

# Gram-Positive Cocci

**A- Gram-positive cocci**

```
graph TD; A["A- Gram-positive cocci"] --- B["I- staphylococci"]; A --- C["II- streptococci"]
```

**I- staphylococci**

**II- streptococci**

# Staphylococci

## ■ General characters:

- Gram Positive Cocci
- Grape-like
- Non Motile
- Non Spore Forming
- Non Capsulated
- Non Fastidious
- Facultative Anaerobes
- Fermentative
- Catalase positive

## ■ Characters of *S. aureus*

- *Production of coagulase*
- *Production of phosphatase*
- *Production of DNase*
- Ferment Mannitol
- Gelatin liquefied
- B-hemolysis on blood agar
- Acidification & clotting of litmus milk

# Species of Staphylococci



- **Three species of staphylococci have medical importance:**
  - ***S. aureus*: Pathogenic & commensally found in nose (nares)**
  - ***S. epidermidis*: non pathogenic & common commensals in nares & skin**
  - ***S. saprophyticus*: Cause UTI in female & occasionally commensally found skin**

# Characters of the genus *Staphylococcus*:

- Staphylococci are Gram positive cocci, 0.5-1.5  $\mu\text{m}$  in diameter, which occurs in irregular "grape-like" clusters.
- 3 None: Non motile, Non spore forming and typically Non encapsulated.
- Catalase positive.
- Facultative anaerobes.
- Grow on simple media (i.e. non fastidious).

- **Culture:**

- **Colonial appearance**

- **A- On nonselective media:**

- The typical 24 h isolated colonies of staphylococci are 1-3 mm in diameter. Colonies of coagulase positive staphylococci i.e. *S. aureus* are **pigmented**, smooth, entire, and **hemolytic** on blood agar. However, colonies of coagulase negative staphylococci (e.g. *S. epidermidis*) are **unpigmented**, smooth, entire, glistening, slightly raised to convex, opaque and **non hemolytic**.

- **B- On selective medium:**

- Mannitol salt agar (MSA) is selective differential medium for staphylococci, which contains mainly on 7.5% NaCl, mannitol and phenol red. The selectivity of MSA is due to staphylococci are able to grow in presence of high salt concentration i.e. staphylococci are halotolerant. However, MSA is differential because contains mannitol



# Virulence factors of *S. aureus*

- **Coagulase:**
  - Converting fibrinogen into fibrin
- **Exfoliative toxin:**
  - Desquamation of skin in case of exfoliative dermatitis in SSSS
- **TSST:**
  - Fever, hypotension, & skin rash followed by desquamation of skin
- **Leucocytin**
  - Kills WBCs
- **Polysaccharide A and Protein A**
  - Antiphagocytic and Adhesion
- **Enterotoxins (A,B,C,D, & E)**
  - Food poisoning (Diarrhea, and Vomiting)
- **Hyaluronidase**
  - Destroy hyaluronic acid (constituent of connective tissues)
- **$\alpha, \beta, \gamma$  and  $\delta$  Toxins**
  - Destroy variety of cells (Polymorph)

# Disease caused by *S. aureus*

- Localized suppurative (Pyogenic) inflammation:
  - Folliculitis → Infection of hair follicles
  - Furuncle → Infection of an obstructed hair follicle
  - Carbuncle → Larger abscess
  - Deep Lesions (Osteomyelitis, Endocarditis & Meningitis)
- Toxigenic infection
  - Scalded Skin Syndrome (SSS)
  - Toxic Shock Syndrome
- Food poisoning
  - Nausea, Vomiting, Diarrhea without Fever within 8 h after ingestion of toxins in the contaminated food

