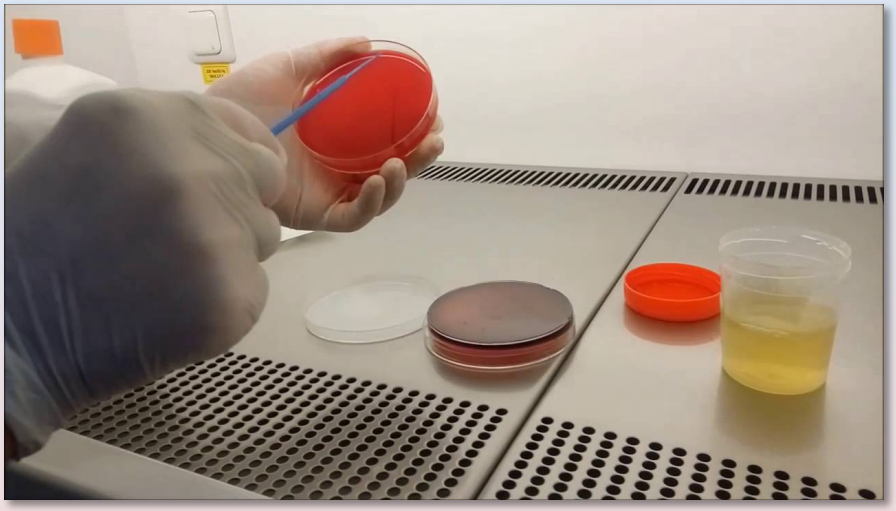
URINARY TRACT INFECTION

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

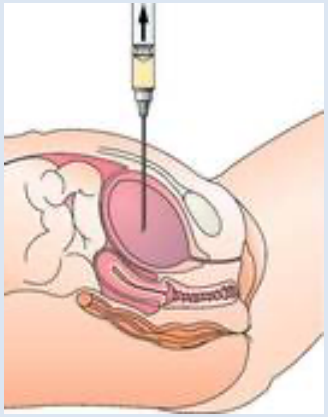
- Midstream urine (MSU)
- Adhesive bag
- Suprapubic Aspiration
- Catheter sample

TYPE OF SPECIMENS

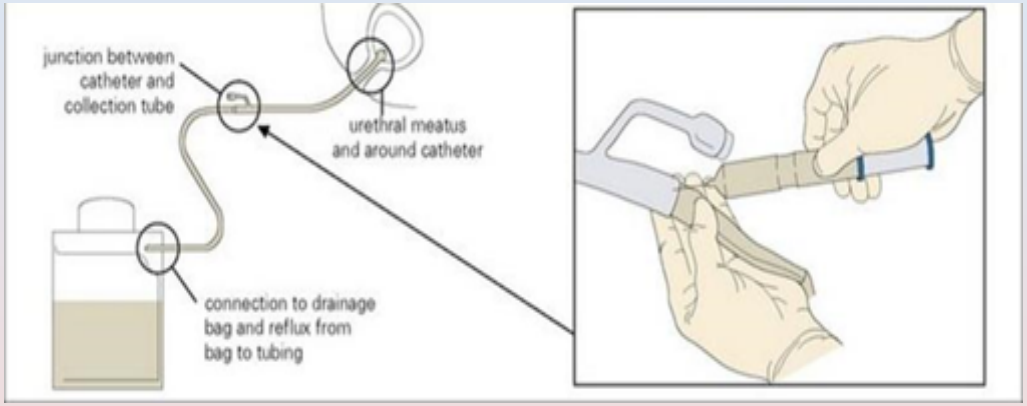
- The urine collected in a wide mouthed container from patients
- A mid stream specimen is the most ideal for processing
- Female patients passes urine with a labia separated and mid stream sample is collected

Midstream Urine (MSU)

Adhesive bag



Suprapubic Aspiration



Catheter sample

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.

Sterile Urine Container

Dip slides

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

SPECIMEN PROCESSING

Specimen processing:

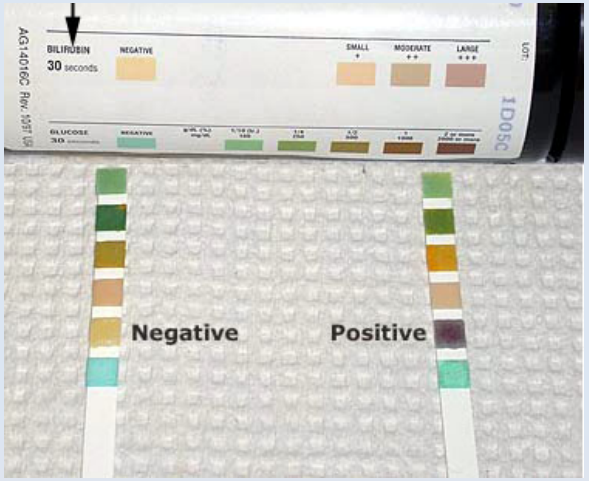
1. Urine analysis
   - Microbiological
   - Biochemical
2. Culture and identification
3. Antimicrobial Susceptibility testing
4. Results interpretation

Urine Analysis

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

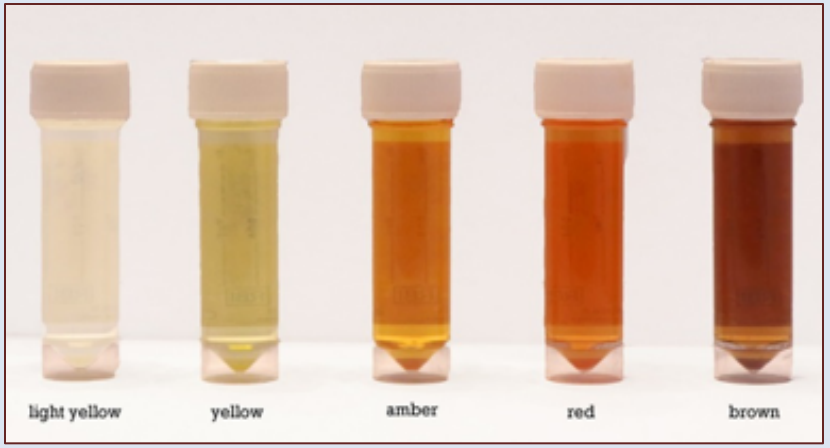
Biochemical Urine Examination (Dip stick)

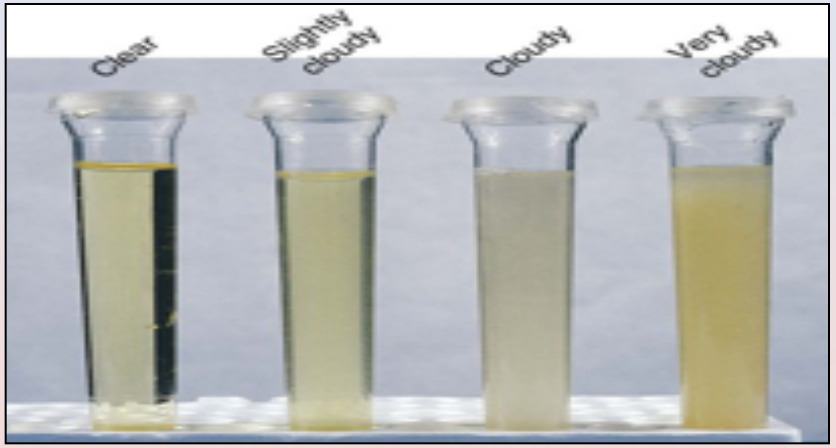
- leukocyte esterase
- Nitrate test
- PH
- Glucose
- Bilirubin



