

Revised by

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MEDICINE
KING SAUD UNIVERSITY

Microbiology

team 436



Lecture : Practical UTI

■ important

■ Extra notes

■ Doctors notes

"لا حول ولا قوة إلا بالله العلي العظيم" وتقال هذه الجملة إذا داهم الإنسان أمر عظيم لا يستطيعه ، أو يصعب عليه القيام به .

Objectives:

- 1- to Know the important steps in specimen collection and transport to the lab.
2. How to process urine Specimens in the lab.
 - Urine microbiological and biochemical analysis.
 - Organisms culture and identification.
 - Antibiotic susceptibility testing.
 - Results interoperation.
3. Know the clinically important etiological Organisms associated with UTI, their identification and susceptibility testing.

Overview :

المحاضرة تتكلم عن أربع محاور أساسية :

- 1- urine collecting
- 2- urine analysis
- 3- the microorganism
- 4- Antimicrobial Susceptibility Testing

- العمل معتمد و شامل على محاضرات الدكتوراة
- لا تخافون من عدد السلايدز , السلايدز خفيفة و غالبية المعلومات معروفة وسهلة إن شاء الله
- في نهاية المحاضرة فيه كيسيز من سلايد الدكتوراة لكن الحل من التيم
- ركزوا على .. **microorganisms and their culture + stain + groth** (وبالتوفيق اجمعين)

Important aspects of Microbiologic Examination of UTI:

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

1- Urine collection

Types of specimens:

Midstream urine (MSU):

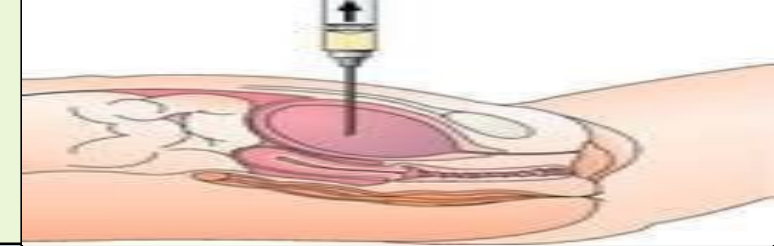
- The urine collected in a wide mouthed container from patients a mid stream specimen is the most ideal for processing
- Female patients pass urine with a labia separated and mid stream sample is collected



Adhesive bag



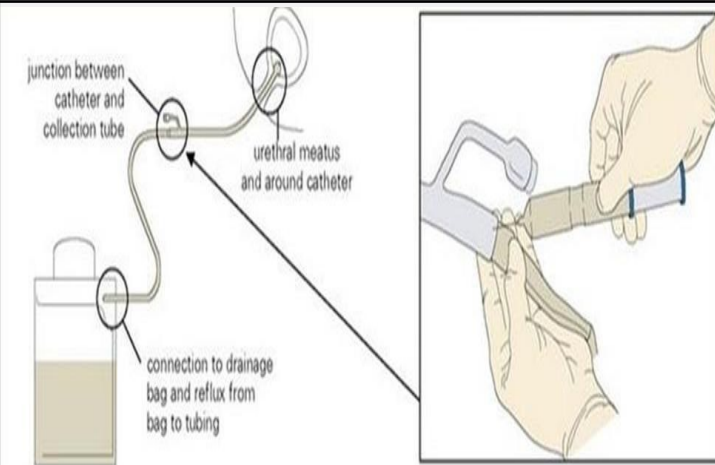
Suprapubic aspiration (for children)



Clean catch

Catheter sample: (used with elderly)

- 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.



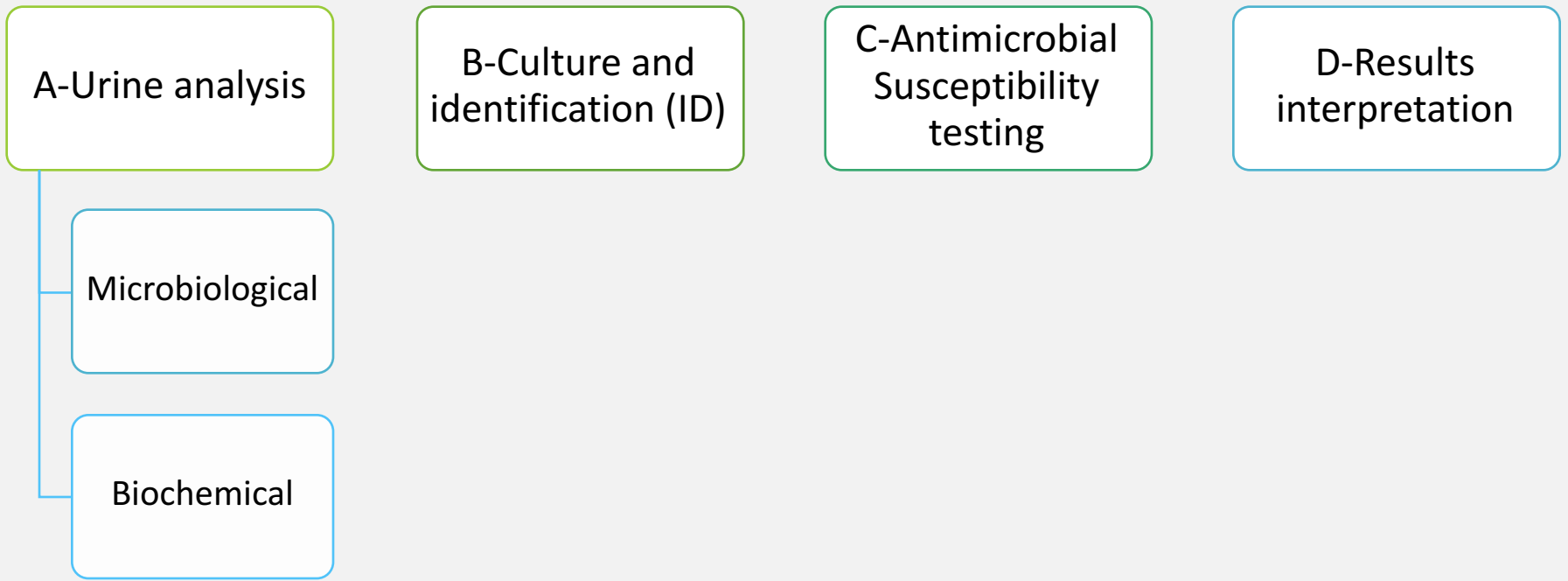
Transport media

Sterile urine container

Dip slide (one side is Cled media and the other is MacConkey agar or blood agar)



SPECIMEN PROCESSING:



2- Urine processing

Urine analysis is subdivided into biochemical and microbiological testing:

A- Urine analysis

Biochemical (dipstick)

leukocyte esterase (next slide)

Nitrate test (next slide)

PH

Glucose

Bilirubin

Protein*

*For kidney impairment

Microbiological

Microscopic

Macroscopic

Microbiological (Microscopic Urine Examination (WET MOUNT))

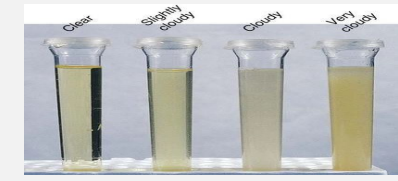
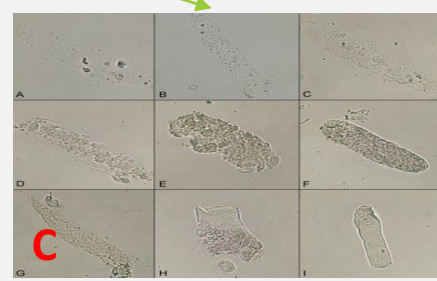
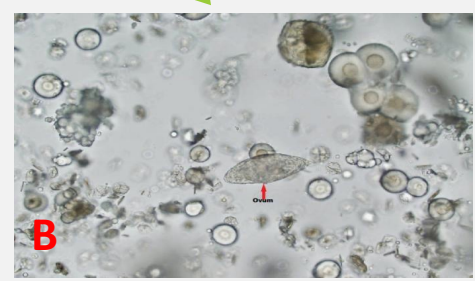
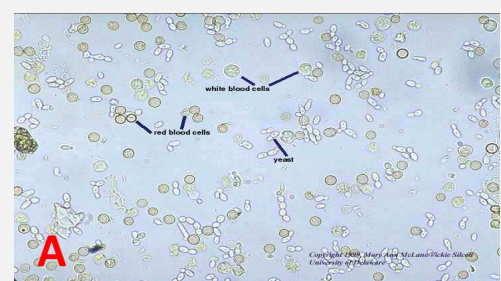
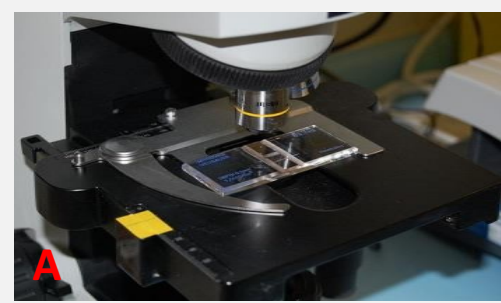
Microscopic:

- A- Cell-counting chamber (WBC, RBC)
- B- Parasite (Ovum, Trichomonas, Yeast)
- C- Casts

Physical (with naked eyes)

Macroscopic:

- Color +(blood)
- Odor
- Turbidity



Testing for UTI:

○ Midstream clean catch with dipstick analysis:

It is negative in case of gram positive bacteria

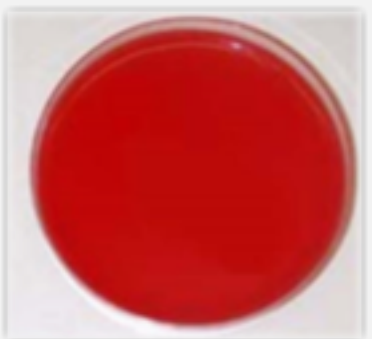
- Nitrite positive → for gram-negative bacteria which can convert nitrate
 - ✓ to nitrite (sensitivity 92-100%, low specificity);
 - ✓ false negative with bacteria that do not reduce nitrate
 - ✓ gram-positive bacteria
 - ✓ excess dietary Vitamin C
 - Leukocyte esterase + → indicates presence of white blood cells,
 - ✓ (sensitivity 75-95%, specificity 94-98%)–
 - ✓ Dipstick results may be affected by medications/dyes, ie pyridium, nitrofurantoin, metronidazole, bilirubin, methylene blue, Vitamin B complex
-

2- Urine processing

B- CULTURE AND IDENTIFICATION:

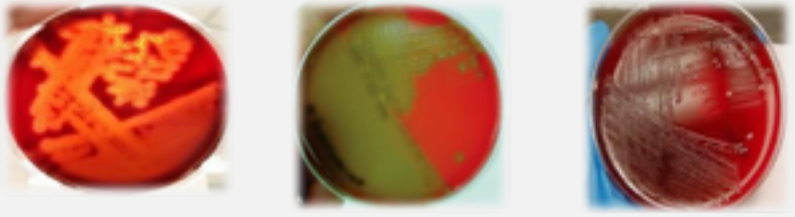
- 1- Culture media
- 2- Urine inoculation and reading of culture
- 3- Identification of cultured organisms

Culture media :

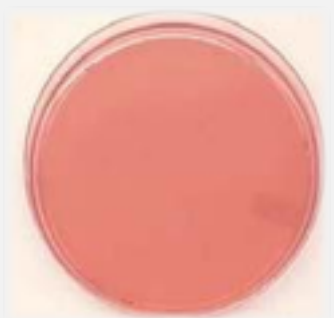


1-Blood Agar

Enriched culture medium, for culturing fastidious* microorganism and observing the hemolytic reaction (Beta,Alpha,Gama).

