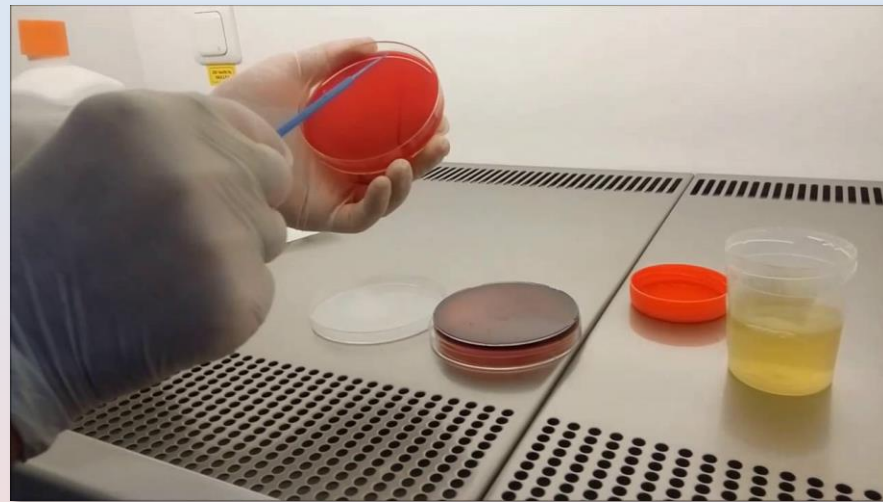


# **URINARY TRACT INFECTION**

## **Microbiology Practical Class**

**Renal System Block  
First Year**



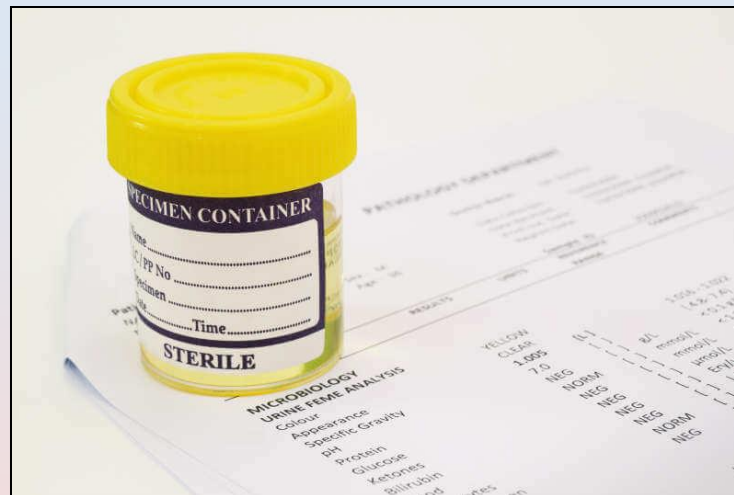
**Dr. Fawzia Al-Otaibi**

# Objectives

**It is expected that by the end of this practical class, students should be able to:**

- 1. 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.**

# SPECIMEN COLLECTION



# **TYPE OF SPECIMENS**

- **Midstream urine (MSU)**
- **Adhesive bag**
- **Suprapubic Aspiration**
- **Catheter sample**

# TYPE OF SPECIMENS

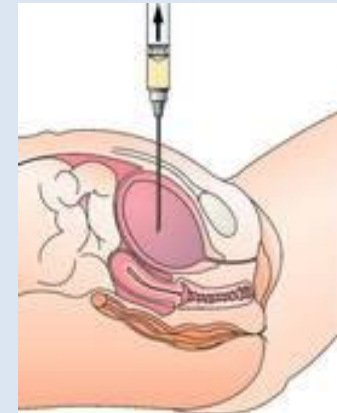
- The urine collected in a wide mouthed container from patients
- A mid stream specimen is the most ideal for processing
- Female patients passes urine with a labia separated and mid stream sample is collected



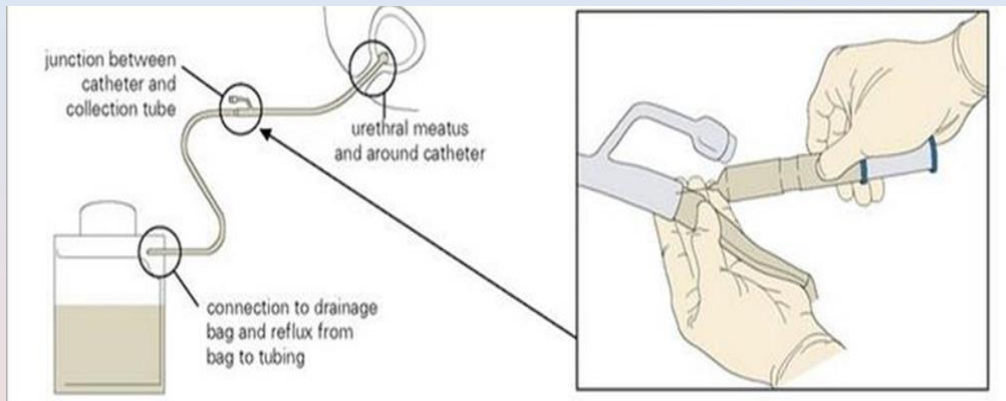
**Midstream Urine (MSU)**



**Adhesive bag**



**Suprapubic Aspiration**



## **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.

**Catheter sample**

# Sterile Urine Container



## Dip slides

One side is CLED media, the other can be MacConkey (MAC) agar or blood agar.