Microbiological Urine Examination

- **Macroscopic:**
  - **Color**
  - **Odor**
  - **Turbidity**

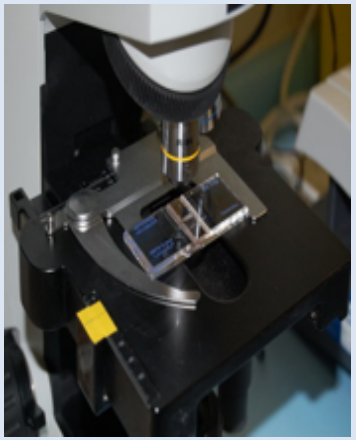




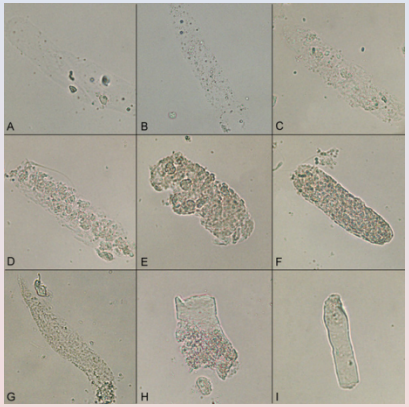
Microbiological Urine Examination

- **Microscopic:**
  - **cell-counting (WBC, RBC)**
  - **Parasite (Ovum, Trichomonas, yeast)**
  - **Casts**

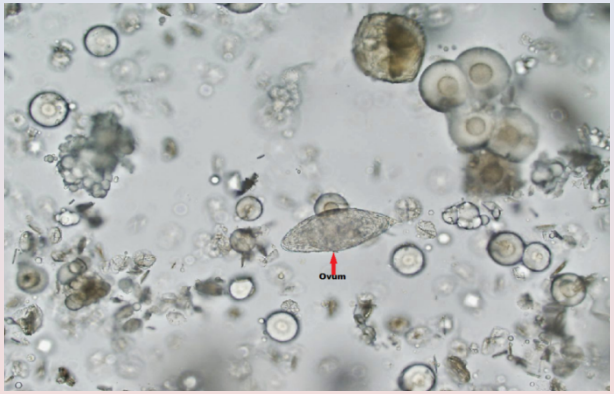
Microscopic Urine Examination (WET MOUNT)



cell-counting (WBC, RBC)



Casts



Parasite

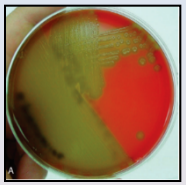
CULTURE AND IDENTIFICATION

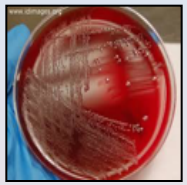
Culture and Identification

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

Culture Media Routinely Used for Urine Culture

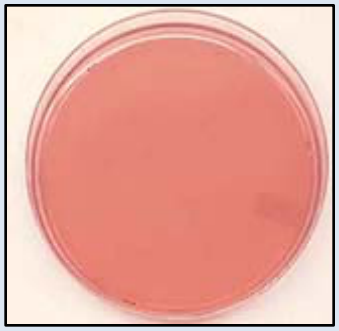
Blood Agar

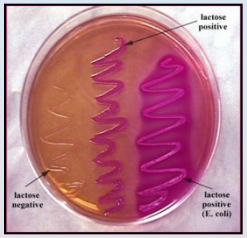


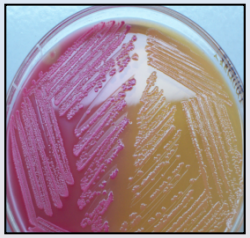


Enriched culture medium, for culturing fastidious organism and observed the hemolytic reaction(Beta,Alpha,Gama).

MacConkey Agar

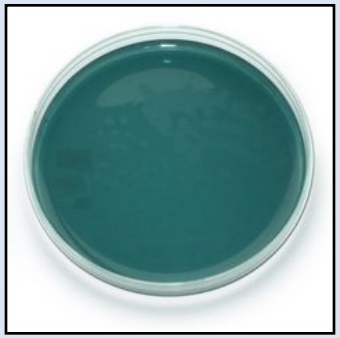


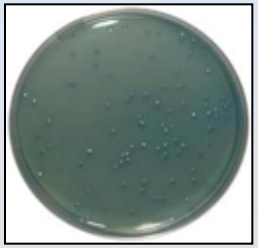


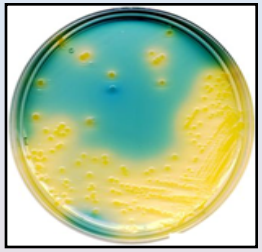


Differential culture medium, showing both lactose and non-lactose fermenting colonies.
LFC = Pink NLFC = Colorless or appear same as the medium.

CLED Agar

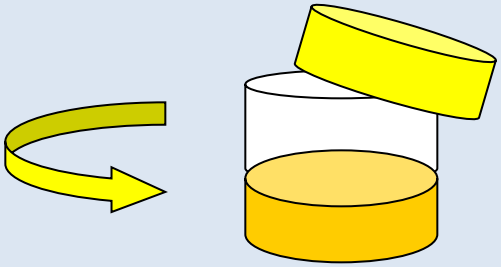




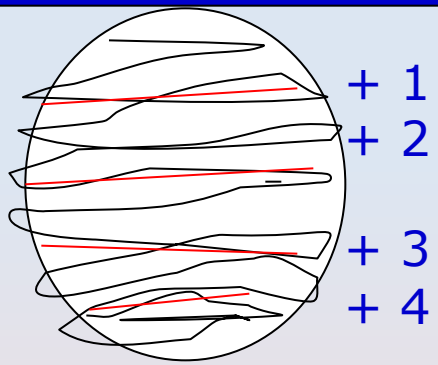


Selective culture medium, for detection and isolation of *E. coli* and coliform bacteria in urine.

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.