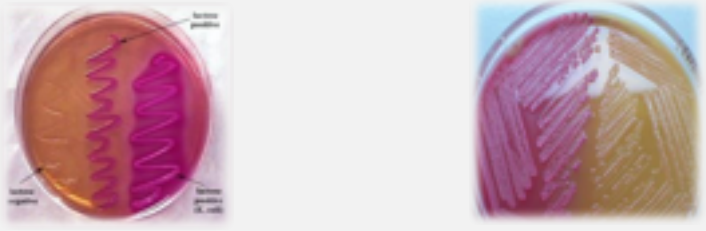


Useful for gram positive

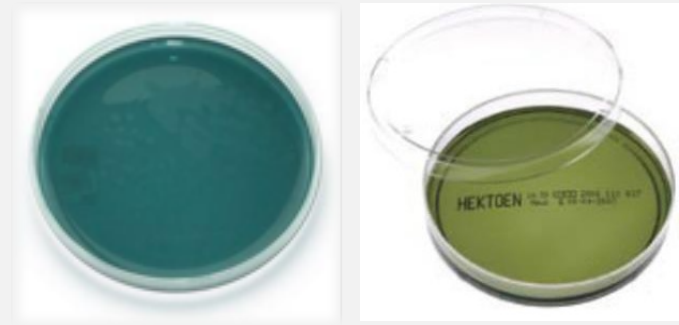


2-MacConkey Agar

Differential culture medium, showing both lactose and non- lactose fermenting colonies. LFC = Pink NLFC = Colorless or appear same as the medium



Useful for gram negative



3-CLED Agar

Selective culture medium, for detection and isolation of E. coli and coliform bacteria in urine.



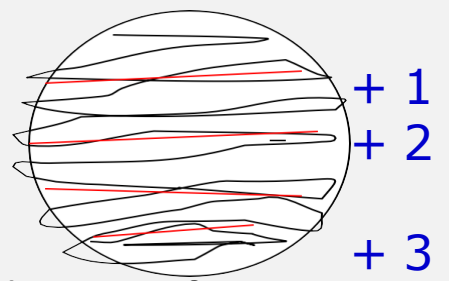
Useful for gram negative

*يعني تتطلب احتياجات خاصة ومعقدة من ناحية الغذاء عشان تنمو.

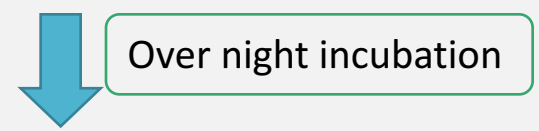
Urine inoculation:

Labartory examination of urin

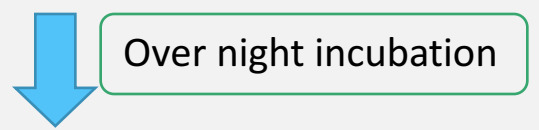
Quantitative (Colony counts):



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

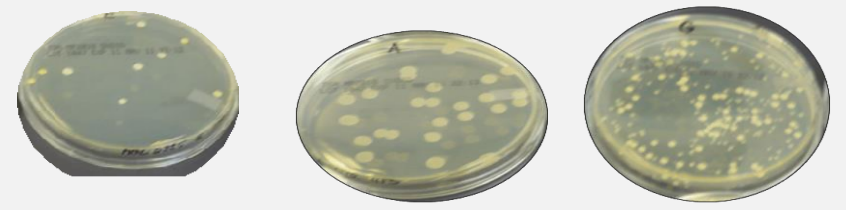
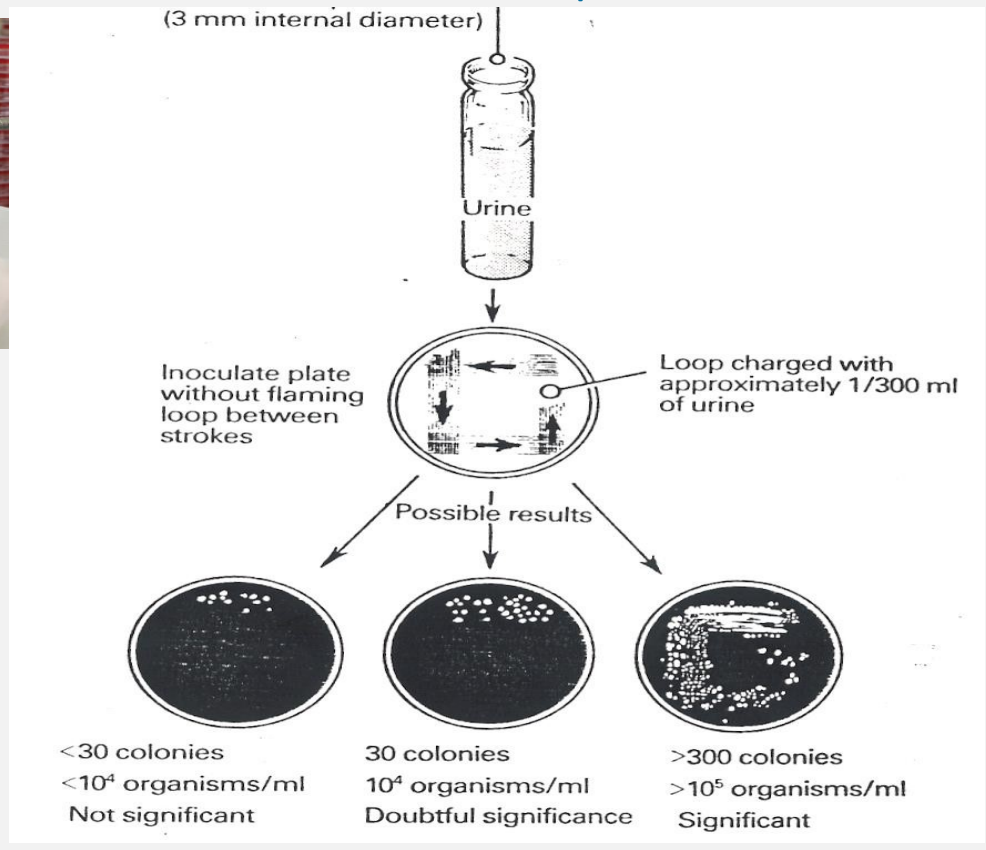


2. Isolation of colonies, Biochemical tests, Drug susceptibility test.



results

Smi-Quantitative Culture of Urine Sample



Quantitative (Colony counts):

1

A urine sample is streaked on surface of blood agar plate and Cled agar/Mc Conkey agar

2

Isolation of colonies








3

Results

ID of cultured organisms

We identify the causative agents by using these methods :

- Biochemical tests
- Type of hemolysis
- Serological tests

Organism	Gram Stain	Culture (colony)	Oxidase			
E.coli	Negative bacilli 	Lactose fermentative (pink)	- Indole +			
Klebsiella		Lactose fermentative (muroid)				
Proteus	Negative bacilli 	Lactose non-fermentative (colorless)	- Urease +			
Pseudomonas	Negative bacilli 	Lactose non-fermentative (greenish)	+			
S.saprophyticus	Cocci in clusters 	White (blood culture) 	catalase	coagulase	novabiocin	Bile Esculin
			+	-	R	N/A
Enterococcus	Cocci in chains 	Gray (blood culture) 	-	N/A	N/A	+

Etiological Agents of UTI:

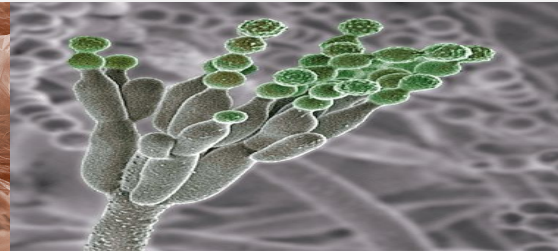
Bacteria



Parasite



Fungi



GRAM NEGATIVE	GRAM POSITIVE	Other
Escherichia coli	Enterococcus	Candida albicans
Klebsiella	Staphylococcus saprophyticus	Schistosoma haematobium
Proteus	Streptococcus agalactiae (group B)	Trichomonas vaginalis
Other Enterobacteriaceae (Enterobacter, Citrobacter....)	Staphylococcus aureus ¹ (Associated with staphylococemia)	
Pseudomonas aeruginosa		

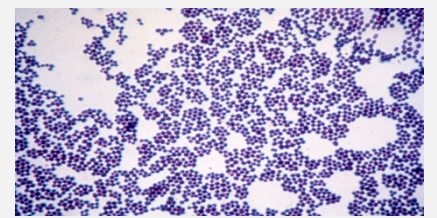
Etiological Agents of UTI:

Causes of UTI's	Outpatients (%)	Inpatients (%)
Escherichia coli	53-72	18-57
Coagulase negative Staphylococcus	2-8	2-13
Klebsiella	6-12	6-15
Proteus	4-6	4-8
Morganella	3-4	5-6
Enterococcus	2-12	7-16
Staphylococcus aureus	2	2-4
Staphylococcus saprophyticus	0-2	0.4
Pseudomonas	0-4	1-11
Candida	3-8	2-26