# SPECIMEN PROCESSING



# **Specimen processing:**

## **1. Urine analysis**

- **Microbiological**
- **Biochemical**

## **2. Culture and identification (ID)**

## **3. Antimicrobial Susceptibility testing**

## **4. Results interpretation**

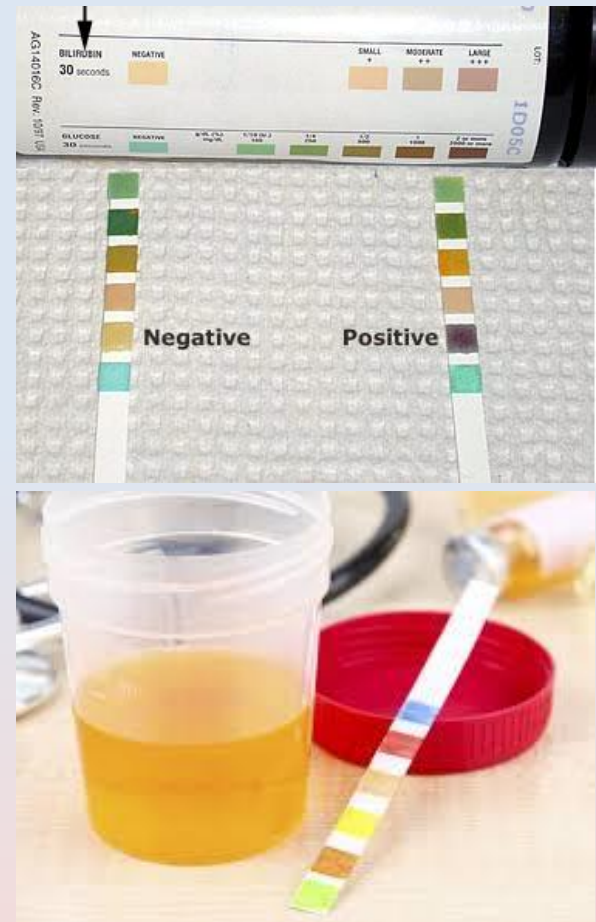


# 1. Urine Analysis

- **Biochemical**
- **Microbiological**
  - Macroscopic**
  - Microscopic**

# ➤ Biochemical Urine Examination (Dip stick)

- leukocyte esterase
- Nitrate test
- PH
- Glucose
- Bilirubin
- Protein



## Testing for UTI

### ❑ **Midstream clean catch with dipstick analysis**

❑ **Nitrite +** → 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

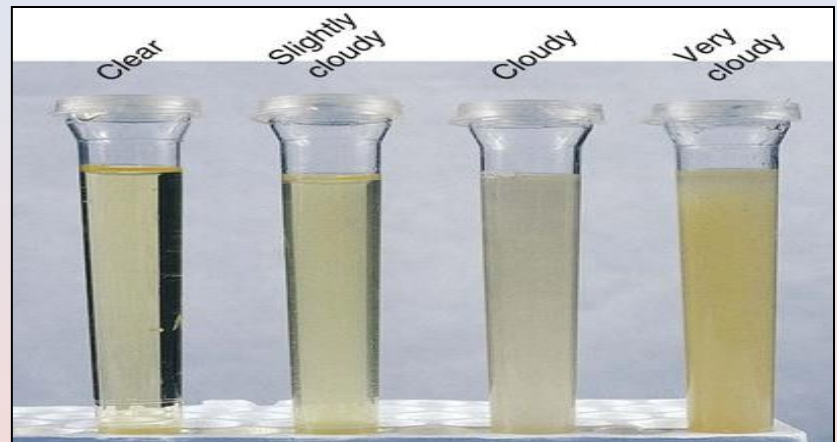
❑ **Leukocyte esterase +** → indicates presence of white blood cells, (sensitivity 75-95%, specificity 94-98%) –

❑ Dipstick results may be affected by medications/dyes, ie pyridium, nitrofurantoin, metronidazole, bilirubin, methylene blue, Vitamin B complex

# ➤ Physical Urine Examination

## ☐ Macroscopic

- Color
- Odor
- Turbidity

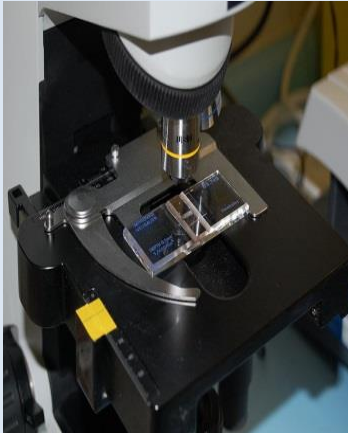


# Microbiological Urine Examination

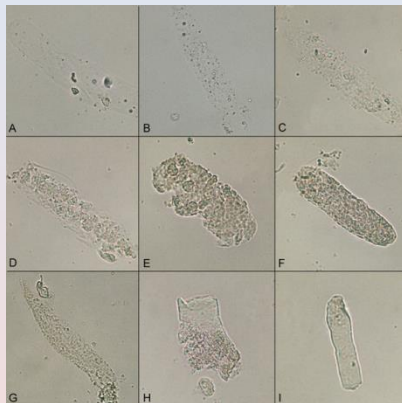
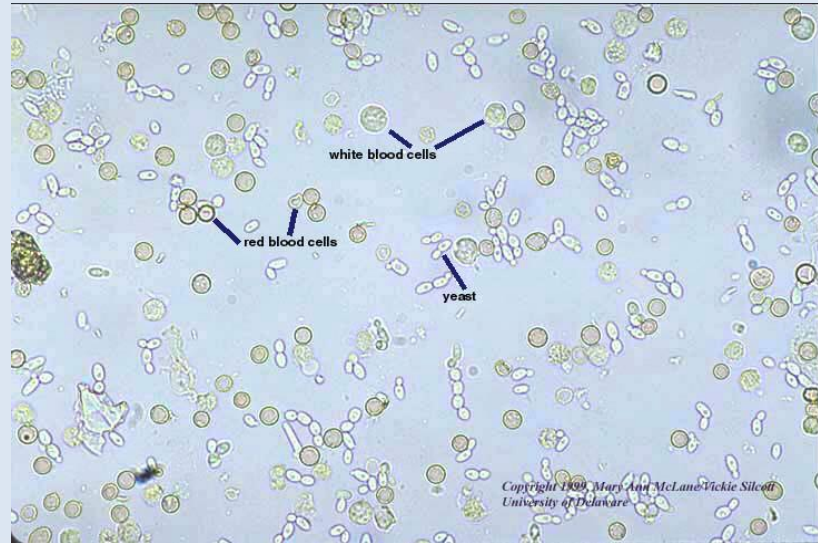
## □ Microscopic:

- **Cell-counting (WBC, RBC)**
- **Parasite (Ovum, Trichomonas, yeast)**
- **Casts**

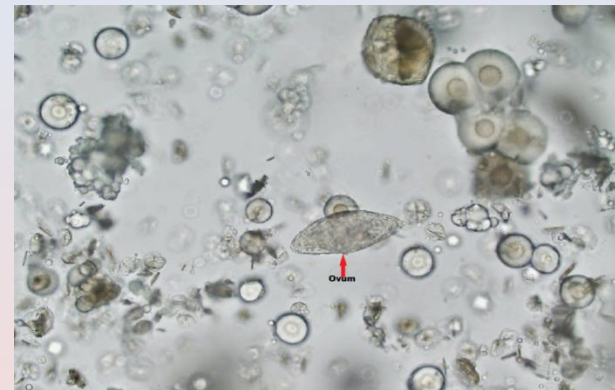
# Microscopic Urine Examination (WET MOUNT)



**cell-counting (WBC, RBC)**



**Casts**



**Parasite**

# 2. CULTURE AND ID



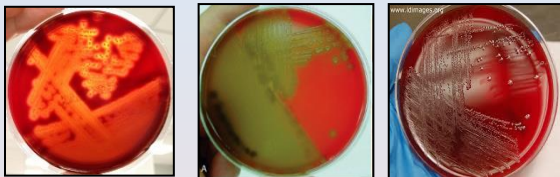
# **Culture and Identification**

- **Culture media**
- **Urine inoculation and reading of culture**
- **Identification of cultured organisms**



# ➤ Culture Media Routinely Used for Urine Culture

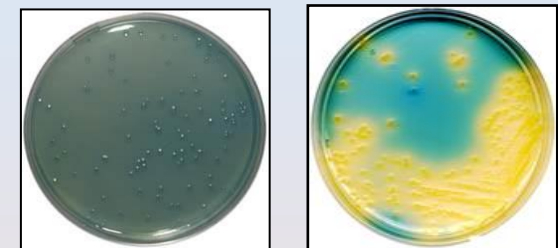
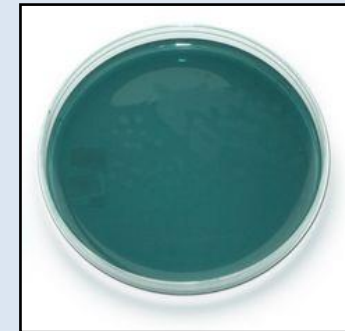
## Blood Agar



## MacConkey Agar



## CLED Agar

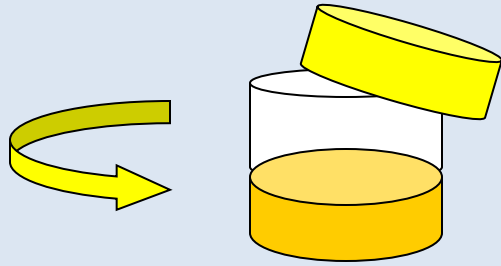


Enriched culture medium, for culturing fastidious organism and observed the hemolytic reaction(Beta,Alpha,Gama).