# Laboratory diagnosis of *Staphylococcus*

## ■ Specimen:

- Pus, Urine, Stool, Blood, CSF

## ■ Gram Stain:

- Gram Positive Cocci, arranged in cluster

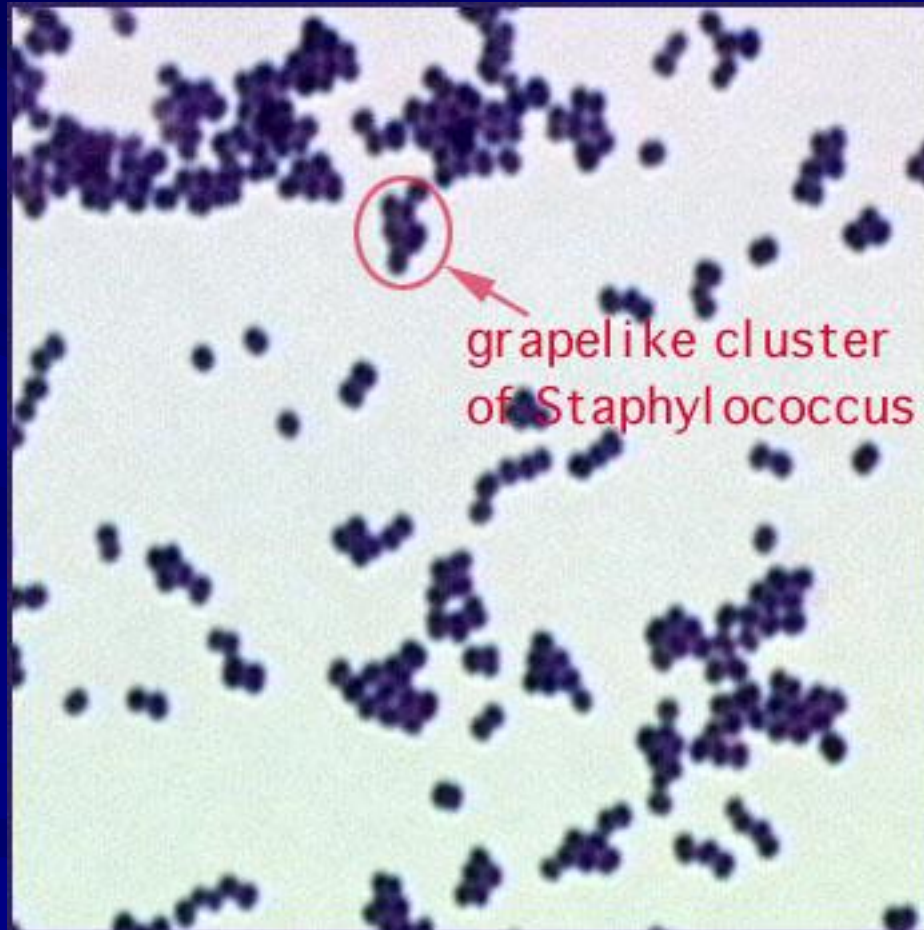
## ■ Culture:

### – Blood agar (Non-Selective Media)

- Coagulase Positive Staphylococci are Pigmented & hemolytic
- Coagulase Negative Staphylococci are non-pigmented & non-hemolytic



# Gram stain of Staphylococcus



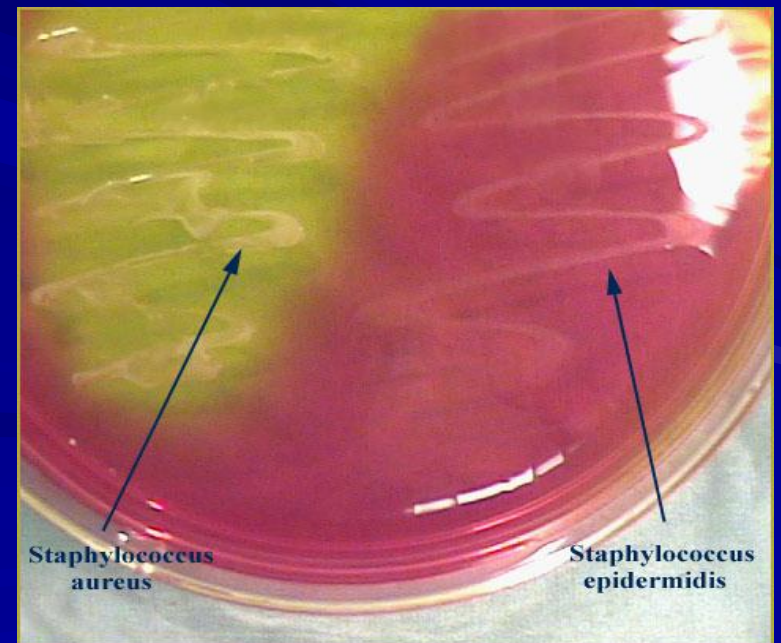
- **MSA** is selective differential medium for staphylococci
  - It contains: NaCl (7.5%), Mannitol, & Phenol Red
  - The cause of **selectivity** due to presence of high salt concentration
  - The cause of **differential** because contains mannitol (sugar) and phenol red (pH indicators turns yellow in acidic pH and turns red in alkaline pH).