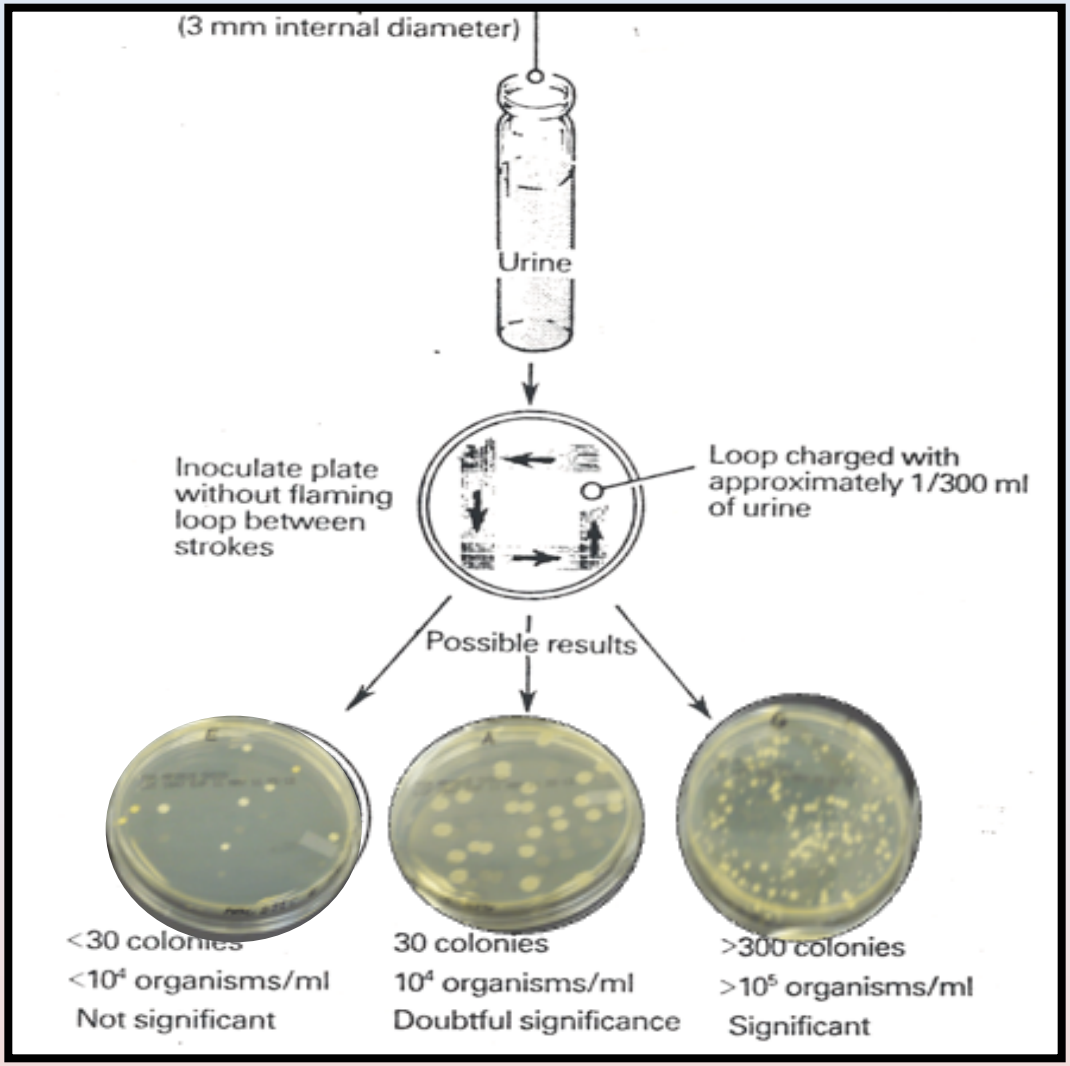
Over night incubation

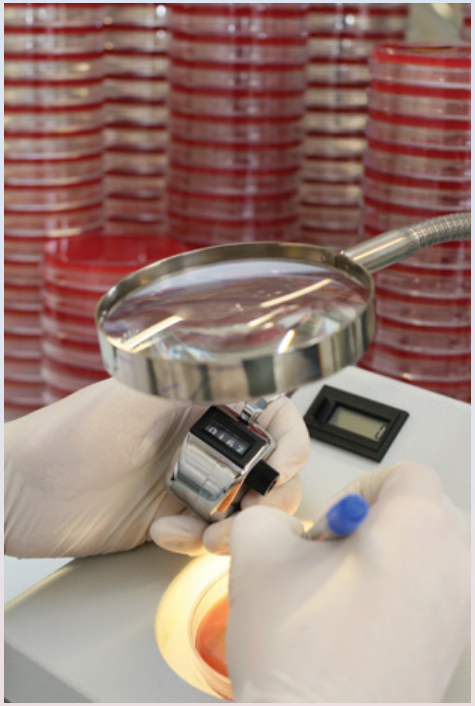
Isolation of colonies,
Biochemical tests,
Drug susceptibility test,

Over night incubation

RESULT

Smi-Qantitative Culture of Urine Sample

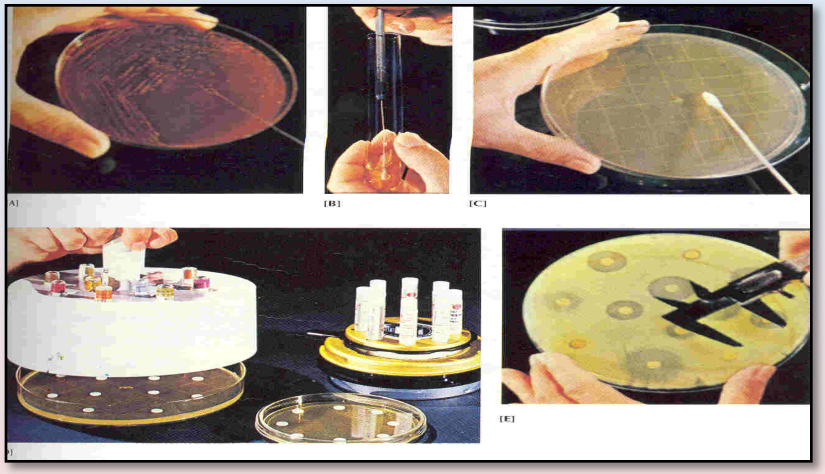




Identification of cultured organisms

- Biochemical tests.
- Type of hemolysis
- Serological tests

Antimicrobial Susceptibility Testing AST



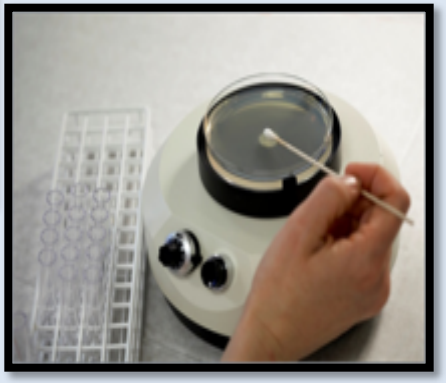
Methods of AST

- Disk diffusion test
- E test

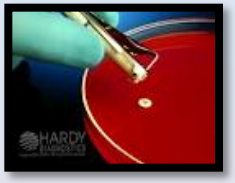
The Antibiotic Sensitivity Testing Method