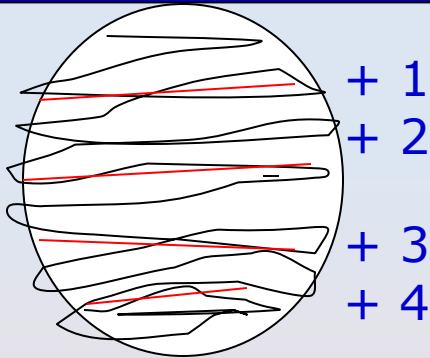
Differential culture medium, showing both **lactose** and **non-lactose fermenting** colonies.  
LFC = **Pink** NLFC = **Colorless** or appear same as the medium.

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

# ➤ Urine Inoculation



## Quantitative (Colony counts)



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.

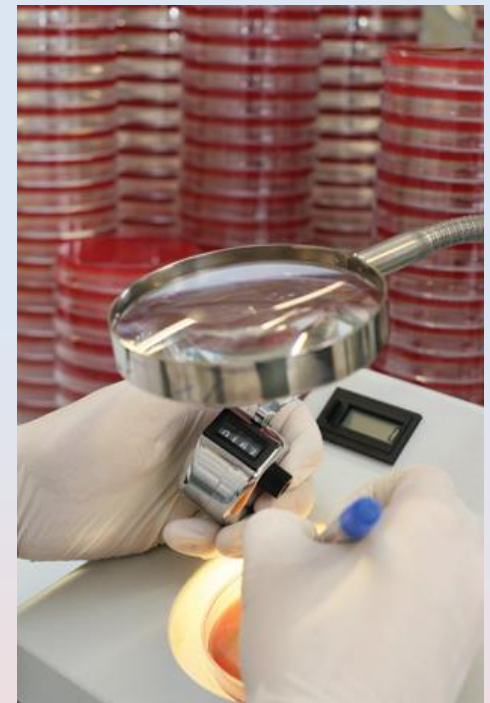
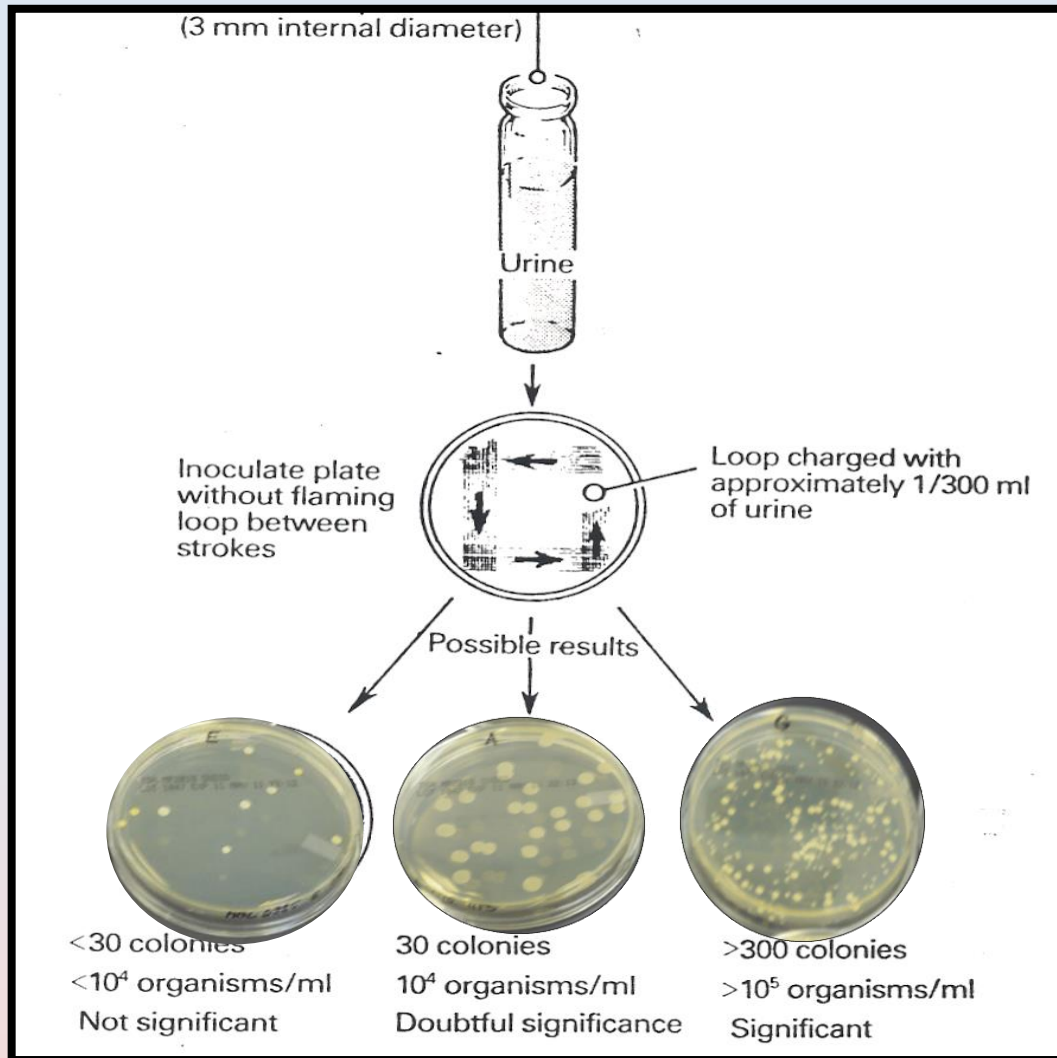
Over night incubation

Isolation of colonies,  
Biochemical tests,  
Drug susceptibility test,

Over night incubation

**RESULT**

# Smi-Quantitative Culture of Urine Sample



# ➤ **ID of cultured organisms**

➤ **Biochemical tests.**

➤ **Type of hemolysis**

➤ **Serological tests**

# CLINICALLY IMPORTANT MICROORGANISMS CAUSING UTI



# **Etiological Agents of UTI**

- **Bacteria**
- **Fungi**
- **Parasites**

# BACTERIA

## ➤ Gram- negative bacilli

- *Enterobacteriace*
- *Non-Enterobacteriace*

## ➤ Gram-positive cocci

### ➤ Staphylococci

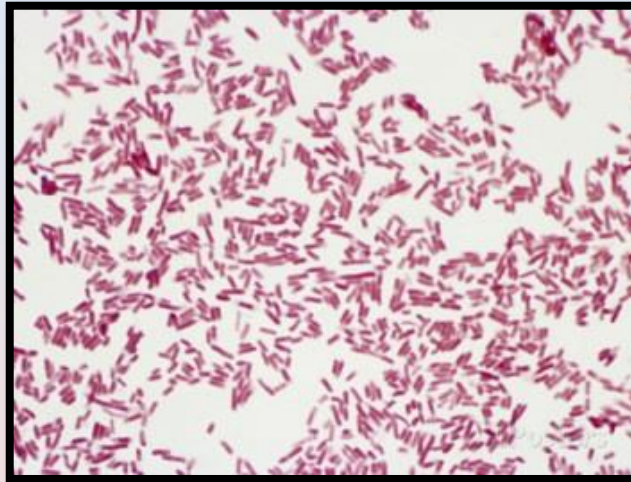
- Coagulase-positive (*Staph. aureus*)
- Coagulase *negative* (*Staph. saprophyticus* , *Staph. Epidermidis*)