### Mannitol fermentation on MSA

Mannitol fermented  
Yellow colonies:  
*S. aureus*

Mannitol nonfermenter  
Red colonies:  
*S. epidermidis* & *S. saprophyticus*

Manal Al Khulaifi



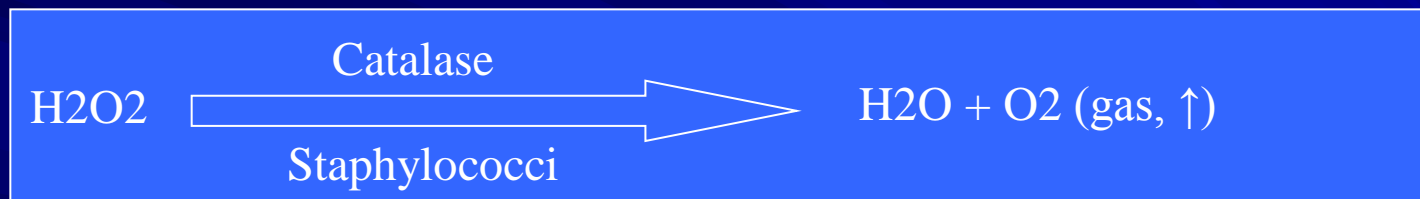
# Mannitol fermentation on MSA

Mannitol fermenter  
Yellow colonies:  
*S. aureus*

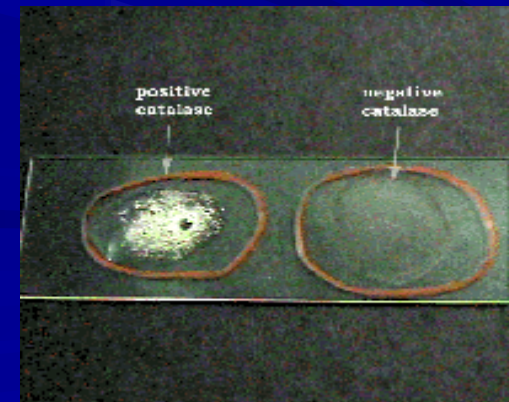
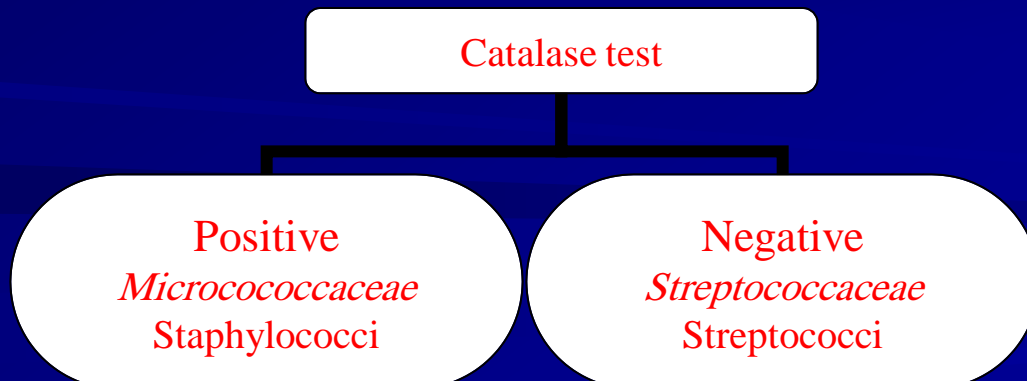
Mannitol nonfermenter  
Red colonies  
*S. epidermidis* & *S. saprophyticus*

# Catalase test

- The catalase test is distinguished streptococci from staphylococci
- flood culture with drops of 3% H<sub>2</sub>O<sub>2</sub>
- Catalase-positive cultures bubble at once



- The test should not be done on blood agar because blood itself will produce bubbles