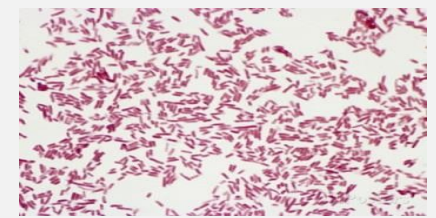
Complicated aetiology of UTI:

The Microorganism	Percentage %
<i>Escherichia coli</i>	21 – 54
<i>Klebsiella pneumoniae</i>	1.9 – 17
<i>Enterobacter species</i>	1.9 – 9.6
<i>Citrobacter species</i>	4.7 – 6.1
<i>Proteus mirabilis</i>	0.9 – 9.6
<i>Providencia species</i>	18
<i>Pseudomonas aeruginosa</i>	2 – 19
<i>Enterococci species</i>	6.1 – 23

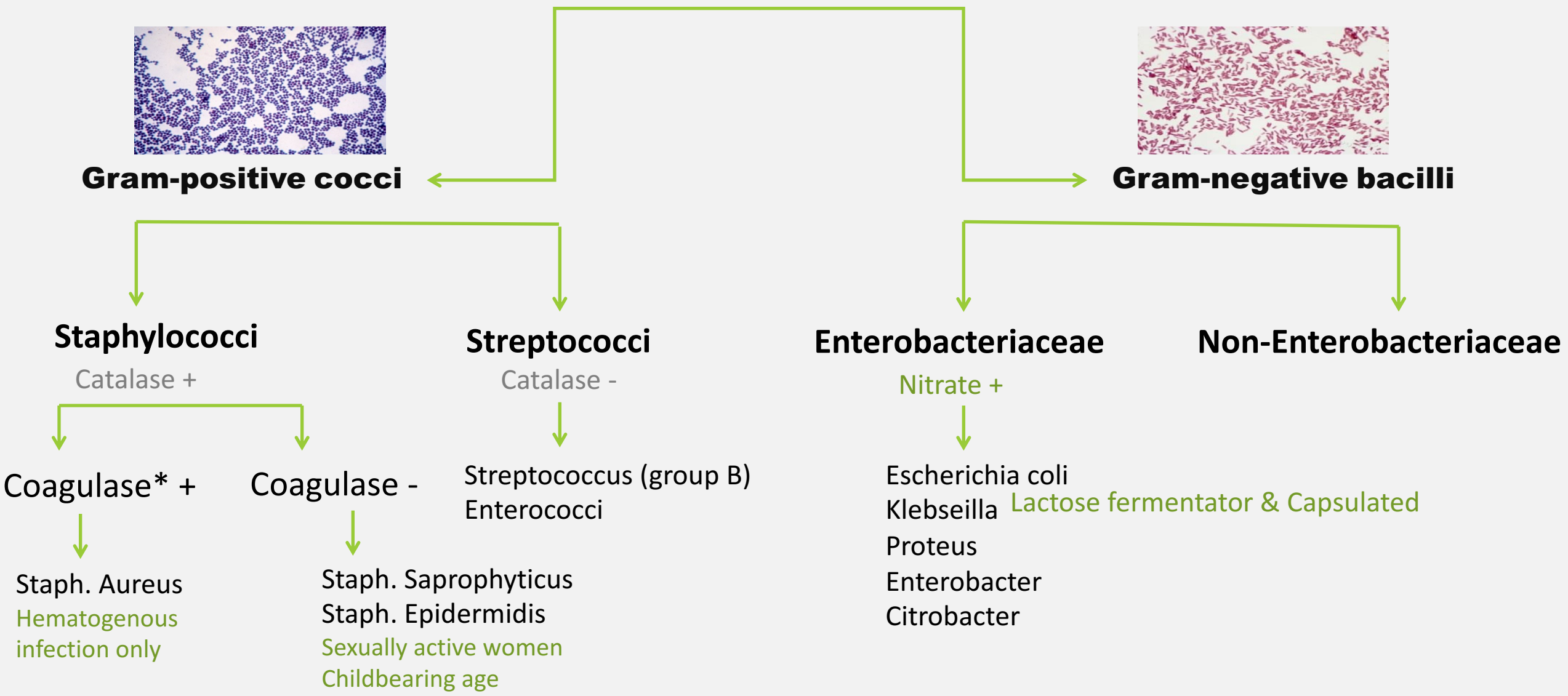
Etiological Agents of UTI:



Gram-positive cocci

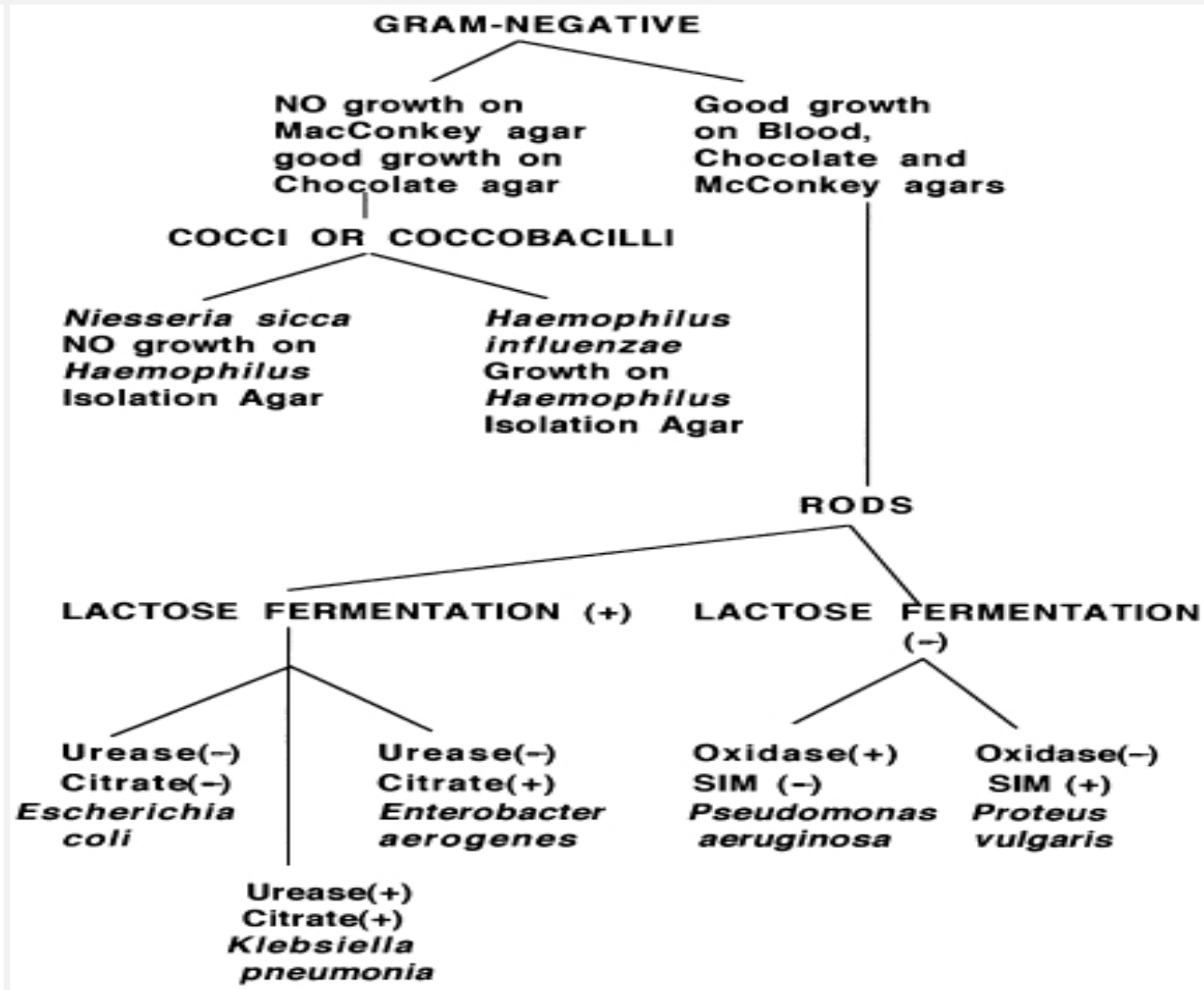
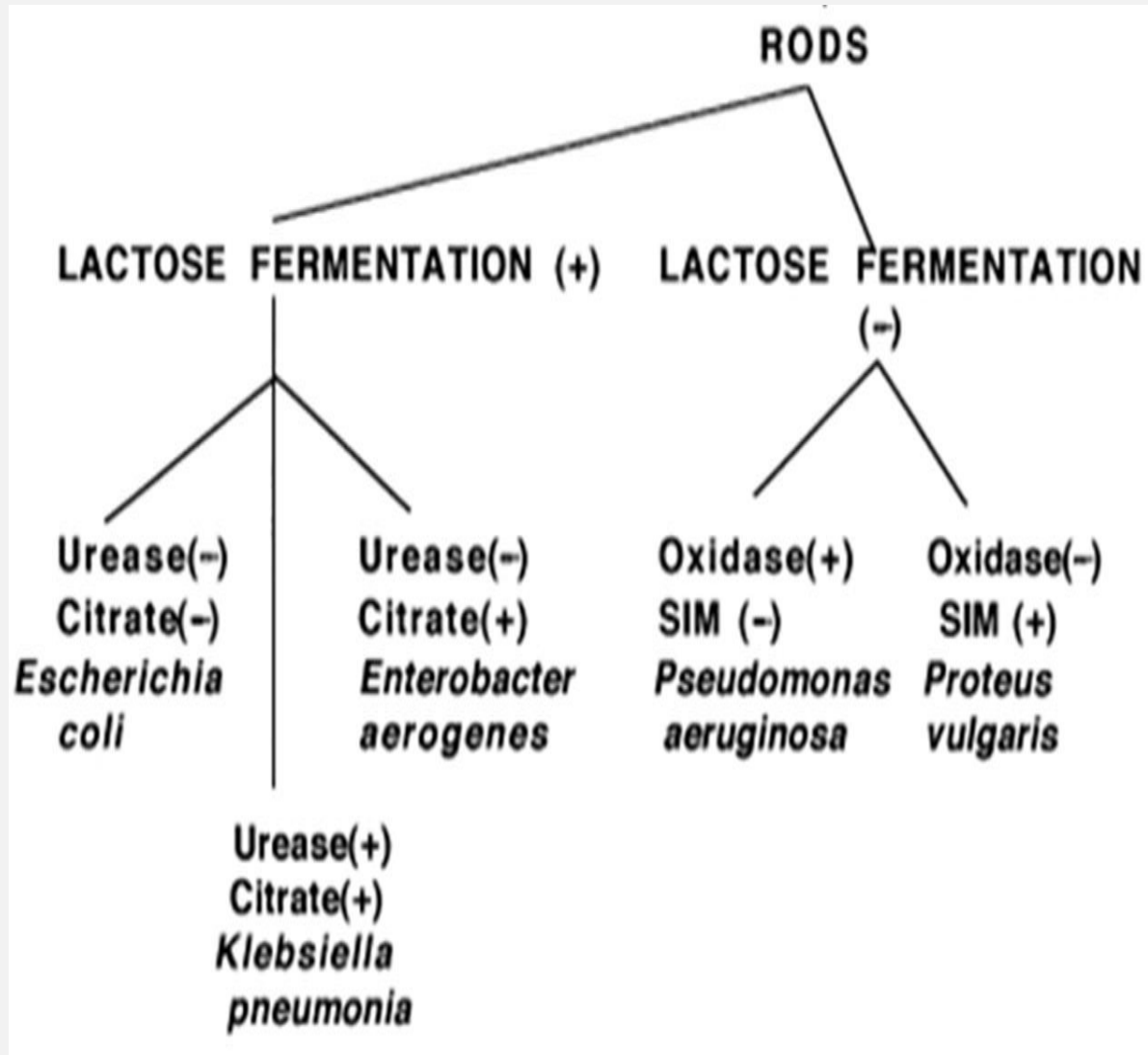