Select well-isolated colonies

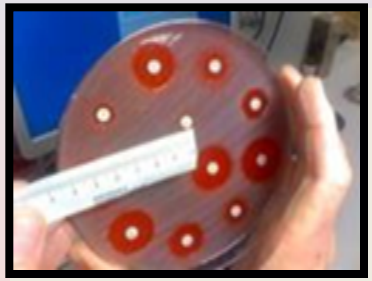
Inoculum suspension

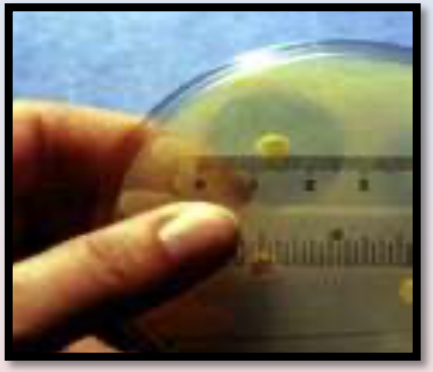


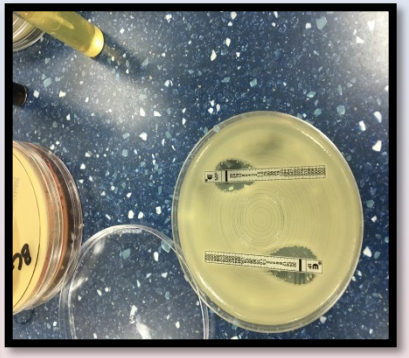
Spread the inoculum



Apply antibiotics disks







Read the result

Disk Diffusion Method

ETIOLOGICAL MICROORGANISMS OF CLINICALLY IMPORTANT PATHOGENS CAUSING UTI

Etiological Agents of UTI

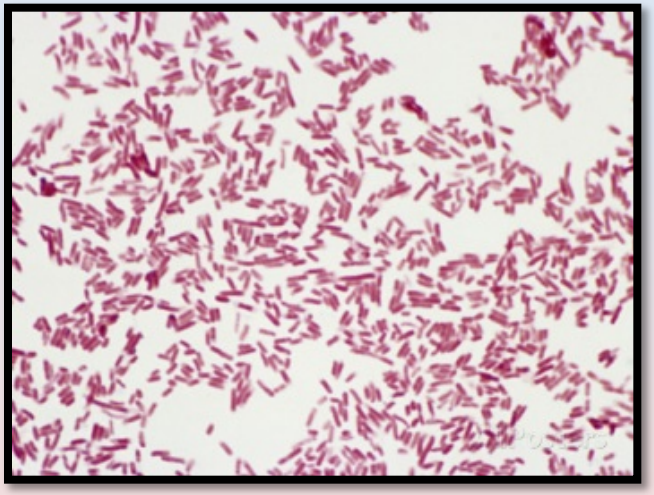
- Bacterial
- Fungal
- Parasites

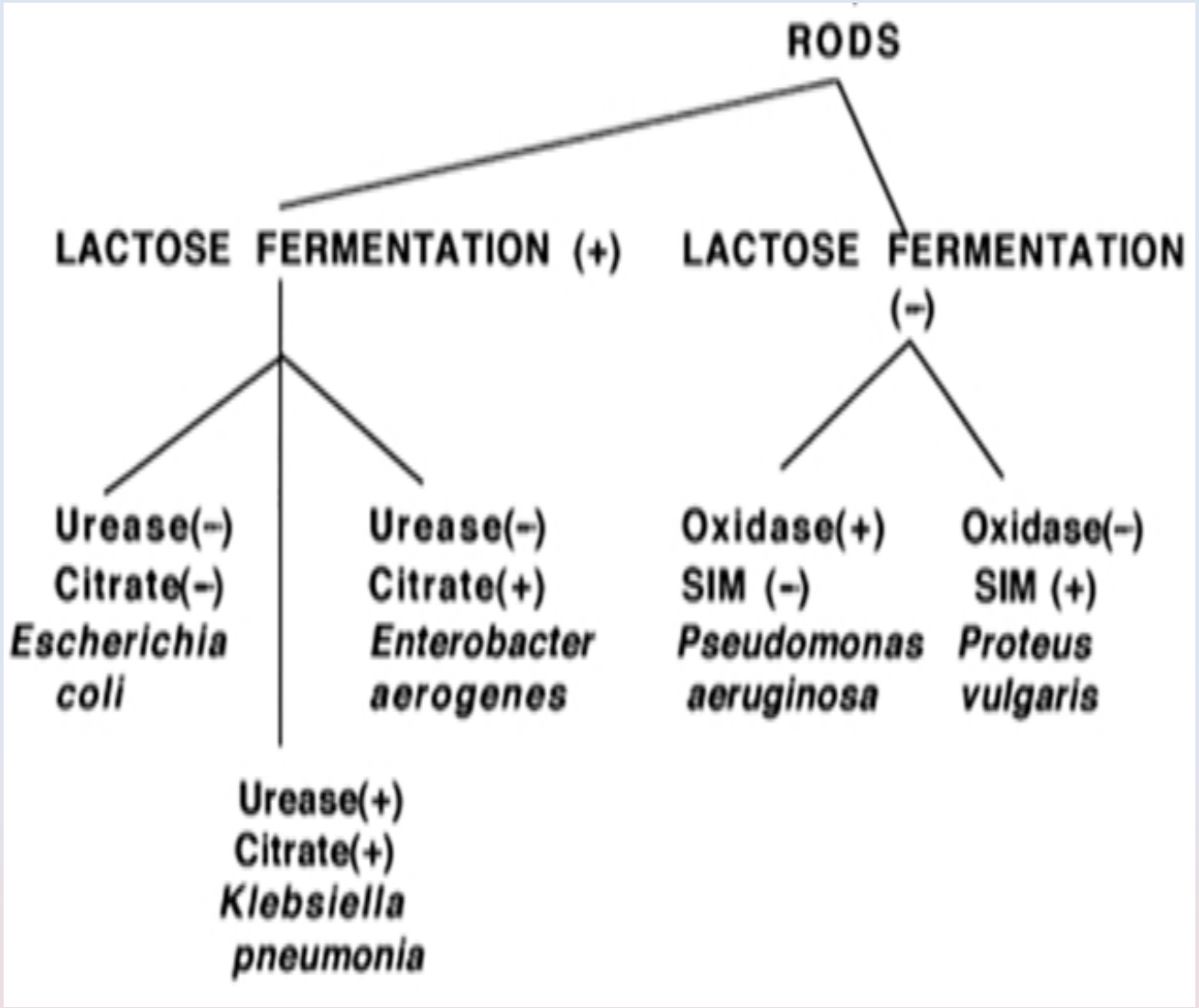
BACTERIA CAUSING UTI

- **Gram- negative bacilli**
  - **Enterobacteriace**
  - **Non-Enterobacteriace**
- **Gram-positive cocci**
- **Staphylococci**
  - **Coagulase-positive (*Staph. aureus*)**
  - **Coagulase negative (*Staph. saprophyticus*)**
  - **Coagulase negative (Staph. epidermidis)**
- ***Streptococcus* (group B)**
- **Enterococci**

Gram Negative Bacilli

- Enterobacteriacae
- Non-Enterobacteriacae

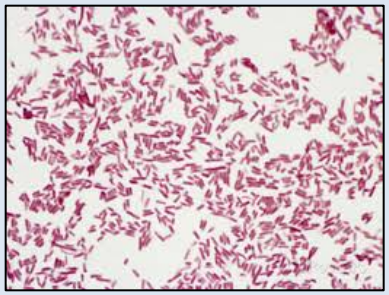
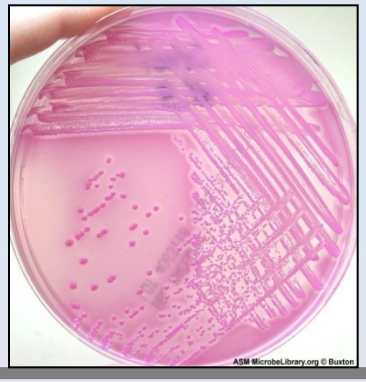
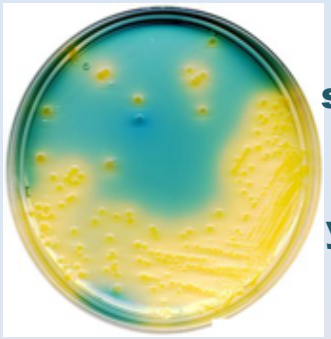