### ➤ Streptococci

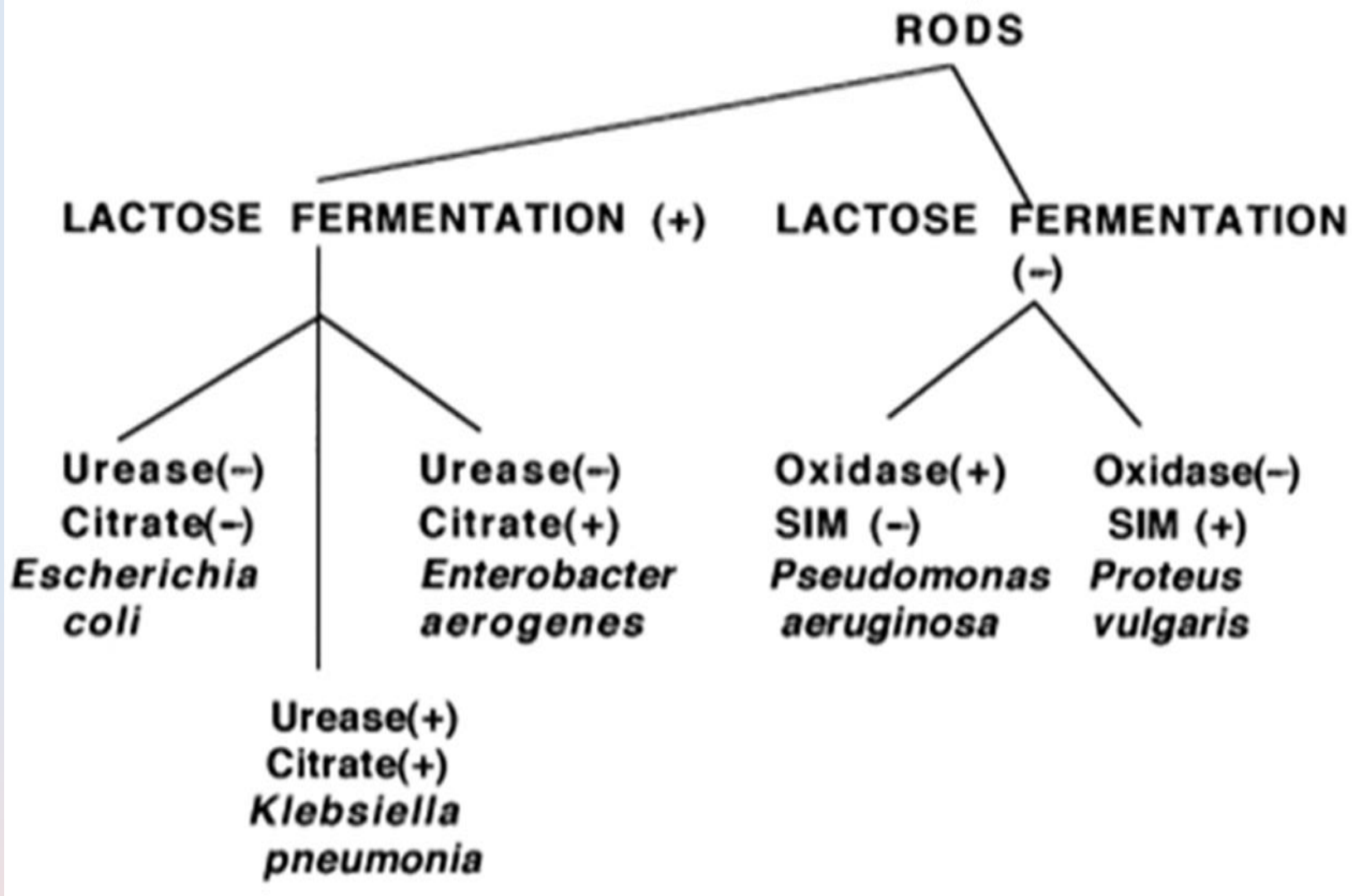
- Streptococcus (group B)
- *Enterococci*

# Gram Negative Bacilli

- **Enterobacteriaceae**
- **Non-Enterobacteriaceae**





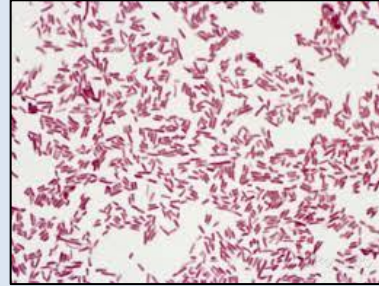


# ➤ **Enterobacteriaceae**

- ***Escherichia coli***
- ***Klebsella***
- ***Proteus***
- ***Enterobacter***
- ***Citrobacter***

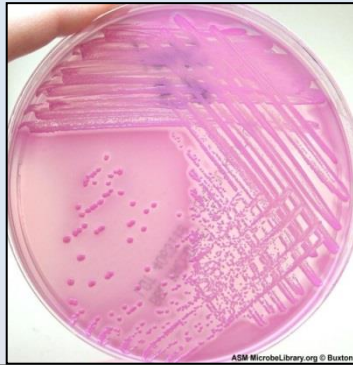
# ■ *E. coli*

## 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

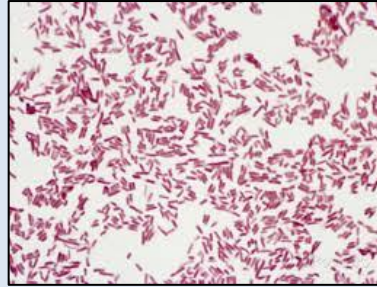


**APE 20 E test**



# ■ *Proteus spp*

## Morphology



Microscopic appearance Gram negative bacilli

## Culture



Blood culture plate showing swarm of *Proteus*



CLED [(Cystine-Lactose-Electrolyte-Deficient) - Inhibits The *Proteus* Swarming

## Identification



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

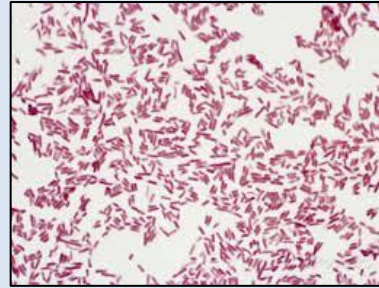


APE 20 E test

# ➤ Non-Enterobacteriaceae

## *Pseudomonas spp*

### Morphology



Microscopic appearance Gram negative bacilli

### Culture



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

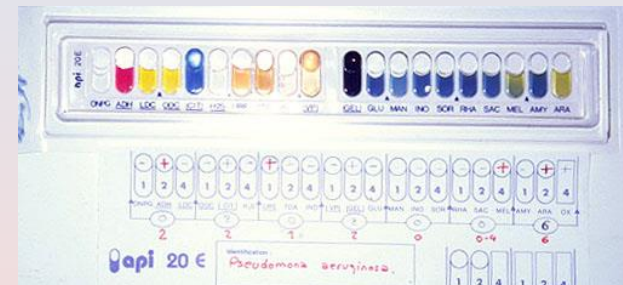


Nutrient Agar showing growth of *Pseudomonas* pigmentation

### Identification



Oxidase positive test

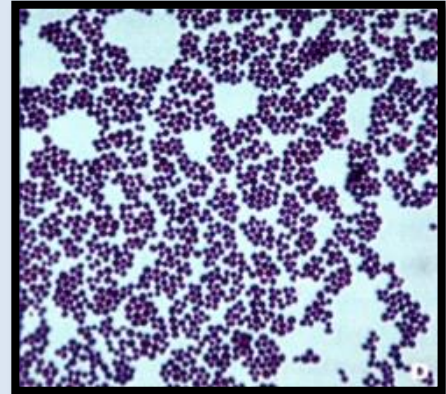


APE 20 E test

# Gram Positive Cocci

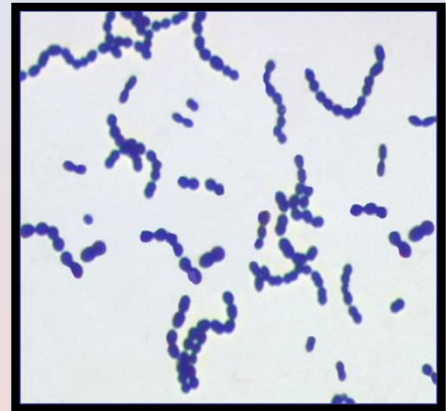
## ➤ Staphylococci

- Coagulase-positive (*Staph. aureus*)
- Coagulase negative (*Staph. saprophyticus*)
- Coagulase negative (*Staph. epidermidis*)