Gram-negative bacilli



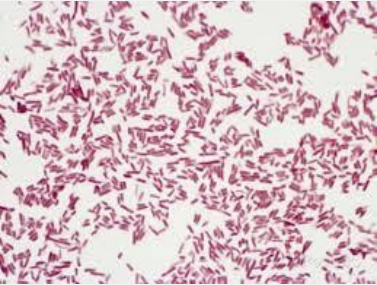
*Coagulase test done only for staph.

3-The Microorganism



Escherichia Coli

Morphology



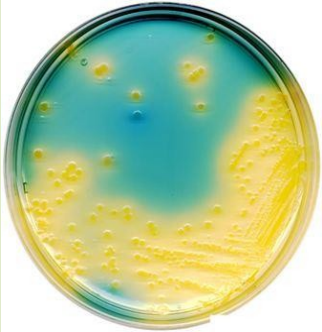
Microscopic appearance Gram negative bacilli

Culture



MacConkey agar showing growth of Lactose fermenter Pink colonies

LFC



CLED* agar showing growth of Lactose fermenter yellow colonies

LFC

Identification

Indole Reactions Test: Positive



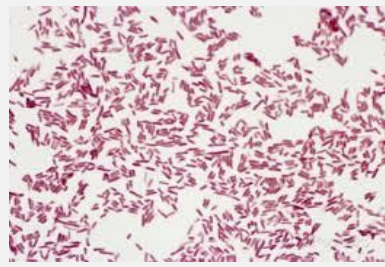
APE 20 E test***

*CLED= Cystine lactose electrolyte deficient agar.

Klebseilla spp.

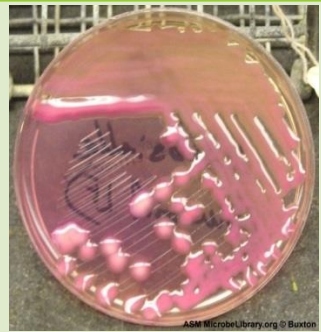
(Klebsiella pneumoniae)

Morphology



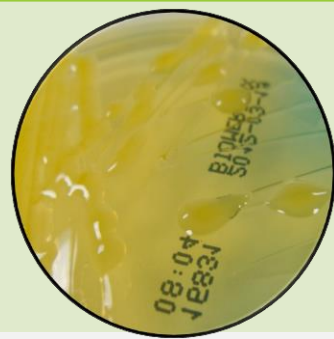
Microscopic appearance Gram negative bacilli

Culture



MacConkey agar showing growth of Lactose Fermenter Mucoid Pink colonies

LFC



CLED agar showing growth of Lactose fermenter Mucoid yellow colonies

LFC

Identification

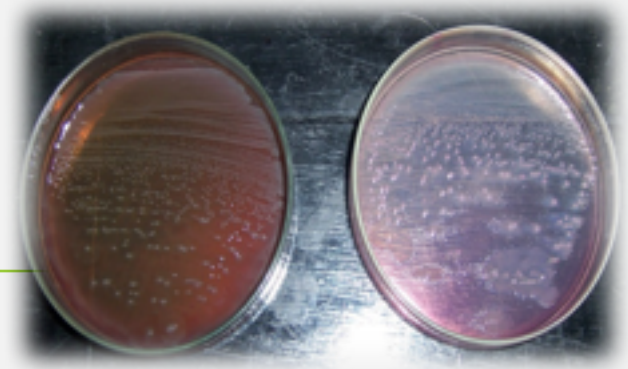
*Indole Reactions Test: Negative



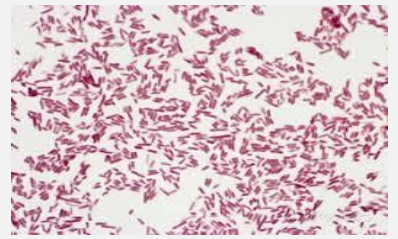
APE 20 E test

*The **indole test** is a biochemical test performed on bacterial species to determine the ability of the organism to convert tryptophan into the indole.

Proteus spp.

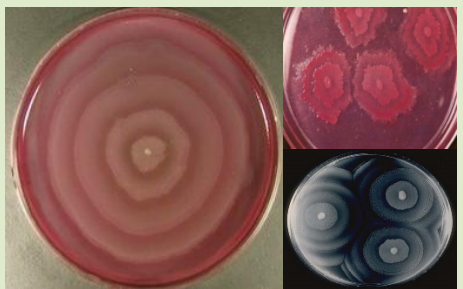


Morphology



Microscopic appearance Gram negative bacilli

Culture



Blood culture plate showing warm of Proteus



CLED {cystine-lactose-electrolyte-deficient} Inhibits The Proteus Swarming

Identification



Proteus is * Urease positive. Urease splits urea into ammonia and alkalizes the urine with production of crystals.

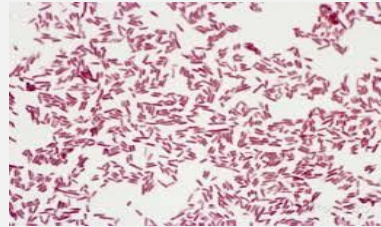


APE 20 E test

*Urease test, is a rapid diagnostic test for diagnosis of Helicobacter pylori. The basis of the test is the ability of H. pylori to secrete the urease enzyme, which catalyzes the conversion of urea to ammonia and carbon dioxide.

Pseudomonas spp

Morphology



Microscopic appearance Gram negative bacilli

Culture

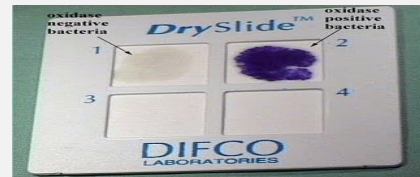


MacConkey agar showing growth of Non-Lactose Fermenter
Mucoid Pink colonies
LFC

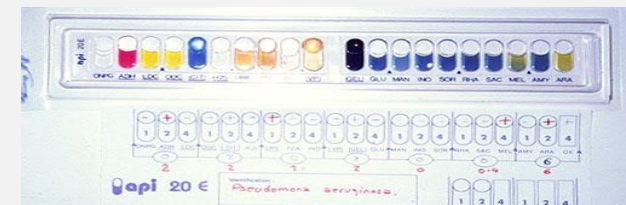


Nutrient Agar showing growth of Pseudomonas pigmentation

Identification



*Oxidase positive test



APE 20 E test

*The **oxidase test** is used to identify bacteria that produce cytochrome c **oxidase**, an enzyme of the bacterial electron transport chain. (note: All bacteria that are **oxidase positive** are aerobic).

Three API 20E strips :

Only in Male's slides

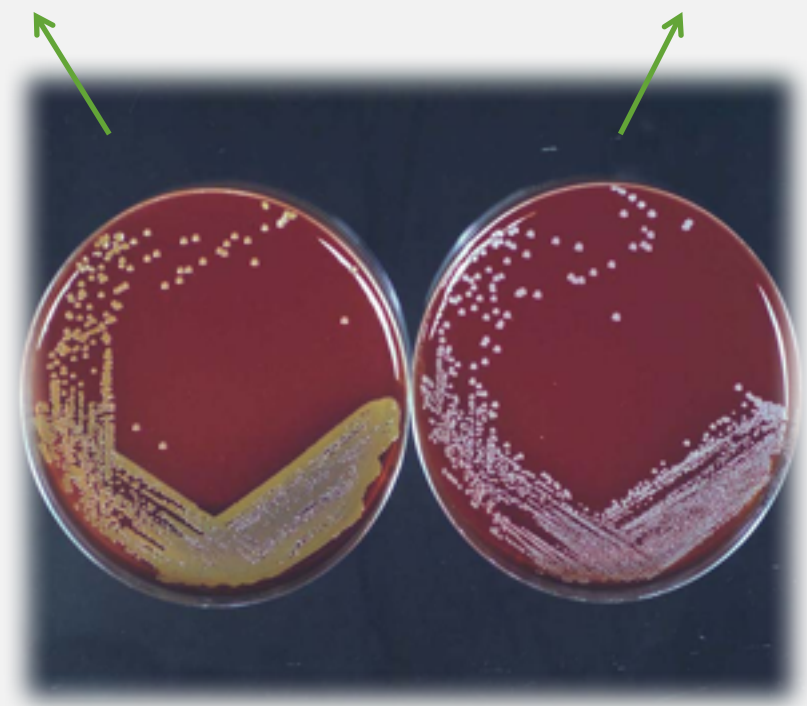


76 Three API 20E strips: (a) immediately after inoculation, (b) after 24 hours incubation, (c) that in (b) after the addition of reagents to certain wells. The organism here is *Escherichia coli*. Here the first carbohydrate well (glucose) is also used for the nitrate reduction test.

