e.coli are lactose fermenters because they showed a pink color on the culture medium. Last picture: there are types of klebsiella that don't produce a mucoid appearance on culture medium while some types produce like the klebsiella pneumoniae.
	**Proteus** 	Proteus can cause UTI ,ear infections ,abscesses and ulcers. Usually proteus infection is secondary which means that it comes with other infections. Proteus is the only bacteria that grow by swarming pattern .. Swarm = تموج Proteus can break down urea to form ammonia that changes the urine ph to alkaline ph=8 (urine normally is acidic ph is from 5-6) It can lead to stones formation.
	***Pseudomonas aeruginosa*** 	Pseudomonas aeruginosa is responsible for nosocomial infections then candida (fungus) then klebsiella. Two things we have to notice: Growth: mucoid and dry. Pseudomonas aeruginosa is the only bacteria that produce biocin صبغة Important test to identify pseudomonas aeruginosa is the oxidase test. Pseudomonas is strictly aerobic (strong production of oxidase) We rarely find pseudomonas causing deep infections like (bone infection) because it is aerobic Pseudomonas causes superficial infections like ear infection.

Three API 20E strips :

a. Immediately after inoculation
b. After 24 hours incubation
c. 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

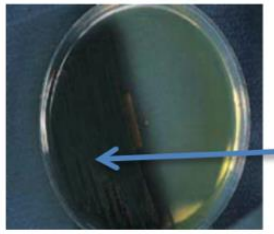
Second: GRAM POSITIVE



Enterococcus
Gram positive cocci in chains

1-Streptococci (GRAM positive cocci in chains)

Catalase test	**Negative**
Enterococcus species	Positive Bile Esculin hydrolysis test Normal flora in colon

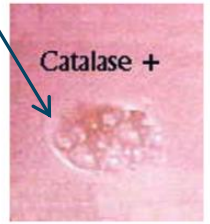


Positive Bile Esculin hydrolysis test
Way to identify enterococcus species

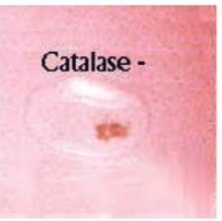
2- Staphylococcus species (GRAM positive cocci in clusters)

Catalase test		**Positive** $2H_2O_2 \rightarrow O_2 + 2H_2O$	Oxygen production is responsible for the bubbles
Types	Coagulase+ Coagulation of plasma	S.aureus (normal flora in the nose)	Golden colonies (yellowish)
	Coagulase- Plasma remains fluid	Novobiocin resistant	s.saprophyticus	white
		Novobiocin sensitive	S.epidermidis	white colonies