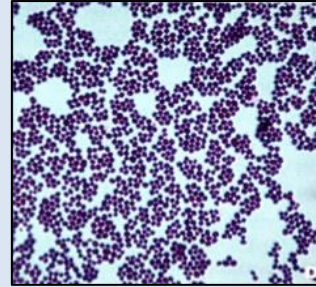
## ➤ Streptococcus (group B)

## ➤ Enterococci



# *Staph. aureus*

## Morphology



Microscopic appearance: Gram positive cocci in clusters

## Culture

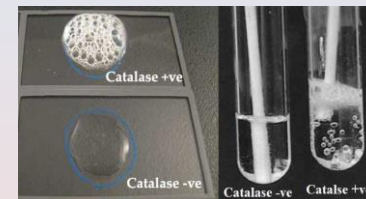


Blood culture plate showing growth of golden yellow colonies

## Identification



Coagulase test = Positive

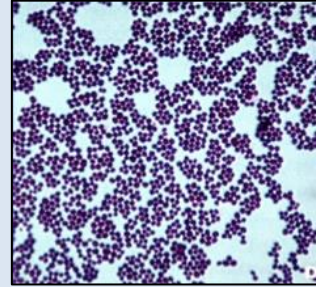


**Catalase**  
 $2\text{H}_2\text{O}_2 \longrightarrow \text{O}_2 + 2\text{H}_2\text{O}$   
Catalase test = Positive  
Streptococci vs. Staphylococci



# *Staph. epidermidis*

## Morphology



Microscopic appearance: Gram positive cocci in clusters

## Culture

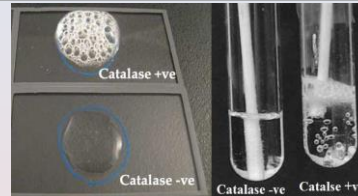


Blood culture plate showing growth of white colonies

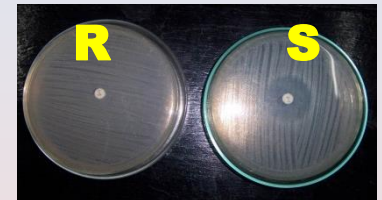
## Identification



*Coagulase test*  
**Negative**



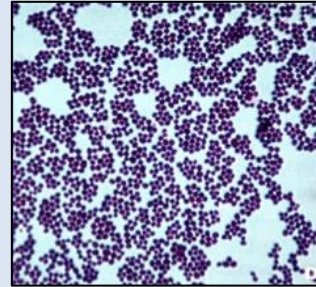
**Catalase**  
 $2H_2O_2 \longrightarrow O_2 + 2H_2O$   
Catalase test = Positive  
**Streptococci vs. Staphylococci**



**Staph. epidermidis vs. Staph. saprophyticus**  
**Novobiocin test**  
**Sensitive**

# *Staph. saprophyticus*

## Morphology



**Microscopic appearance: Gram positive cocci in clusters**

## Culture

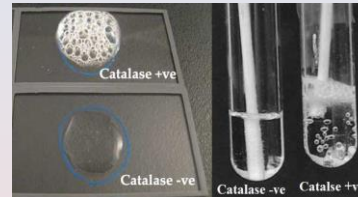


**Blood culture plate showing growth of white colonies**

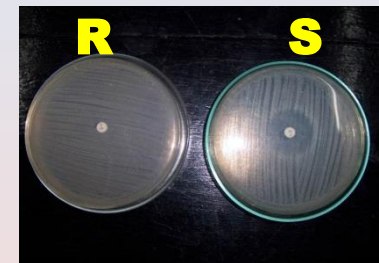
## Identification



**Coagulase test = Negative**



**Catalase**  
 $2\text{H}_2\text{O}_2 \longrightarrow \text{O}_2 + 2\text{H}_2\text{O}$   
Catalase test = Negative  
**Streptococci vs. Staphylococci**



**Novobiocin Test Resistant**

# ***Strept. agalactiae* (group B)**

## **Morphology**



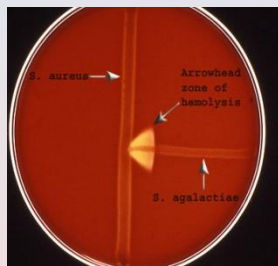
**Microscopic appearance: Gram positive cocci in chains**

## **Culture**

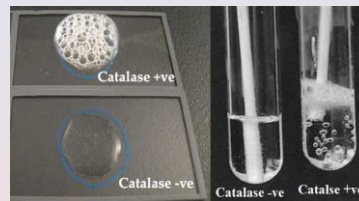


**Blood culture plate showing growth of Beta-haemolytic colonies**

## **Identification**



**CAMP test  
Positive**



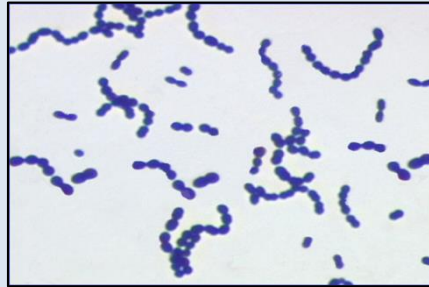
**Catalase**  
 $2\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}_2\text{O}$   
Catalase test = **Negative**  
**Streptococci vs. Staphylococci**



**Streptics**  
**Mix bacterial colony with various group-specific antisera on a slide**

# Enterococci

## Morphology



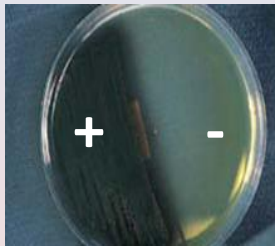
Microscopic appearance: Gram positive cocci in chains

## Culture

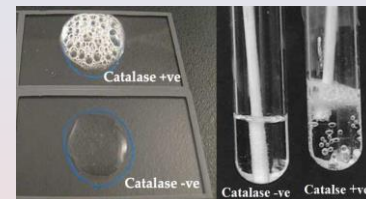


Blood culture plate showing growth of Beta-haemolytic colonies

## Identification



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



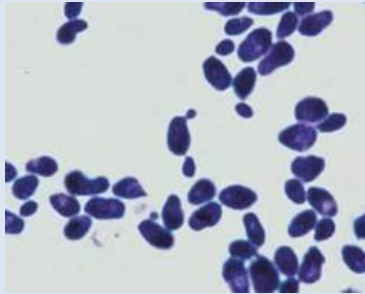
**Catalase**  
 $2\text{H}_2\text{O}_2 \xrightarrow{\text{Catalase}} \text{O}_2 + 2\text{H}_2\text{O}$   
Catalase test = Negative  
**Streptococci vs. Staphylococci**