- 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.

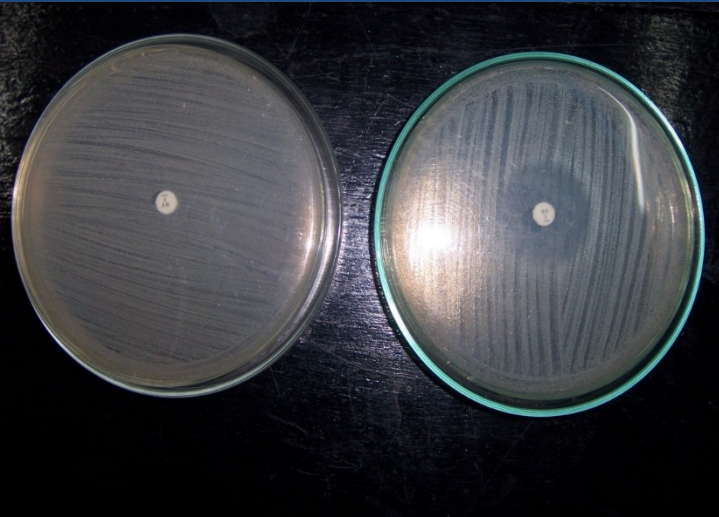
Staphylococcus aureus
Golden colonies (yellowish)

Staphylococcus epidermidis
white colonies



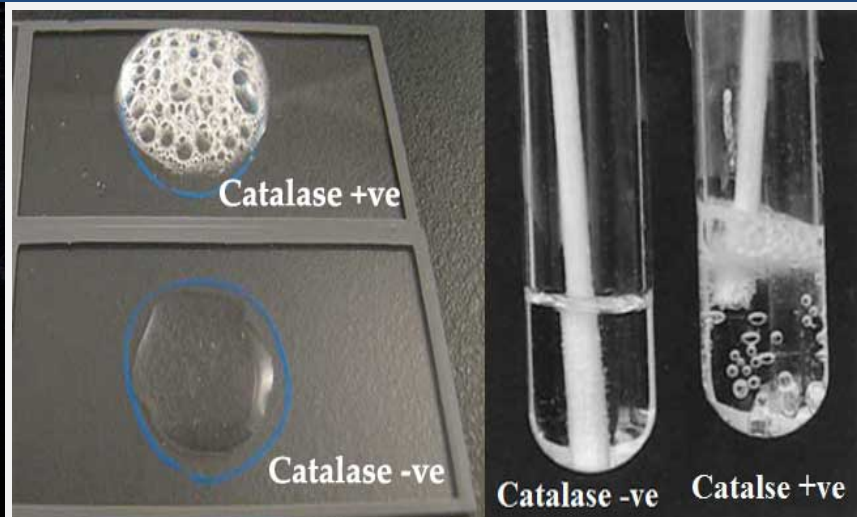
Staphylococcus spp.

NOVOBIOCIN TEST



Staphylococcus saprophyticus (resistant- Novobiocin) **Staphylococcus epidermidis (sensitive- Novobiocin)**

CATALASE TESTextra**



Catalase +ve **Catalase -ve**

COAGULASE TEST



S. aureus

COAGULASE TEST

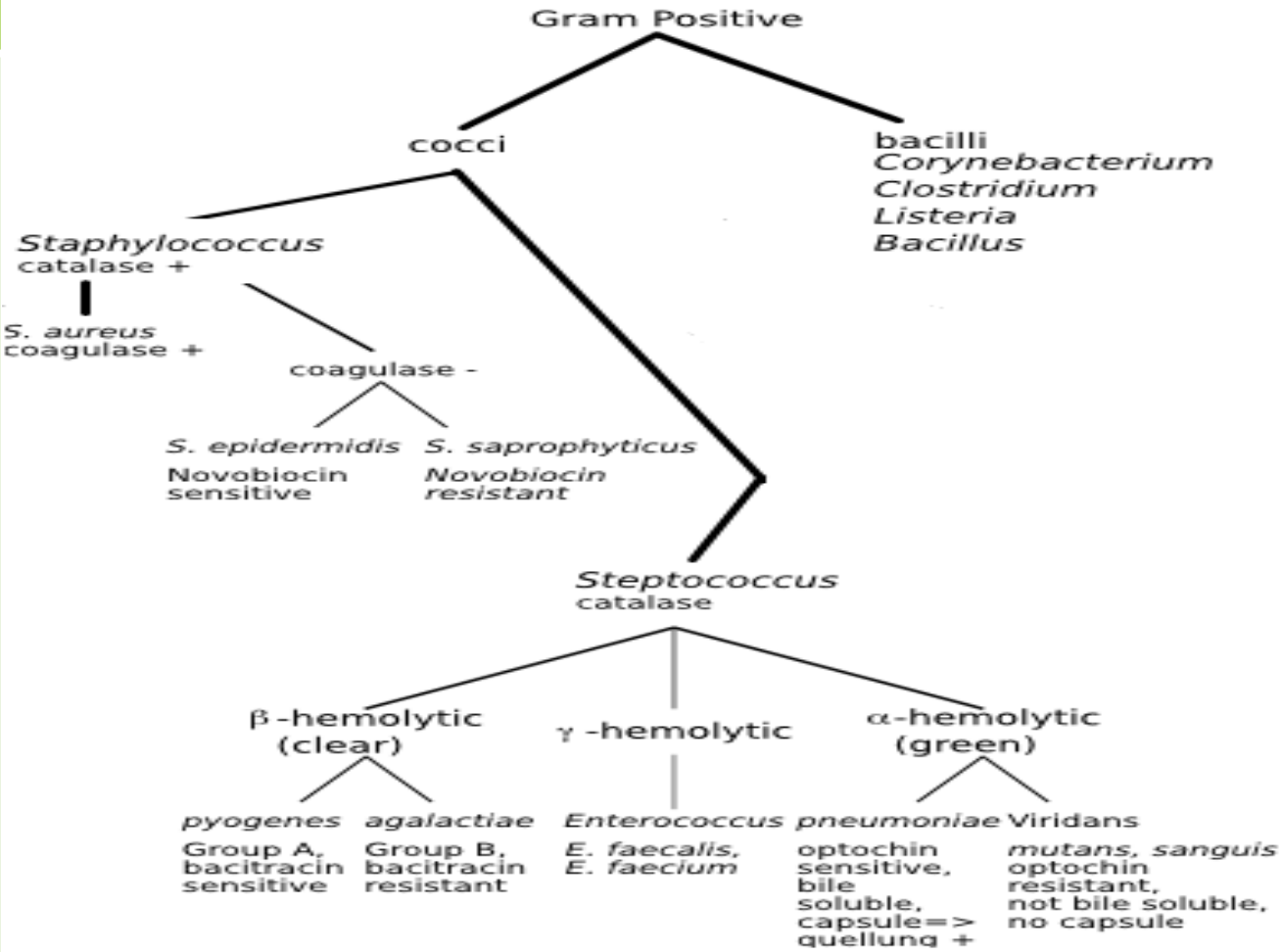
POSITIVE **NEGATIVE**

Coagulase is a protein enzyme made by several *microorganisms* that enables the conversion of fibrinogen to fibrin. In the laboratory, it is used to distinguish protein enzyme produced between different types of *Staphylococcus* isolates. Importantly, *S. aureus* is generally coagulase-positive, meaning that coagulase negativity usually excludes *S. aureus*. **extra

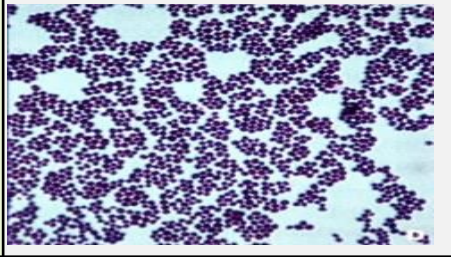


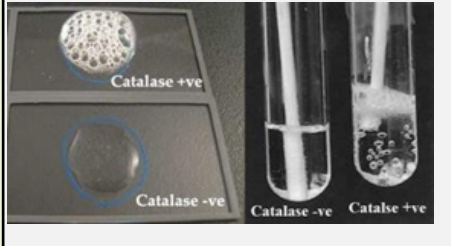
*Coagulase test is used to differentiate *Staphylococcus aureus* (positive) from *Coagulase Negative Staphylococcus* (CONS). *Coagulase* is an enzyme produced by *S. aureus* that converts (soluble) fibrinogen in plasma to (insoluble) fibrin.

Gram Positive Cocci

Gram Positive Cocci		
staphylococci	Streptococcus (group B)	enterococci
Coagulase- positive (Staph. aureus) very dangerous		
Coagulase negative (Staph. saprophyticus)		
Coagulase negative (Staph. epidermidis)		

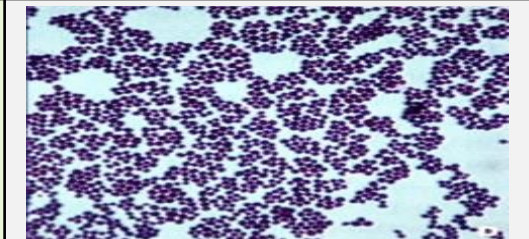
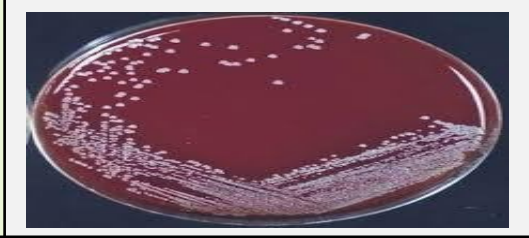