Catalase test



Staphylococcus

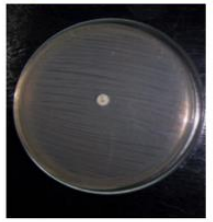


Streptococci

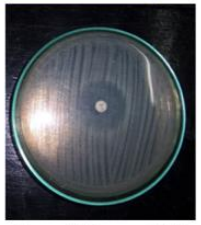
S.aureus

S.epidermidis

Novobiocin test



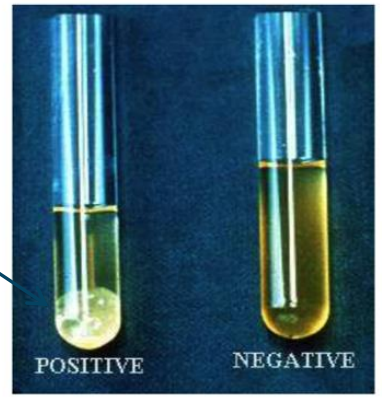
s.saprophyticus



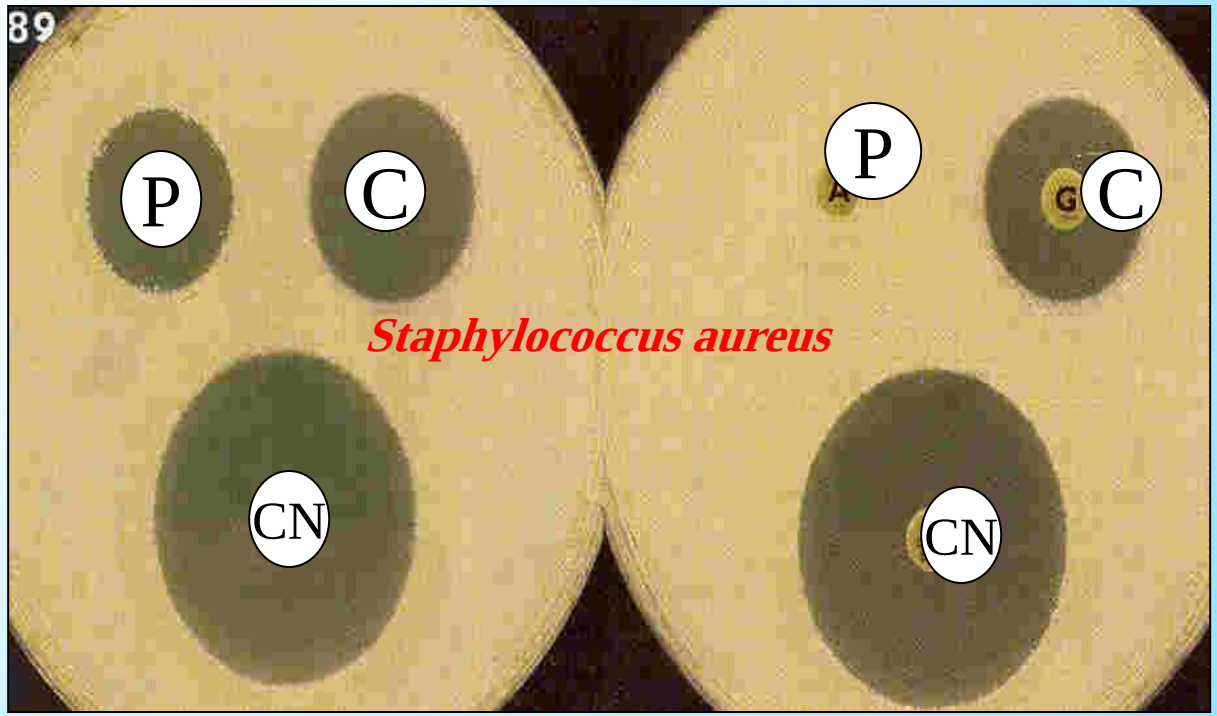
S.epidermidis

Coagulase test

We see clumping if the bacteria is coagulase positive



Antibiotic sensitivity test: Agar diffusion method



We determine the sensitivity based on the area of inhibition of growth.

R (Resistant) ; S(Sensitive)

According to spreading of antibiotic we get different diameters of the zones of inhibition of growth.

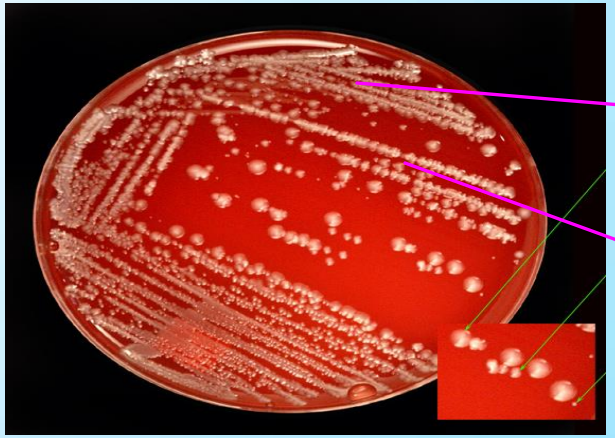
Gentamisin (CN) : 12 - 15
Chloramphenicol (C) : 12 - 18
Penicilin (P) : 28 – 29

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.