RODS
LACTOSE FERMENTATION (+)
LACTOSE FERMENTATION (-)
Urease(-)
Citrate(-)
Escherichia coli
Urease(-)
Citrate(+)
Enterobacter aerogenes
Oxidase(+)
SIM (-)
Pseudomonas aeruginosa
Oxidase(-)
SIM (+)
Proteus vulgaris
Urease(+)
Citrate(+)
Klebsiella pneumonia

Enterobacteriacae

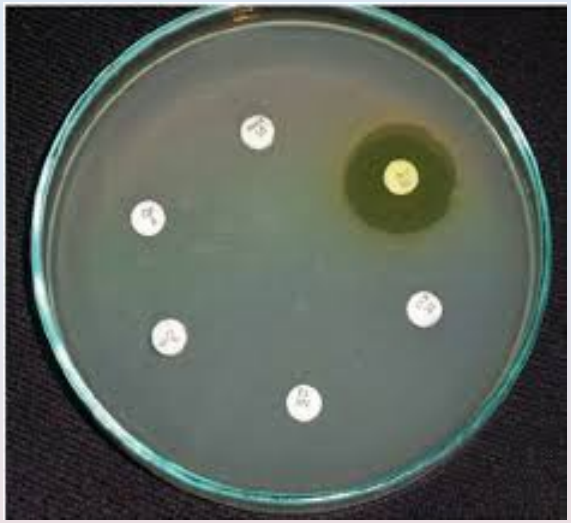
- ***Escherichia coli***
- ***Klebseilla***
- ***Proteus***
- ***Other Enterobacteriaceae***
  (Enterobacter,Citrobacter….)
- ***Pseudomonas aeruginosa***

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**

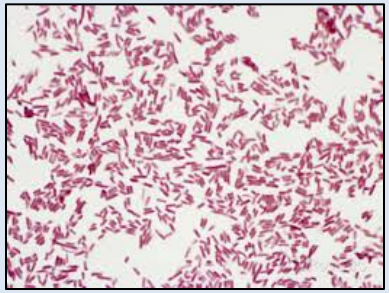
E. coli

Antibiotic Susceptibility Test



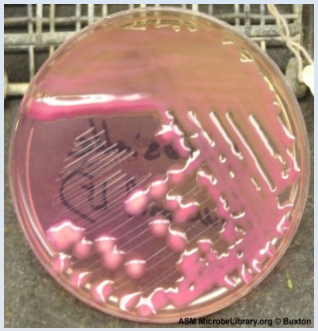
Klebseilla spp

Morphology



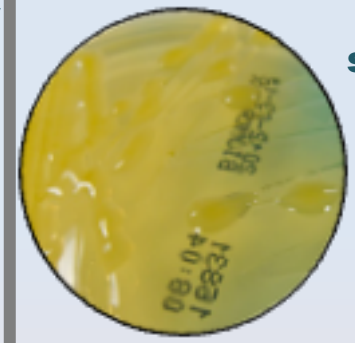
Microscopic appearance Gram negative bacilli

Culture



MacConkey agar showing growth of Lactose Fermenter Mucoid Pink colonies

LFC



CLED agar showing growth of Lactose fermenter Mucoid yellow colonies

LFC

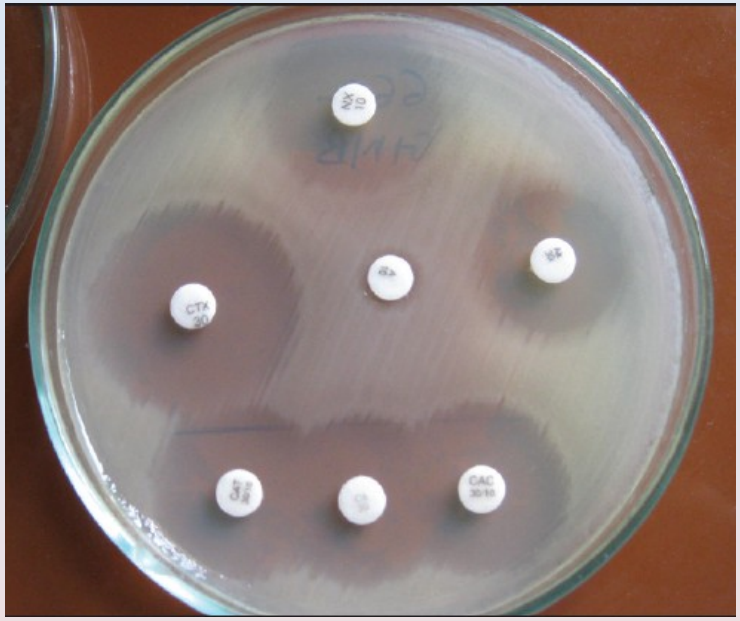
Identification

Indole Reactions Test: Negative

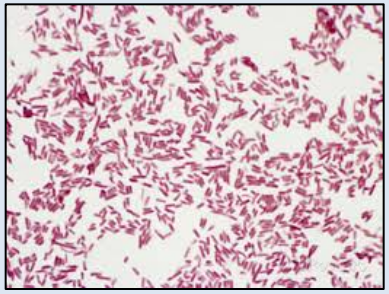
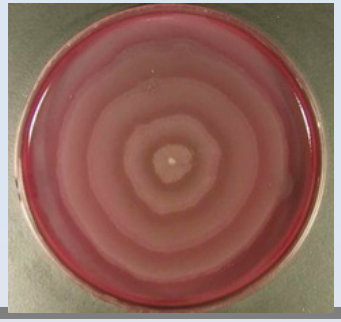
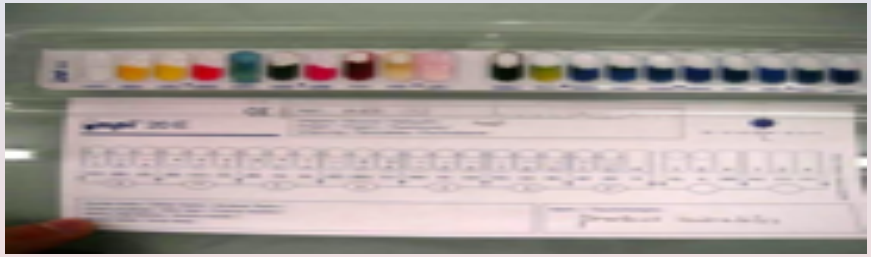
APE 20 E test