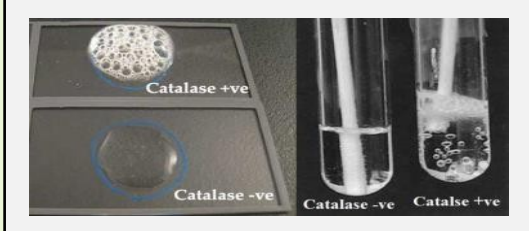



Staph. aureus

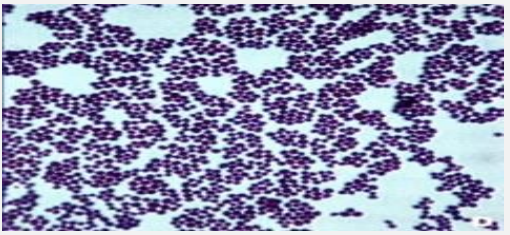


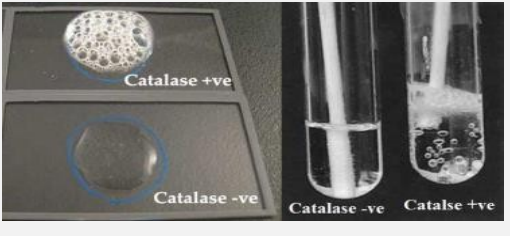
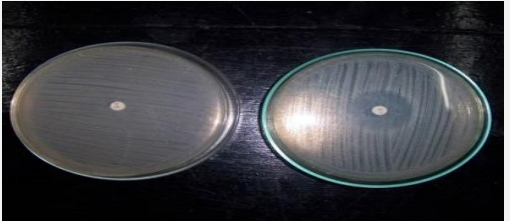
Microscopic appearance		Gram positive cocci in clusters
culture		Blood culture plate showing growth of <u>golden yellow</u> colonies
tests		Coagulase test = Positive
		Catalase test = Positive

Staph. epidermidis


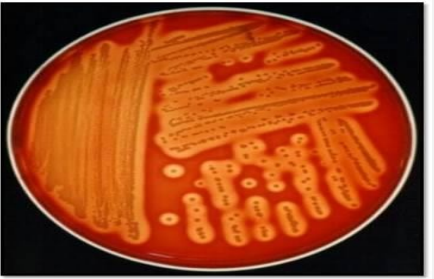
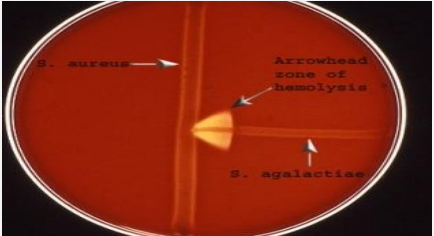
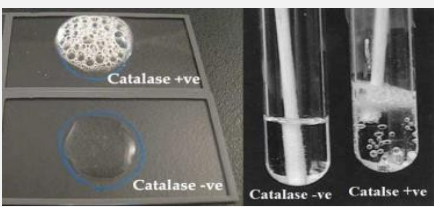

Rarely causes UTI

Microscopic appearance		Gram positive cocci in clusters
culture		Blood culture plate showing growth of <u>white</u> colonies
tests		Coagulase test Negative
		Catalase test = Positive
		Novobiocin test Sensitive

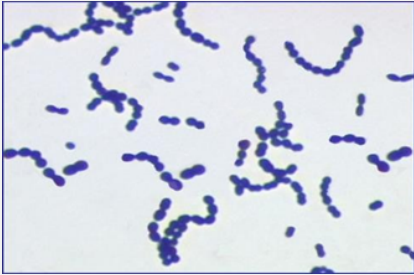

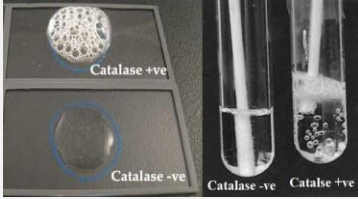

Staphylococcus saprophyticus

Microscopic appearance		Gram positive cocci in clusters
culture		Blood culture plate showing growth of white colonies
tests		Coagulase test = Negative
		Catalase test = positive
		Novobiocin Test Resistant أهم اختبار لأنه يفرقها عن ستاف ابديرمس

Streptococcus agalactiae (group B) Seen in pregnant and neonate

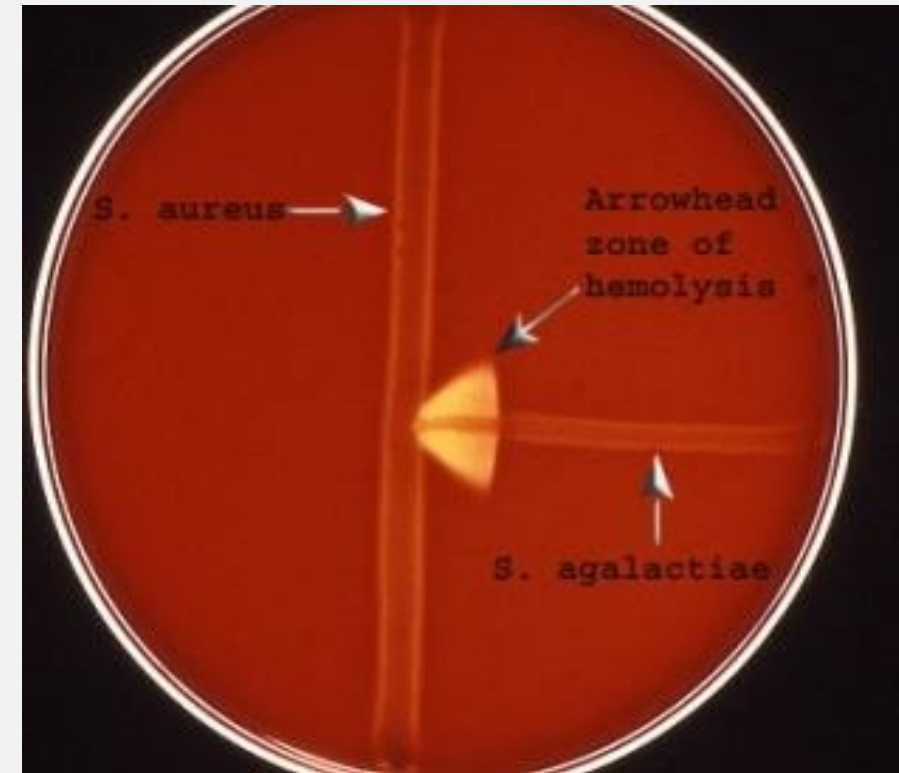
Microscopic appearance		Gram positive cocci in chains
culture		Blood culture plate showing growth of Beta-haemolytic colonies
tests		CAMP test positive
		Catalase test = Negative
		Streptics: Mix bacterial colony with various group-specific antisera on a slide

Enterococci

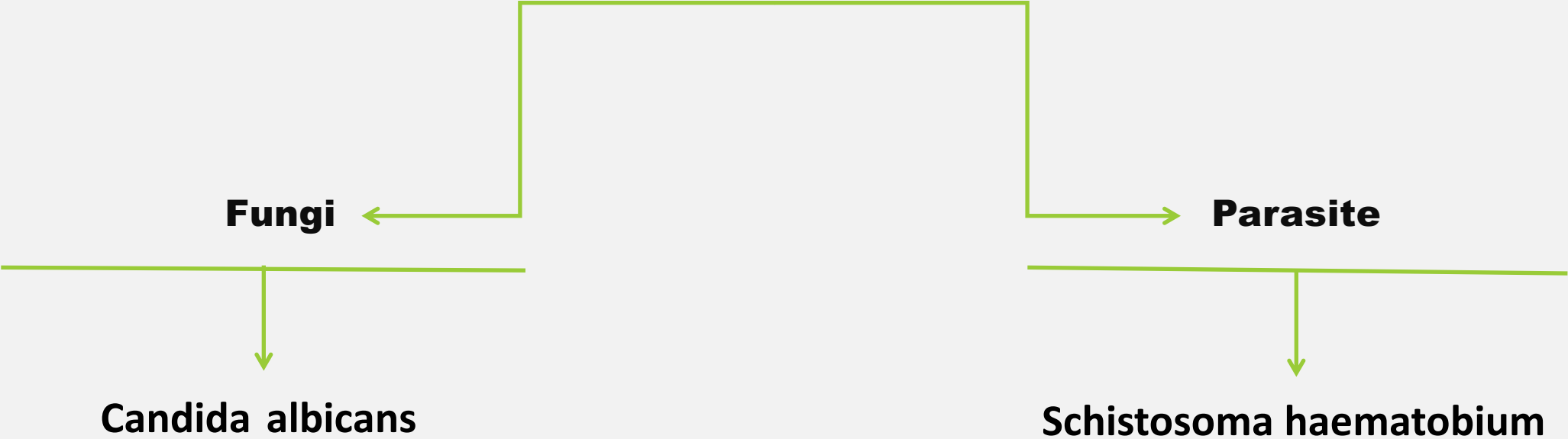
Microscopic appearance		Gram positive cocci in chains
culture		Blood culture plate showing growth of Beta-haemolytic colonies
tests		Catalase test = Negative
		positive bile Esculin hydrolysis test

Both Group D streptococci and enterococci produce a positive (left) bile Esculin hydrolysis test.

From the previous slide: * to see it better*



Fungi and Parasites causing UTI:



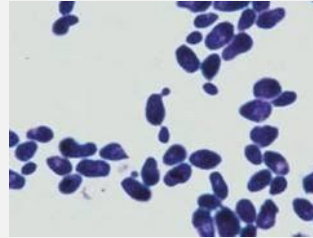
It is a type of yeast that is dimorphic fungus since it grows both as yeast and filamentous cells.



(urine: eggs 115-170 x 45-65 micrometres)
(primates)

Candida albicans

Morphology



Gram positive cocci in chains

Culture



Candida albicans on blood agar



Candida albicans on Sabouraud's Dextrose Media (SDA)

Identification



Chlamydo-spore= test Positive



Germ tube test Positive*

*The bacteria elongates after applying the test.