Q:IdenIfy the colonies on the blood agar plates and photographs. The photographs show the results of the Gram stain of each colony type.

CLED plate



Blood agar

Gram stain

Answer:

- Large colonies are Gram NegaIve
- small colonies are Gram posiIve .

This case to show that UTI can be caused by 2 microorganism

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

Q1. What is the likely this pathogen?
Q2. How would you confirm the idenIty of this pathogen?
Q3.What is the role of this organism in forming stones?

CLED

Blood agar

A1. Proteus
A2.swarming + urease test à positive
A3. Ureaseàact on ureaàsplits ammoniaàalkalinizes the urine (change the PH of urine)àproduction of crystals

Complication and treatment of UTI

Organism	E.Coli	proteus	Pseudomonas	Entrococcus	Group B	Staph.saprophyticus
Gram stain	Negative bacilli	Negative bacilli	Negative bacilli	Positive chain	Positive chain	Positive cluster
Culture	LF	NLF	NLF	SWC	SWC	SWC
Biochemical test to confirm	Indol positive	Urea positive	Oxidase positive	PBEHT	Catalase negative	Coagulase negative (novobiocin resistant)
Treatment			Ciprofloxacin	Vancomycin		
	Ampicillin, sulfonamide, Nitrofurnatoin, ciprofloxacin					

Key:

LF: lactose fermentative
NLF: non lactose fermentative
SWC: small white colony
PBEHT: Positive bile esculin hydrolysis test

Complications of UTI :

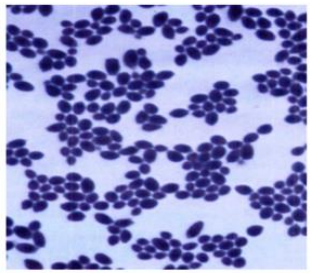
1. Endocarditis
2. Bacteremia
3. renal stone
4. pyelonephritis

Third: Other organisms

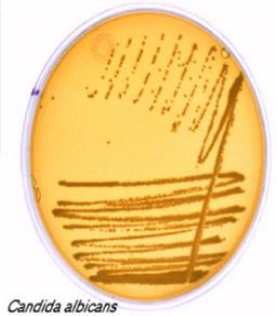
1-Candida albicans (fungi)	
Grow on	1. Sabouraud's Dextrose Media 2.Blood agar
identification tests	1.Chlamydospore 2.Germ tube test
2-Schistosoma haematobium (Parasites)	

Giemsa stain is for fungi

It has an oblique(oval) shape + we can see the budding.



1-Candida albicans

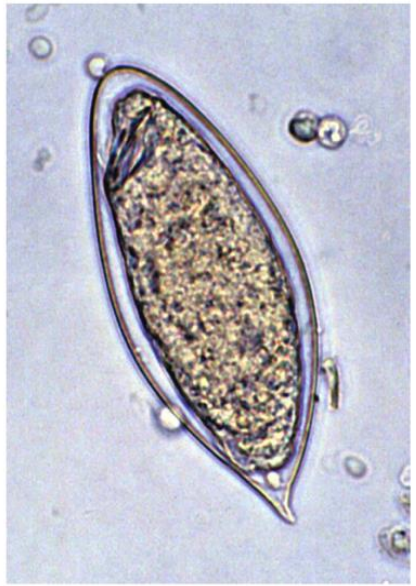


Sabouraud's Dextrose Media

Candida albicans on bloo d agar;