Klebseilla spp

Antibiotic Susceptibility Test



Proteus spp

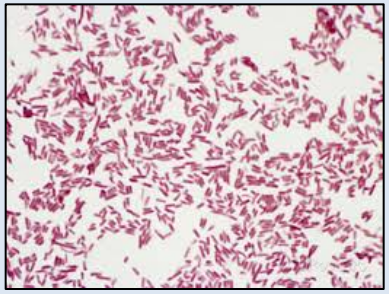
Morphology	 **Microscopic appearance Gram negative bacilli**	
Culture	 **Blood culture plate showing swarm of *Proteus***	 **CLED [(Cystine-Lactose-Electrolyte-Deficient) - Inhibits The *Proteus* Swarming**
Identification	 ***Proteus* is Urease positive Urease splits urea into ammonia; and alkalinizes the urine with production of crystals**	 **APE 20 E test**

Proteus spp

Antibiotic Susceptibility Test

Pseudomonas spp

Morphology

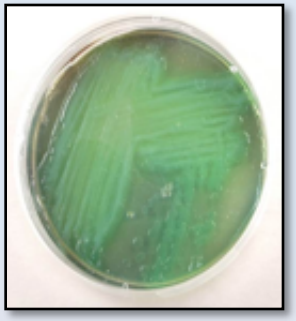


Microscopic appearance Gram negative bacilli

Culture

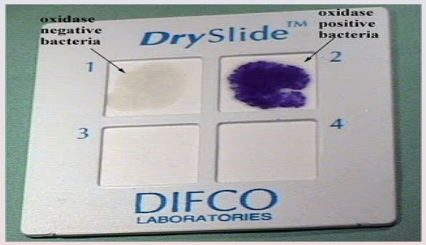
MacConkey agar showing growth of Non-Lactose Fermenter Mucoid Pink colonies

NLFC



Nutrient Agar showing growth of *Pseudomonas pigmentation*

Identification

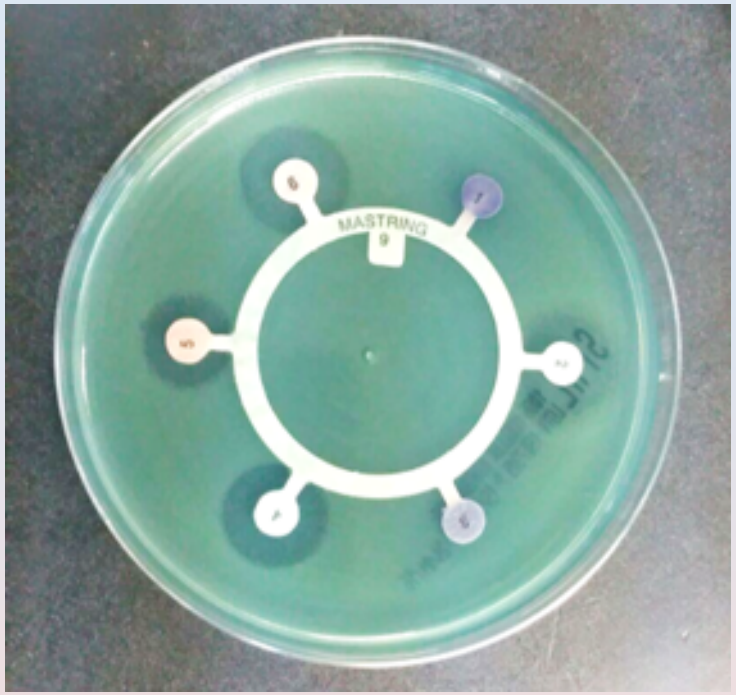


Oxidase positive test

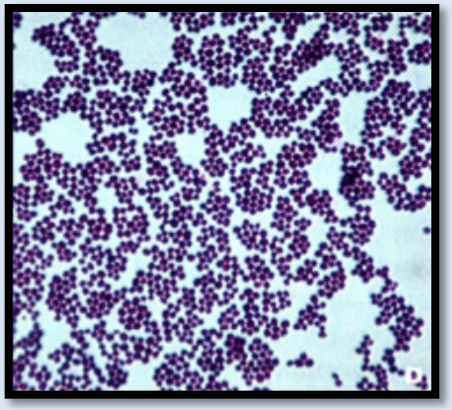
APE 20 E test

Pseudomonas spp

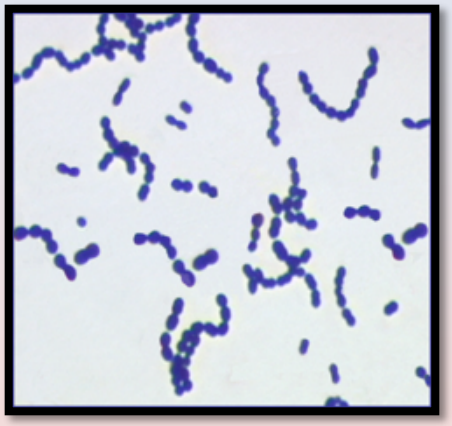
Antibiotic Susceptibility Test



Gram Positive Cocci



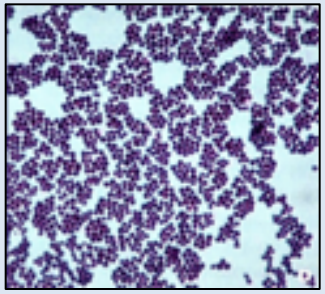
- **Staphylococci**
  - **Coagulase-positive (*Staph. aureus*)**
  - **Coagulase negative (*Staph. saprophyticus*)**
  - **Coagulase negative (Staph. epidermidis)**



- ***Streptococcus* (group B)**
- **Enterococci**

Staph. aureus

Morphology



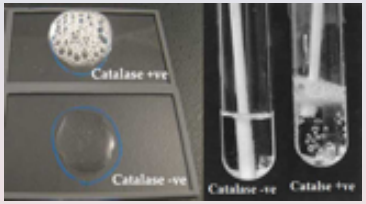
Microscopic appearance: Gram positive cocci in clusters

Culture

Blood culture plate showing growth of golden yellow colonies

Identification

Coagulase test = Positive



Catalase

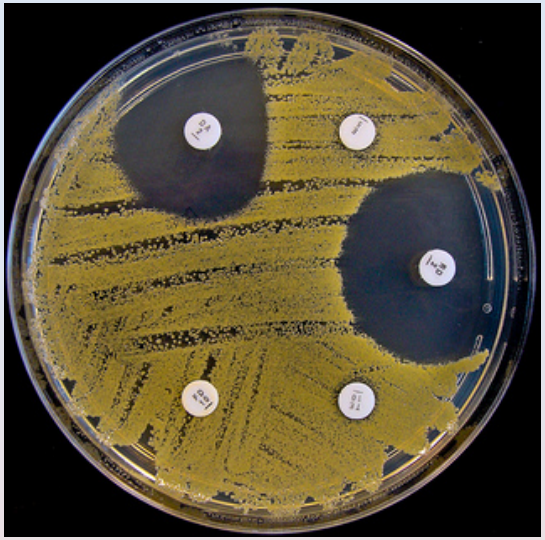
$2H_2O_2 \rightarrow O_2 + 2H_2O$

Catalase test = Positive

Streptococci vs. Staphylococci

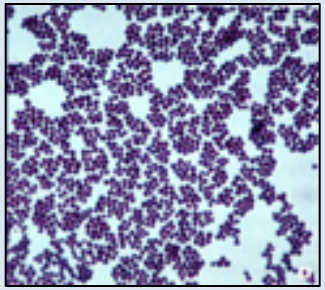
Staph. aureus

Antibiotic Susceptibility Test



Staph. saprophyticus

Morphology



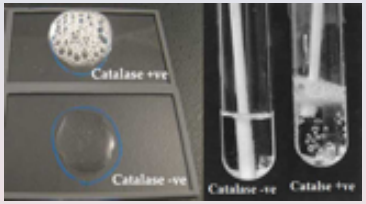
Microscopic appearance: Gram positive cocci in clusters