Antimicrobial Susceptibility Testing (AST):

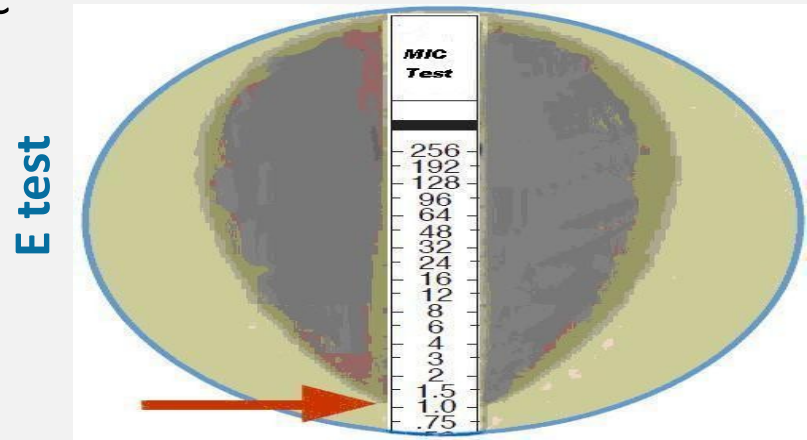
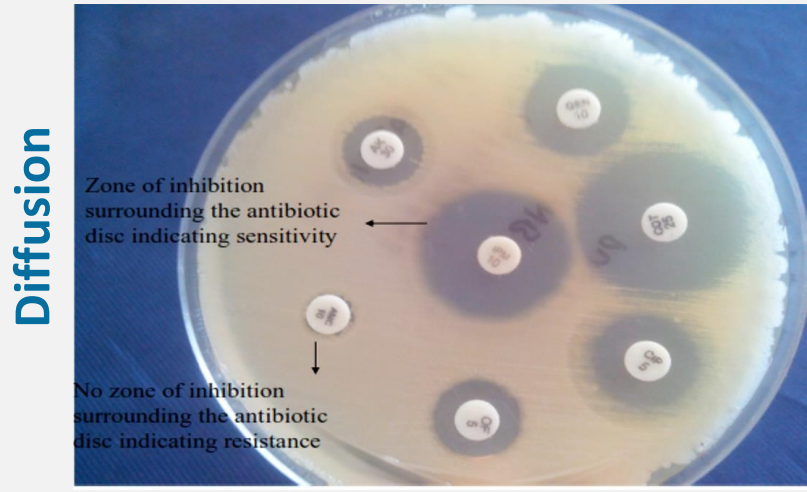
Methods of AST:

○ Disk (agar) Diffusion Method:

هذا الاختبار ببساطة فيه سترب في النص يحتوي على انتي بايوتك بتكيز معين اللي هو 1 وكل ما ارتفع التركيز عن رقم واحد معناه ان الاورقانزم أكثر ريزيستانس + هذا الاختبار دقيق جدا

○ E test:

- Is a well-established method for antimicrobial resistance testing in microbiology laboratories.
- Consists of a predefined **gradient of antibiotic concentrations** on a plastic strip
- Used to determine the Minimum Inhibitory Concentration (MIC) of
 - Antibiotics
 - Antifungal agents
 - Antimycobacterial agents



يحتوي على antibiotic واحد لكن ب concentration مختلف

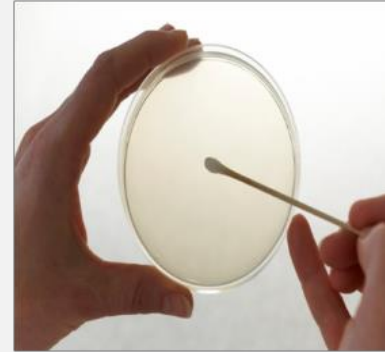
The Antibiotic Sensitivity Testing Method:



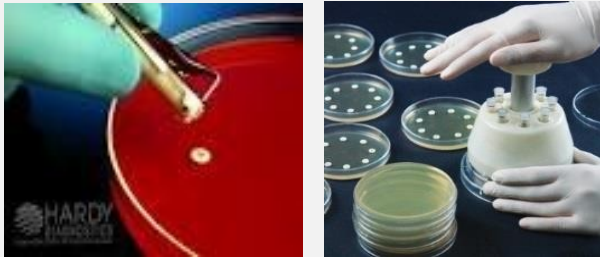
1- Select well-isolated colonies



2- Inoculum suspension



3- Spread the inoculum

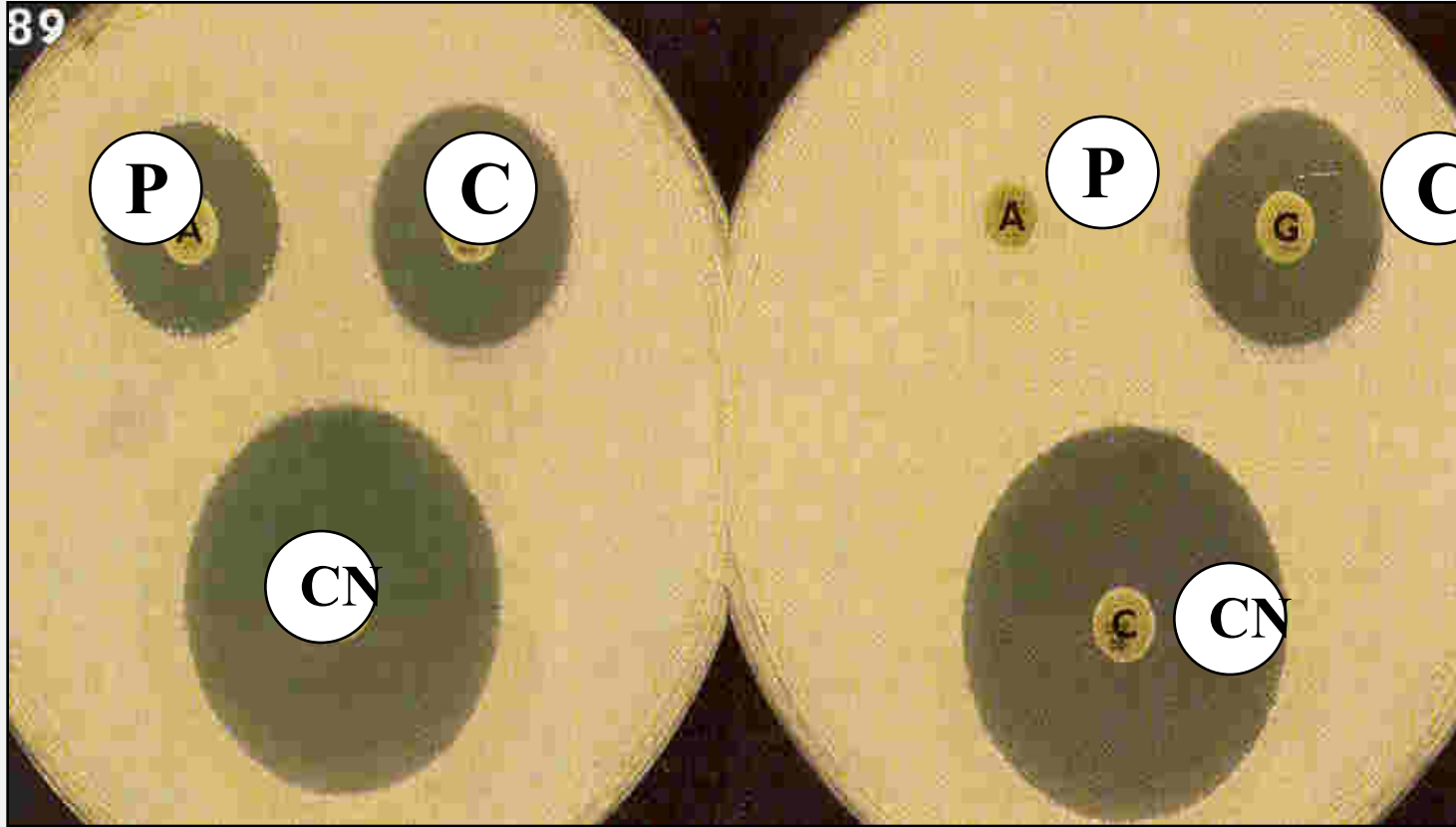


4-Apply antibiotics disks



5- Read the result

Staphylococcus aureus



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

R (Resistant) ; S (Sensitive)

الدكتورة قالت : احنا مراح ندخلكم بالانتي بايوتكس , بيكون describe the stain + the growth
بس مراح يكون دييب But maybe we will ask about the drug of choice

Antibiotic Susceptibility Test:

- Enterobacteriaceae:
- First line UTI treatment:
 - Ampicillin
 - TMP/SMX
 - Ciprofloxacin
 - Gentamicin
 - Amikacin
 - Nitrofurantoin

***You can READ it**

- Pseudomonas spp:

Anti-pseudomonal antibiotics

■ Beta-lactams

- Cefepime
- Piperacillin/tazobactam
- Imipenem
- Meropenem
- Aztreonam
- Ceftazidime
- Ticarcillin/clavulanate

■ Fluoroquinolones

- Ciprofloxacin (PO) (IV)
- Levofloxacin (PO) (IV)

■ Aminoglycosides

- Gentamicin
- Tobramycin

Green- formulary item, no restrictions

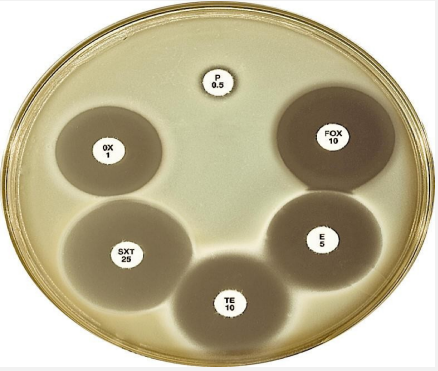
Yellow- formulary but requires ID approval

Red- non-formulary item



Antibiotic Susceptibility Test:

- Staph. Aureus: *You can READ it



Methicillin Sensitive Staph Aureus (MSSA)

www.microbiologyinpictures.com

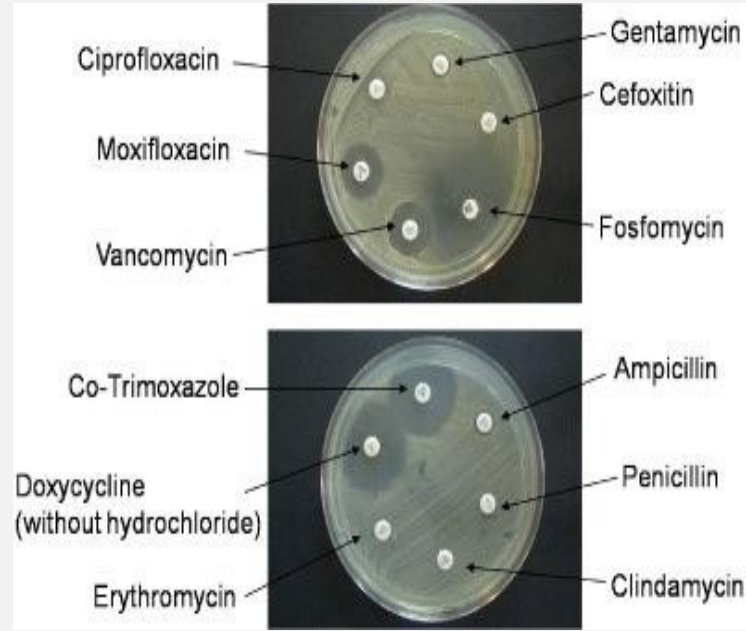


strain A
Staphylococcus aureus

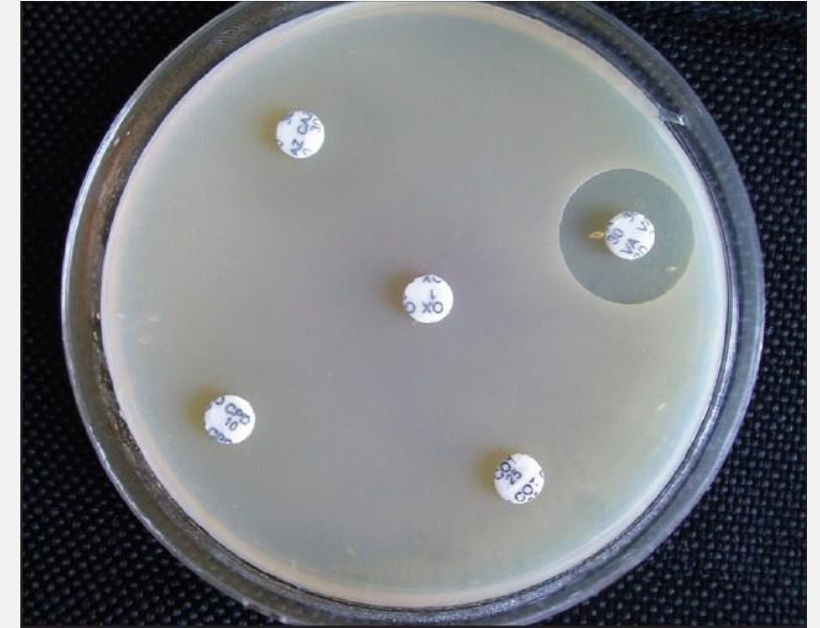


strain B
Staphylococcus aureus
methicillin-resistant
MRSA

- MRSA *You can READ it



- Staph. saprophyticus



Novobiocin Test Resistant

Bacterial Resistance to UTI Antibiotics:

- **Nitrofurantoin**
 - ✓ E. coli <5%
 - ✓ Other uropathogens 15-20%
 - ✓ Not active against:
 - Proteus,
 - some Enterobacter
 - Klebsiella
- **TMP-SMX (Bactrim)**
 - ✓ 10-22% varies geographically
- **Fluoroquinolones (Cipro)**
 - ✓ therapy for acute uncomplicated cystitis
- **Ampicillin**
 - ✓ 30% of E. coli resistant

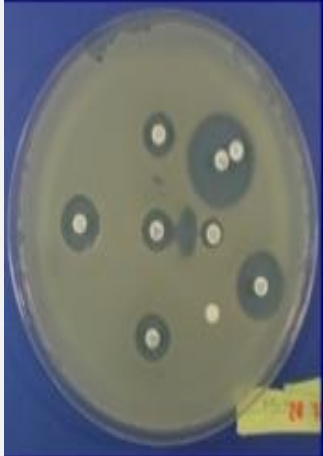
Results interpretation:

Only in female's slides

*You can READ it

- high probability of UTI requiring treatment
- If midstream clean catch positive for
 - ✓ pyuria
 - ✓ microscopic hematuria, with or without positive nitrites
 - ✓ + UTI symptoms
- Presence of pyuria without bacteria on culture (sterile pyuria) rule out:
 - ✓ Tuberculosis
 - ✓ Interstitial cystitis (IC)
 - ✓ Chlamydia urethritis
 - ✓ Kidney disease (stone, glomerulonephritis)

ESBL Confirmatory Methods

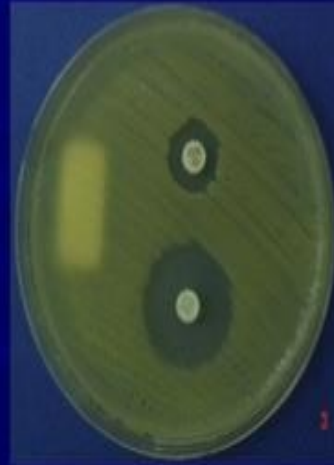


Kirby-Bauer disc
diffusion synergy



E-test
OR MIC

≥ 4 -fold increase



Kirby-Bauer disc
augmentation

$\Delta \geq 5$ mm

Examples of Bacteria of Clinical Importance

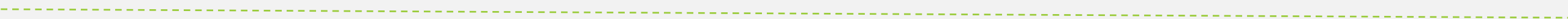
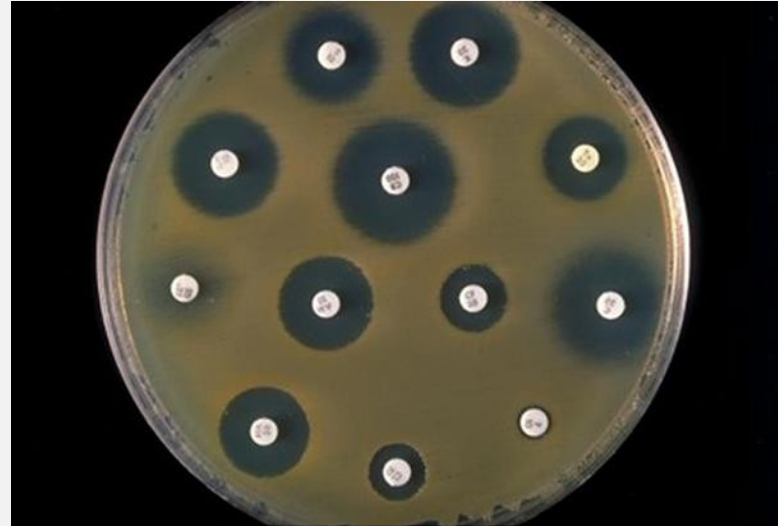
- MRSA - methicillin/oxacillin-resistant *Staphylococcus aureus*
- VRE - vancomycin-resistant enterococci
- ESBLs - extended-spectrum beta-lactamases (which are resistant to cephalosporins and monobactams)
- PRSP - penicillin-resistant *Streptococcus pneumoniae*

■ Dr.T.V.Rao MD

Emerging resistant to Ampicillin and TMP/SMX *You can READ it

Antibiotic	MIC	Interpretation
Ampicillin	≥ 32	R
Cephalexin	≤ 4	S
TMP/SMX	≥ 2	R
Gentamicin	≤ 8	S
Imipenem	≤ 1	S

Organism E coli
 Source urine



Extended spectrum β -lactamase (ESBL) producing E coli:

*You can READ it



Antibiotic	MIC	Interpretation
Ampicillin	≥ 32	R
Cephalexin	≥ 46	R
TMP/SMX	≥ 2	R
Gentamicin	≤ 8	S
Ceftriaxone	≥ 46	R
Ceftazidime	≥ 46	R

ESBL positive E coli

*You can READ it

Extended Spectrum Beta Lactamase [ESBL]

- Enzymes that are produced by Gram negative bacteria
 - Confer resistance to Cephalosporins, Penicillins and Monobactam (Aztreonam) by opening the beta lactam ring inactivating the antibiotic
 - Cannot attack cephamycins (cefoxitin, cefotetan) or the carbapenems (imipenem, meropenem, ertapenem, doripenem)
 - Generally susceptible to beta-lactamase inhibitors (tazobactam)
- Plasmid mediated TEM, SHV, CTX-M beta lactamases are the most common
- Therapy for ESBL producing gram negative rods:
 - Carbapenems: Imipenem, Meropenem, Doripenem, Ertapenem
 - Piperacillin/Tazobactam – Tazobactam blocks beta lactamase action

Case 1

Only in Male's slides

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.

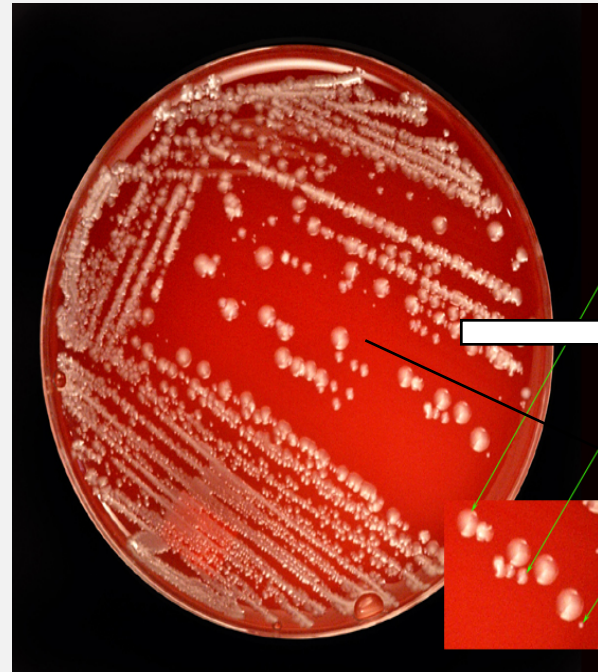
- Identify the colonies on the blood agar plates and photographs.

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

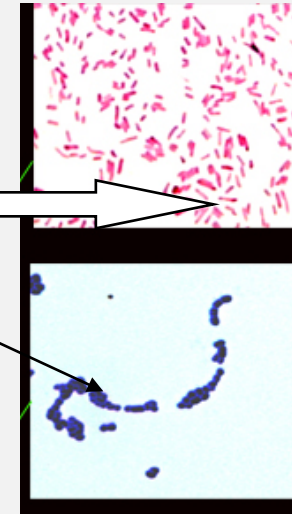
- Large colonies are Gram **(Negative)** .and small colonies are Gram **(positive)**



CLED plate



Blood agar



Gram stain

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 mirabilis

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

Urease test

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

Urease splits urea into ammonia; and alkalinizes the urine with production of crystals

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

A) Bacteria

B) Parasites

C) Fungi

Only in Male's slides



GOOD LUCK!

MICROBIOLOGY TEAM:

- Nawaf alkhudhayri (leader)
- Yousef aljebrin
- Saud alshenafi
- Mohammed alghandour
- Meshal alayidi
- Shrooq Alsomali (leader)
- Hanin bashaikh
- Nada aldakheel

[The Editing File](#)

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Reem alshathri - Heba alnasser

We are waiting for your feedback



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