Chlamydospore

Germ tube test shows the budding



2-Schistosoma haematobium

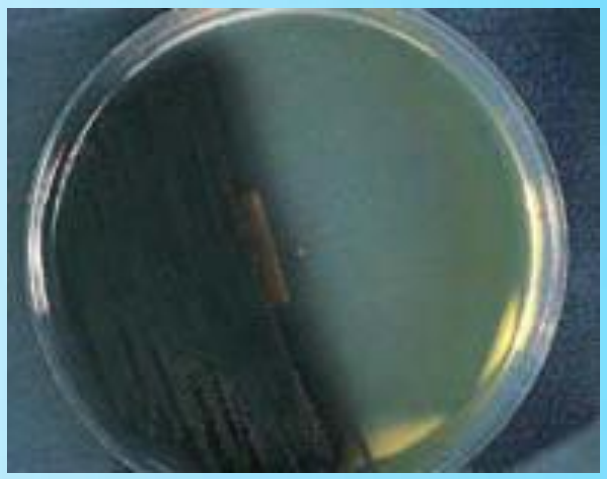
Gram positive cocci in chains

Swarming on blood agar

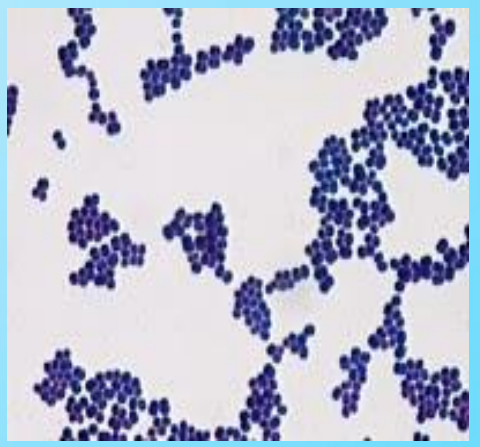
Catalase test

Urea test

Gram Stain



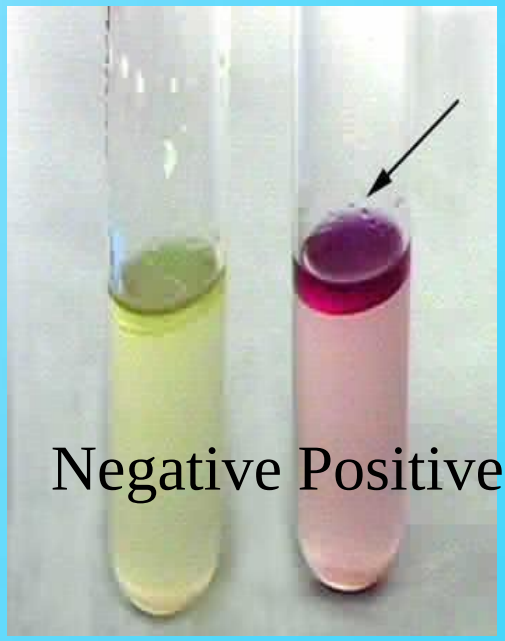
Esculin positive



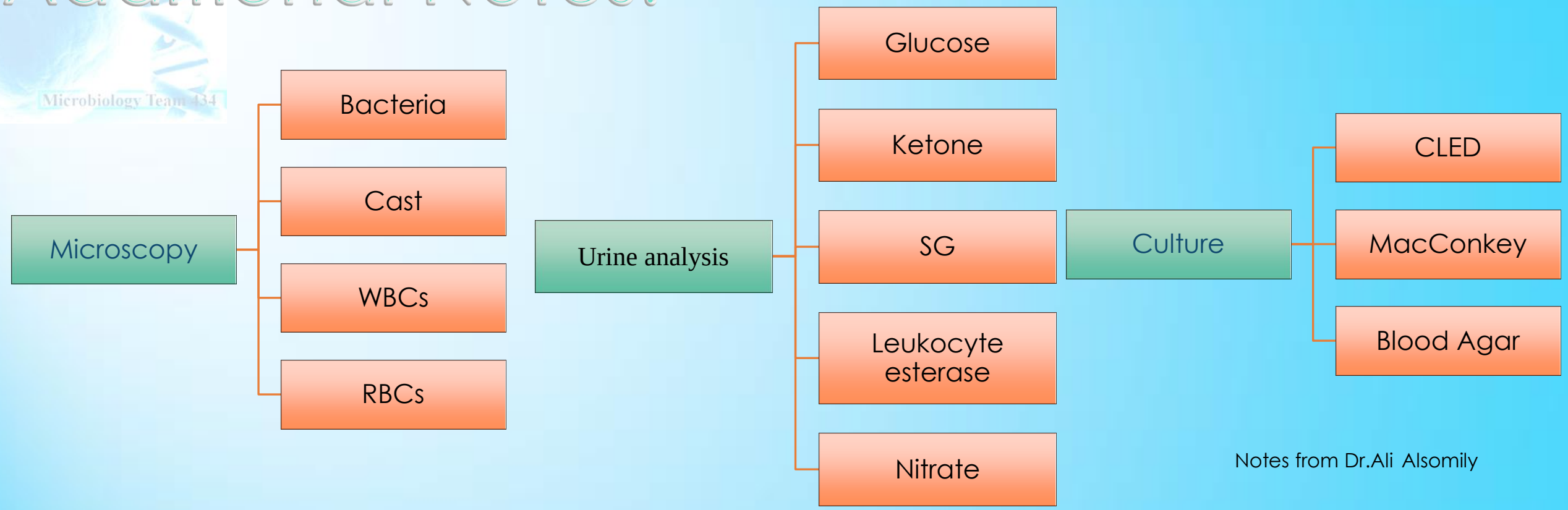
Gram Stain

Coagulase test

Indole test



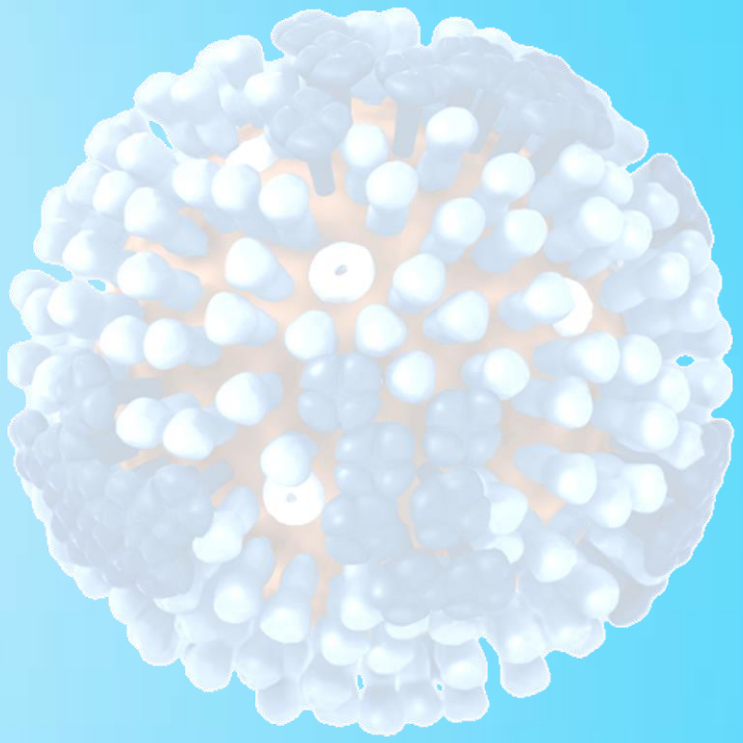
Additional Notes:



Notes from Dr.Ali Alsomily

- Saprophyticus is a pathogen in UTI while epidermidis is not.
- Novobiocin is an antibiotic that is used only in vitro (lab) = not on humans because it is toxic.
- If bacteria is coagulase negative we then investigate if it is novobionic sensitive or resistant.
- Saprophyticus is associated with honeymoon UTI.

Microbiology Team 434



THANK YOU

Microbiology team