Culture

Blood culture plate showing growth of white colonies

Identification

Coagulase test = Negative

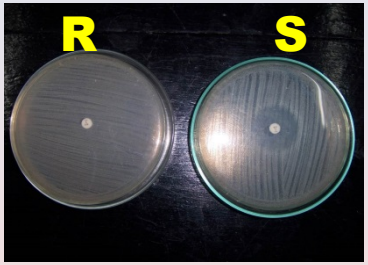


Catalase

$2H_2O_2 \rightarrow O_2 + 2H_2O$

Catalase test = Positive

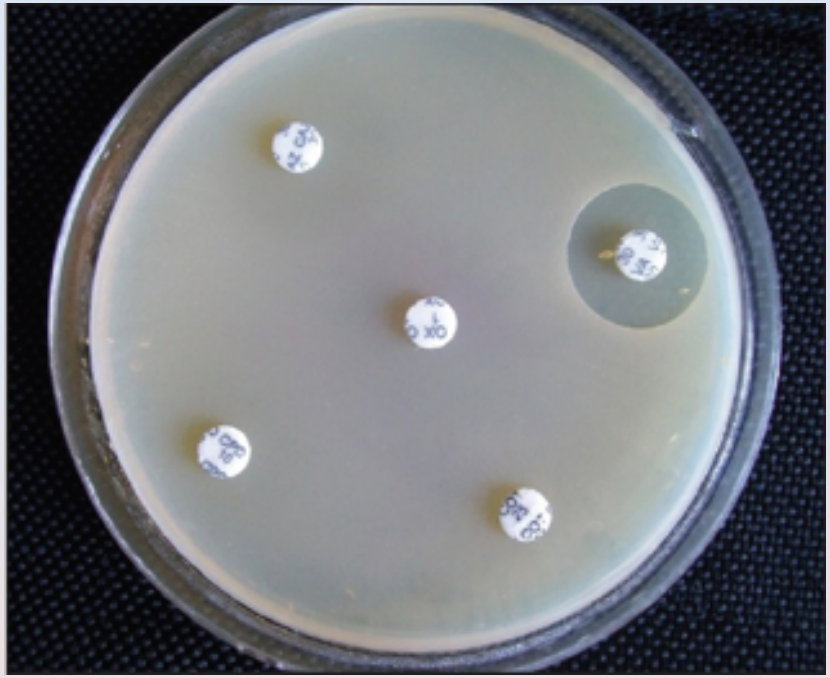
Streptococci vs. Staphylococci



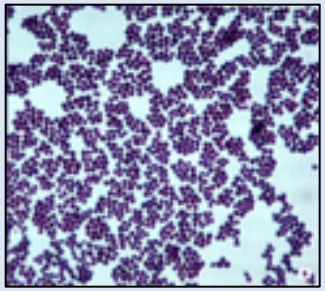
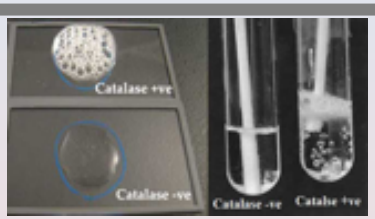
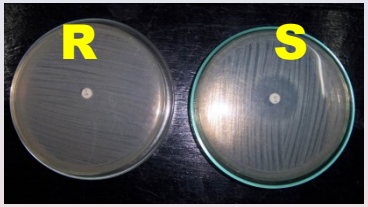
Novobiocin Test Resistant

Staph. saprophyticus

Antibiotic Susceptibility Test

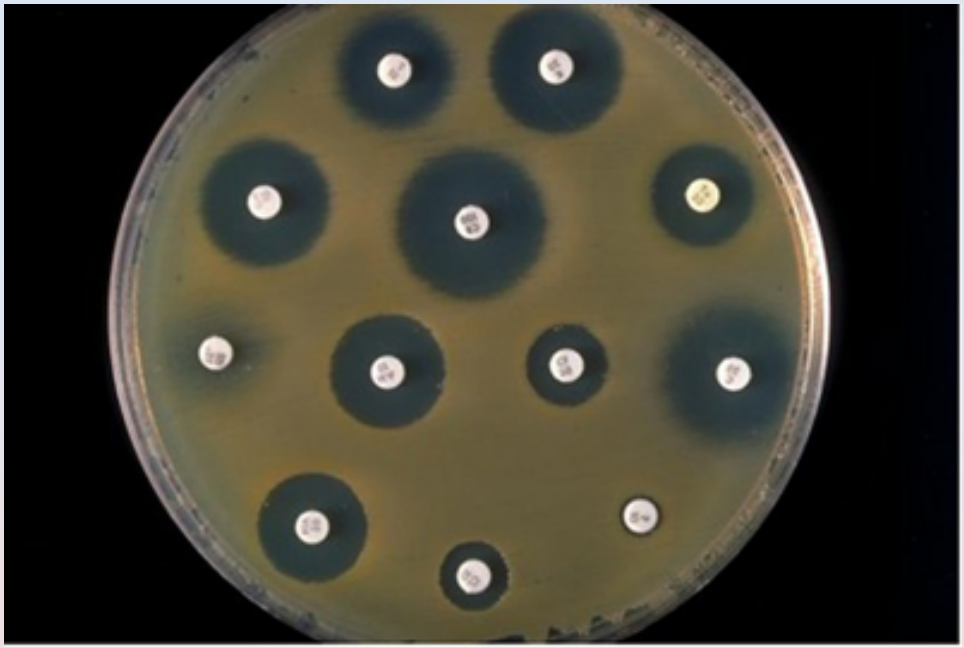


Staph. epidermidis

Morphology	 **Microscopic appearance: Gram positive cocci in clusters**		
Culture	 **Blood culture plate showing growth of white colonies**		
Identification	 ***Coagulase test Negative***	 **Catalase** $2H_2O_2 \rightarrow O_2 + 2H_2O$ **Catalase test = Positive** **Streptococci vs. Staphylococci**	 **Staph.epidermidis vs. Staph. saprophyticus** **Novobiocin test Sensitive**

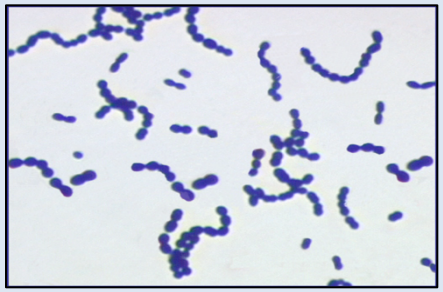
Staph. epidermidis

Antibiotic Susceptibility Test



Strept. agalactiae (group B)