# FUNGI



# *Candida albicans*

## Morphology



Microscopic appearance: Gram positive cocci in chains

## Culture



*Candida albicans*  
on blood agar

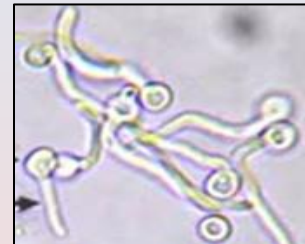


*Candida albicans* on (SDA)  
Sabouraud's Dextrose Media

## Identification



Chlamydo-spore test  
Positive



Germ tube test  
Positive

# PARASITES CAUSING UTI

➤ *Schistosoma haematobium*

# FUNGI CAUSING UTI

➤ *Candida albicans*

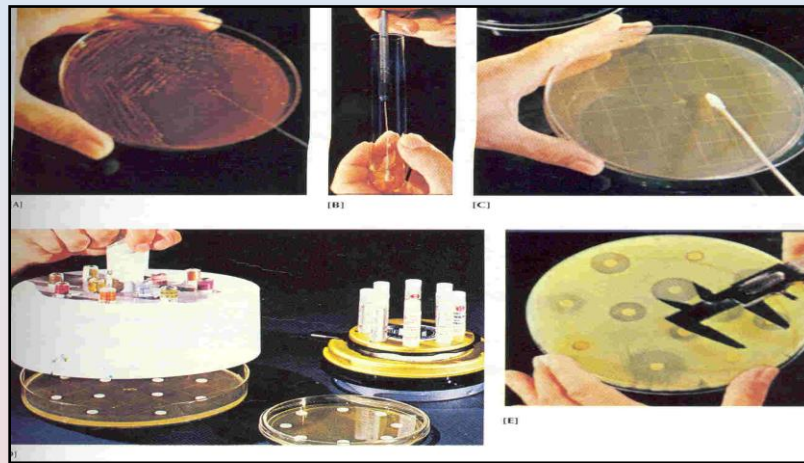


# *Schistosoma haematobium*



**(urine; eggs 115-170 x 45-65 micrometers)  
(primates)**

# 3. Antimicrobial Susceptibility Testing (AST)



# Methods of AST

- **Disk diffusion test**
- **E test**

# The Antibiotic Sensitivity Testing Method



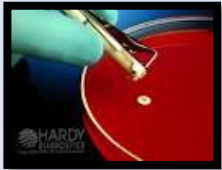
Select well-isolated colonies



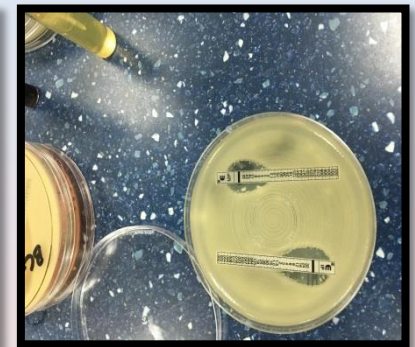
Inoculum suspension



Spread the inoculum

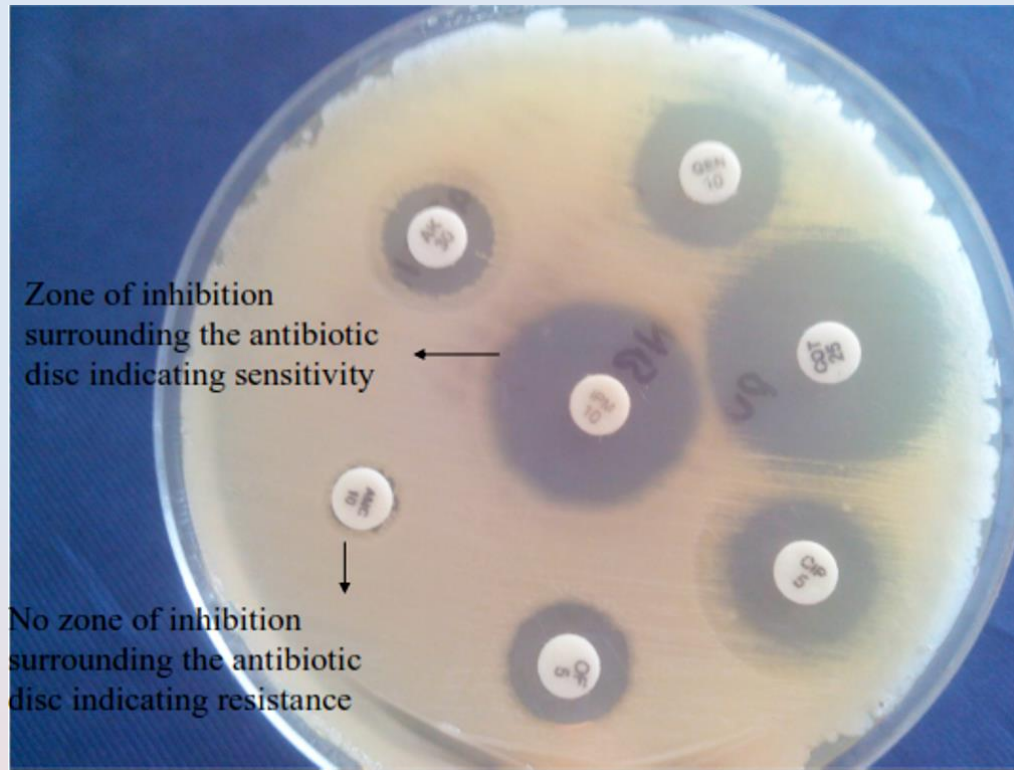


Apply antibiotics disks



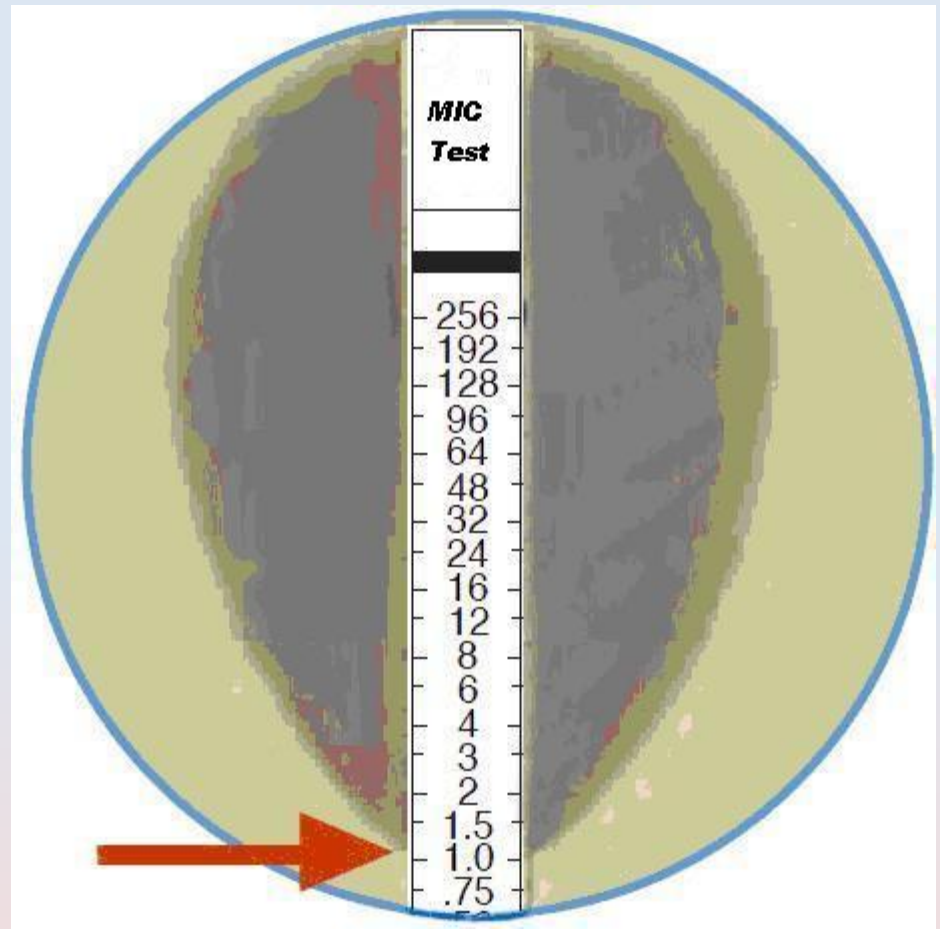
Read the result

# ➤ Disk Diffusion Method



# ➤ E TEST

- 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



# **Enterobacteriaceae**

## **Antibiotic Susceptibility Test**

- **First line UTI treatment:**
  - **Ampicillin**
  - **TMP/SMX**
  - **Ciprofloxacin**
  - **Gentamicin**
  - **Amikacin**
  - **Nitrofunatoin**

# 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**

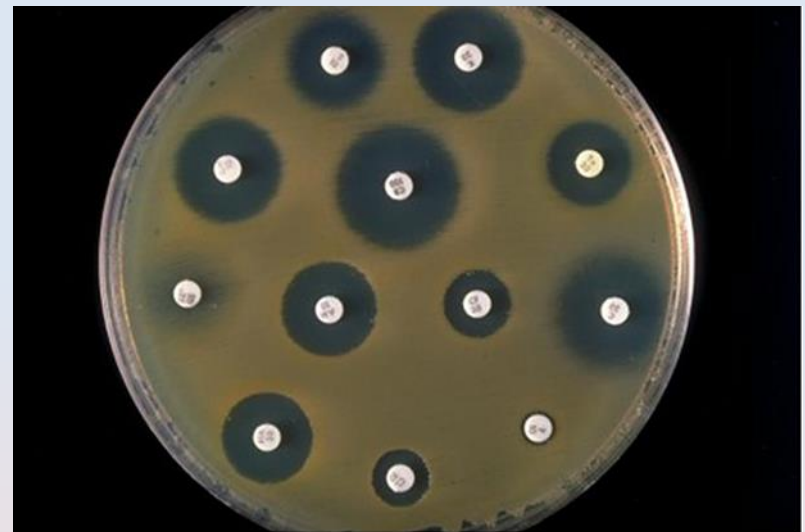
## ❑ Ampicillin

- **30% of E. coli resistant**



# Emerging resistant to Ampicillin and TMP/SMX

Antibiotic	MIC	Interpretation
Ampicillin	$\geq 32$	R
Cephalexin	$\leq 4$	S
Ciprofloxacin	$\leq 4$	S
TMP/SMX	$\geq 2$	R
Nitrofurantoin	$\leq 16$	S
Gentamicin	$\leq 8$	S
Ceftriaxone	$\leq 1$	S
Imipenem	$\leq 1$	S



Organism    E coli  
Source      urine

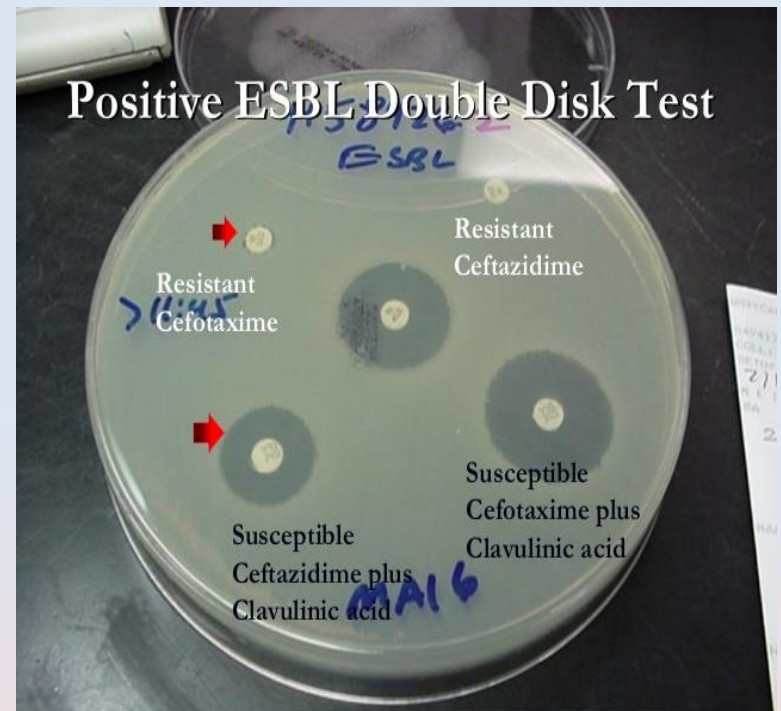
# Extended Spectrum Beta Lactamase [ESBL]

- 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

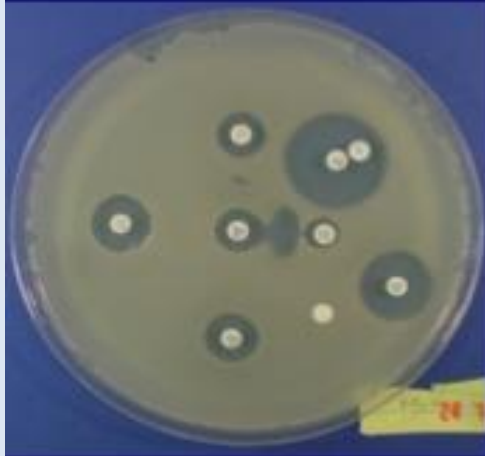
# Extended spectrum $\beta$ -lactamase (ESBL) producing E coli

Antibiotic	MIC	Interpretation
Ampicillin	$\geq 32$	R
Cephalexin	$\geq 46$	R
Ciprofloxacin	$\leq 4$	S
TMP/SMX	$\geq 2$	R
Nitrofurantoin	$\leq 16$	S
Gentamicin	$\leq 8$	S
Ceftriaxone	$\geq 46$	R
Ceftazidime	$\geq 46$	R
Imipenem	$\leq 1$	S