Morphology

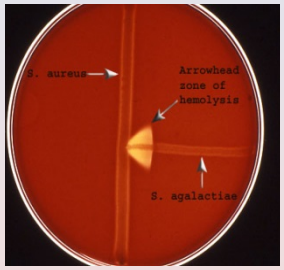


Microscopic appearance: Gram positive cocci in chains

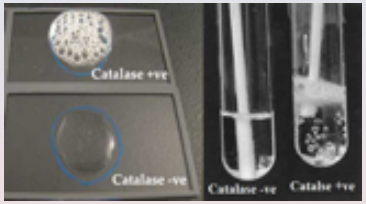
Culture

Blood culture plate showing growth of Beta-haemolitic colonies

Identification



CAMP test Positive



Catalase

$2H_2O_2 \rightarrow O_2 + 2H_2O$

Catalase test = Negative

Streptococci vs. Staphylococci

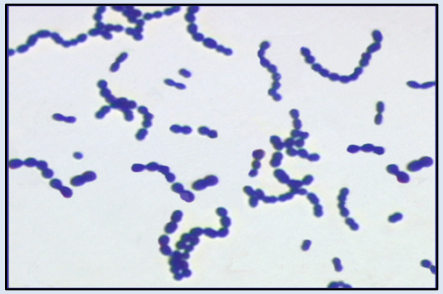
Streptics
Mix bacterial colony with various group-specific antisera on a slide

Strept. agalactiae (group B)

Antibiotic Susceptibility Test

Enterococci

Morphology

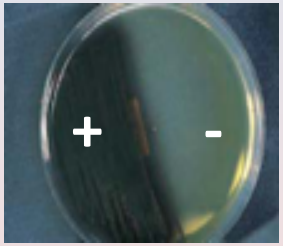


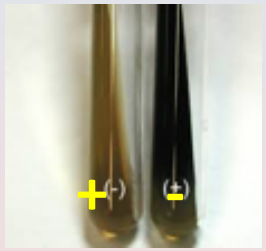
Microscopic appearance: Gram positive cocci in chains

Culture

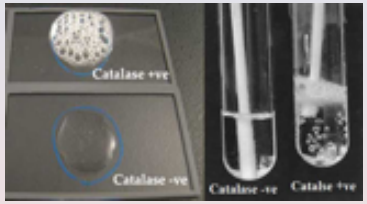
Blood culture plate showing growth of Beta-haemolitic colonies

Identification





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



Catalase

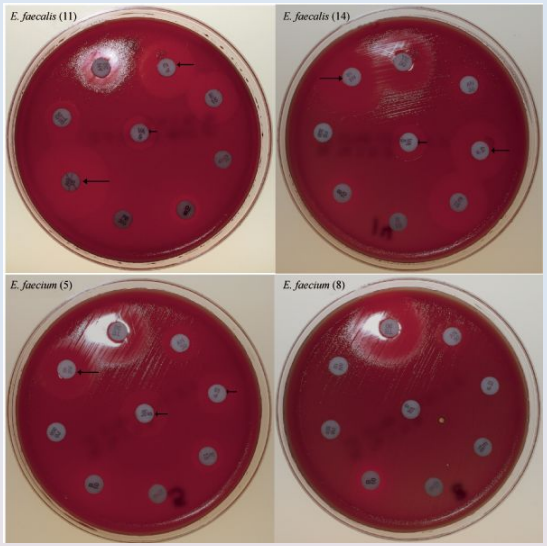
$2H_2O_2 \rightarrow O_2 + 2H_2O$

Catalase test = Negative

Streptococci vs. Staphylococci

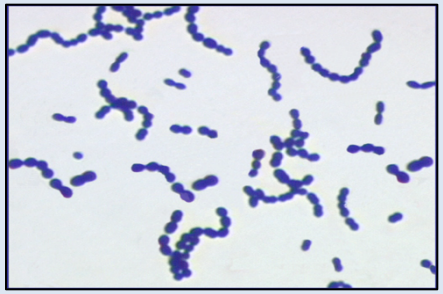
Enterococci

Antibiotic Susceptibility Test



Enterococci

Morphology

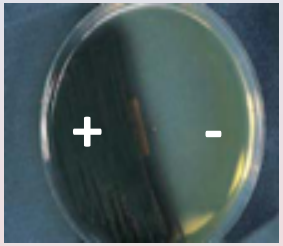


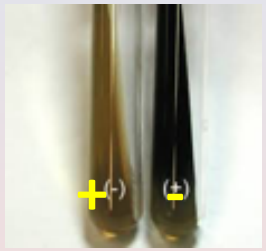
Microscopic appearance: Gram positive cocci in chains

Culture

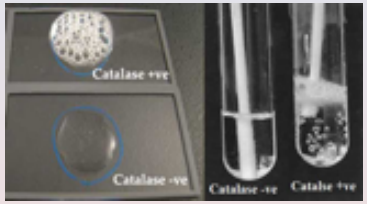
Blood culture plate showing growth of Beta-haemolitic colonies

Identification





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



Catalase

$2H_2O_2 \rightarrow O_2 + 2H_2O$

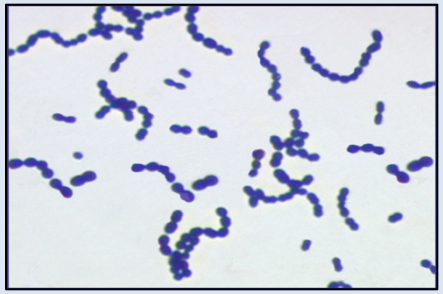
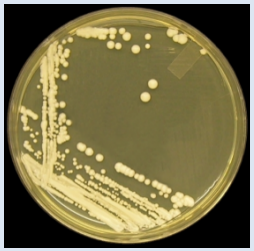
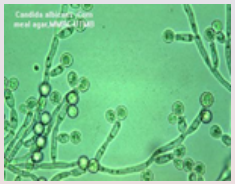
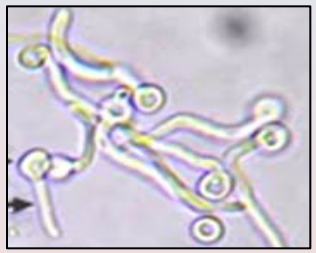
Catalase test = Negative

Streptococci vs. Staphylococci

FUNGI CAUSING UTI

- ***Candida albicans***

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	  **Chlamydospore test Positive**	  **Germ tube test Psitive**

PARASITES CAUSING UTI

- ***Schistosoma haematobium***

Schistosoma haematobium

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

GRAM NEGATIVE	GRAM POSITIVE
Escherichia coli	*Enterococcus*
Klebsiella	*Staphylococcus saprophyticus*
Proteus	*Streptococcus agalactiae* (group B)
Other *Enterobacteriaceae (Enterobacter,Citrobacter….)*	*Staphylococcus aureus*[1] *(*Associated with staphylococcemia
Pseudomonas aeruginosa	

- Other organisms ;
  - *Candida albicans*
  - *Schistosoma haematobium*
  - *Tricomonas vaginalis*