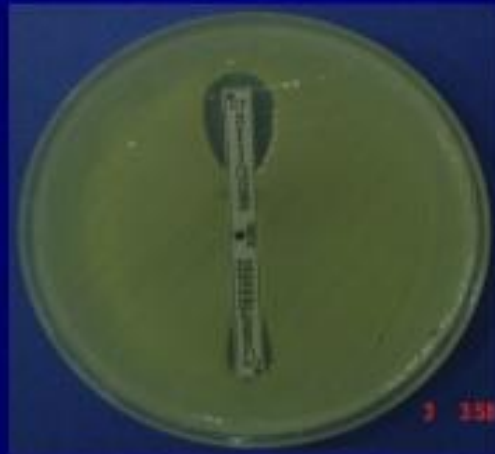
ESBL positive E coli



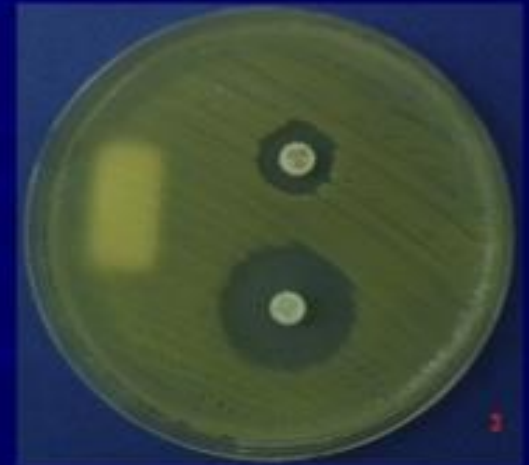
# ESBL Confirmatory Methods



Kirby-Bauer disc  
diffusion synergy



E-test  
OR MIC  
 $\geq 4$ -fold increase



Kirby-Bauer disc  
augmentation  
 $\Delta \geq 5$  mm

# *Pseudomonas spp*

## Antibiotic Susceptibility Test



# Anti-pseudomonal antibiotics

## ■ Beta-lactams

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

## ■ Fluoroquinolones

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

## ■ Aminoglycosides

- Gentamicin
- Tobramycin

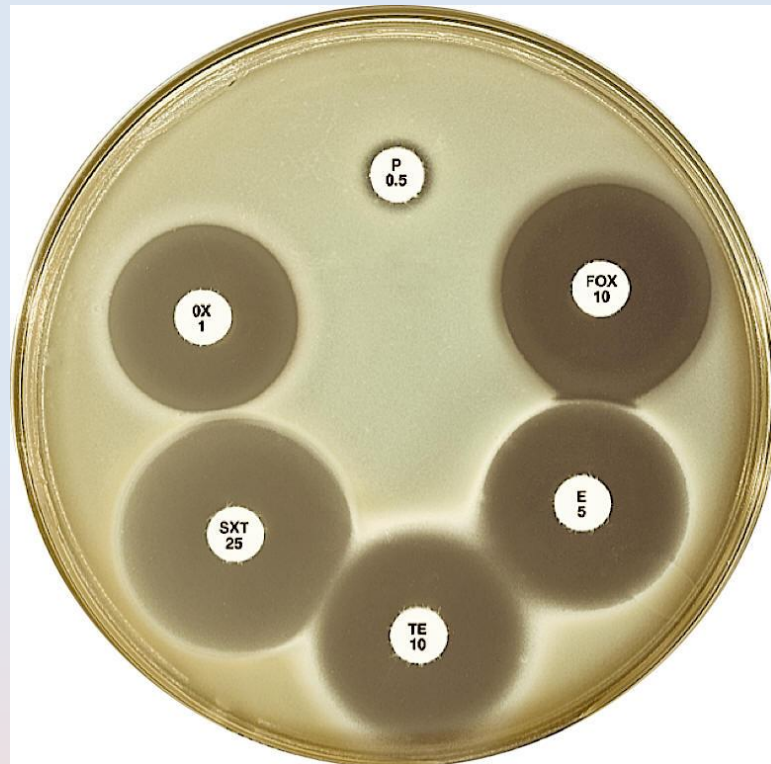
Green- formulary item, no restrictions

Yellow- formulary but requires ID approval

Red- non-formulary item

# *Staph. aureus*

## Antibiotic Susceptibility Test



**Methicillin Sensitive Staph Aureus (MSSA)**



**strain A**  
*Staphylococcus aureus*



**strain B**  
*Staphylococcus aureus*  
methicillin-resistant  
MRSA



# MRSA

Ciprofloxacin

Moxifloxacin

Vancomycin

Gentamycin

Cefoxitin

Fosfomycin

Co-Trimoxazole

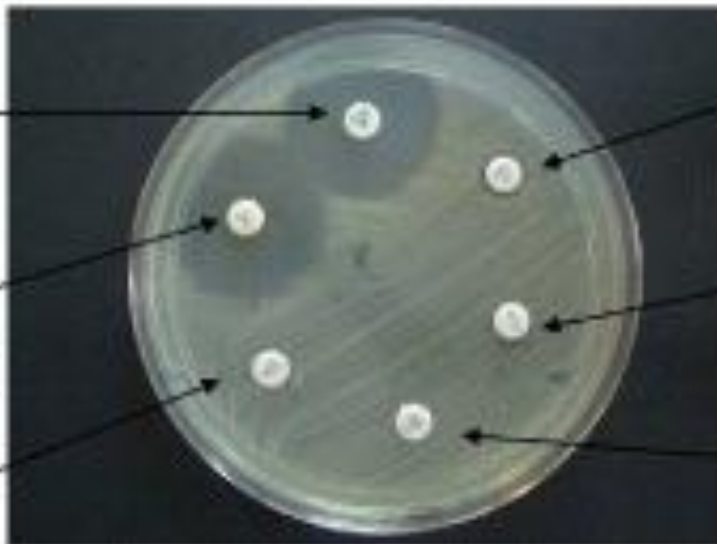
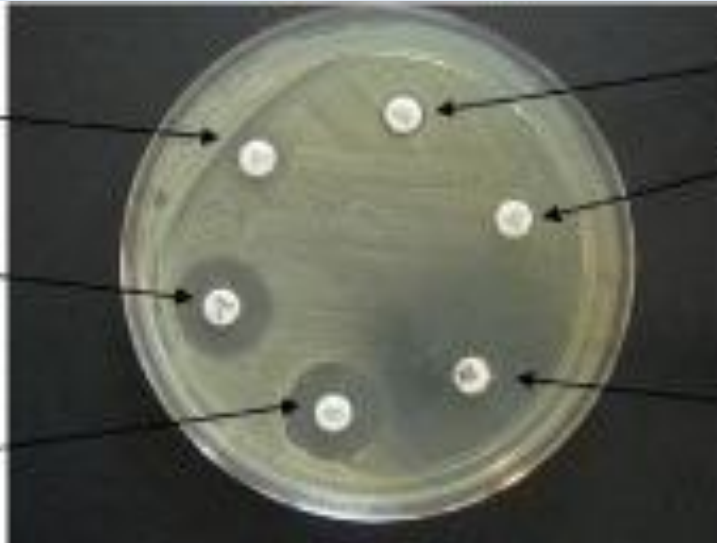
Doxycycline  
(without hydrochloride)

Erythromycin

Ampicillin

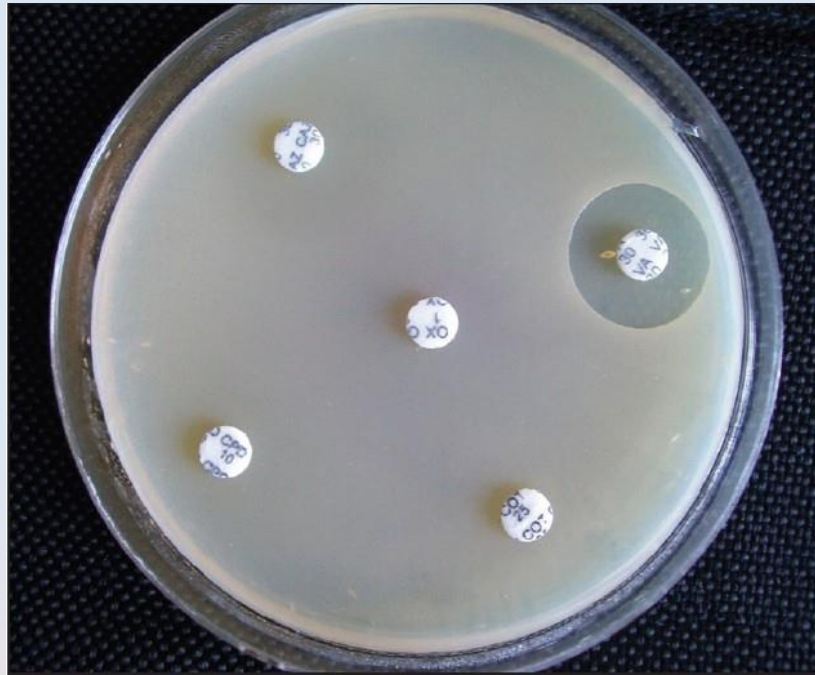
Penicillin

Clindamycin



# ***Staph. saprophyticus***

## **Antibiotic Susceptibility Test**



**Novobiocin Test  
Resistant**

# 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

# Results interpretation

- ❑ **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)**