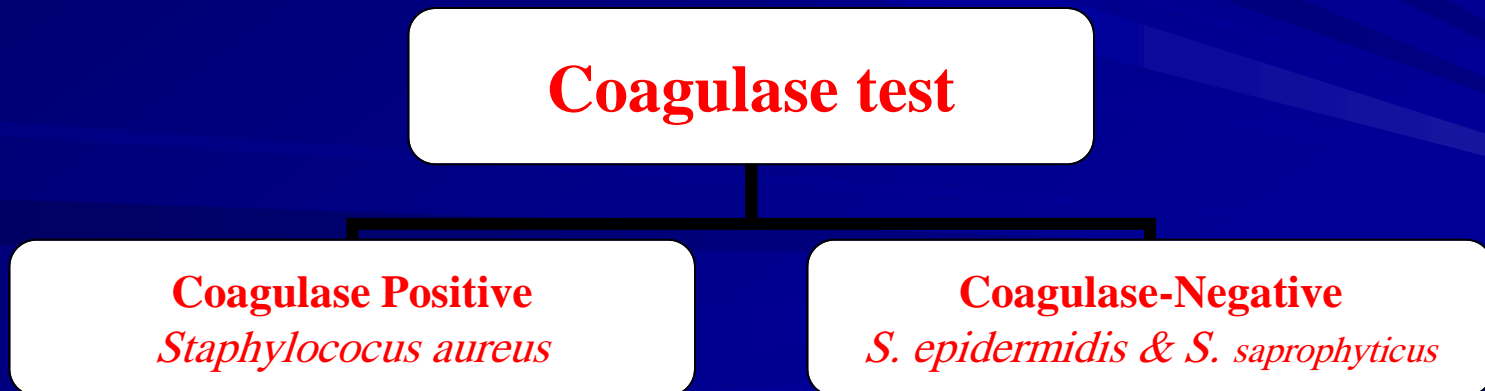
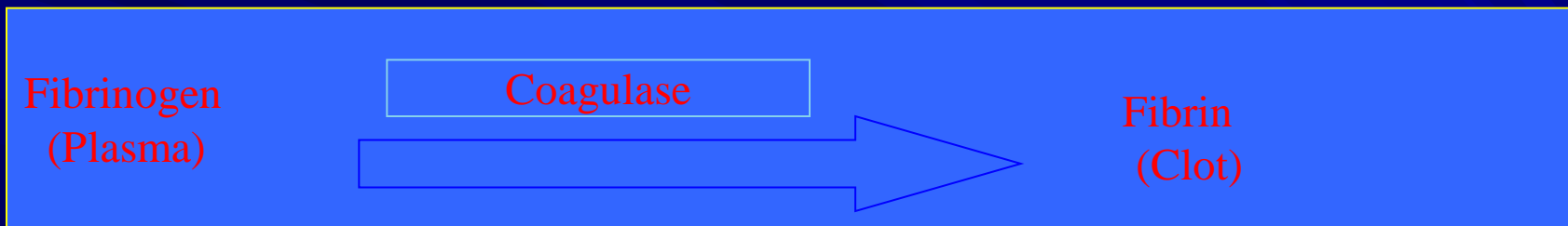




# Coagulase Test

## Principle:

- This test used to differentiate between *S. aureus* (CPS) & other *Staphylococcus* species (CNS)



# Coagulase Test

## ■ The tube coagulase test (Free):

### ■ Procedure:

- Mix 0.1 ml of culture + 0.5 ml of plasma
- Incubate at 37C for 4 h
- Observing the tube for clot formation
- Any degree of clotting constitutes a positive test

### ■ Advantage

- More accurate

### ■ Disadvantage

- Time consumed



*S. aureus*

*S. epidermidis*

# Coagulase Test

- **Two Methods:**
  - **The slide Method**
  - **Tube Method**
- **The slide coagulase test**
  - Used to detect bound coagulase or clumping factor
  - Add one drop heavy bacterial suspension and one drop of plasma on clean slide
  - Mixing well and observing for clumping within 10 seconds
- **Advantage**
  - Rapid diagnosis
- **Disadvantage**
  - Less accurate



# Deoxyribonuclease (DNAase) test

## DNase test

### Positive

*Staphylococcus aureus*

### Negative

*S. epidermidis* & *S. saprophyticus*

## ■ Principle:

- DNA is insoluble in acid
- DNA is hydrolyzed into oligonucleotides by the action of DNase
- Nucleotides soluble in acid

# DNase test

```
graph TD; A[DNase test] --> B[Positive  
Staphylococcus aureus]; A --> C[Negative  
S. epidermidis  
S. saprophyticus];
```

Positive  
*Staphylococcus aureus*

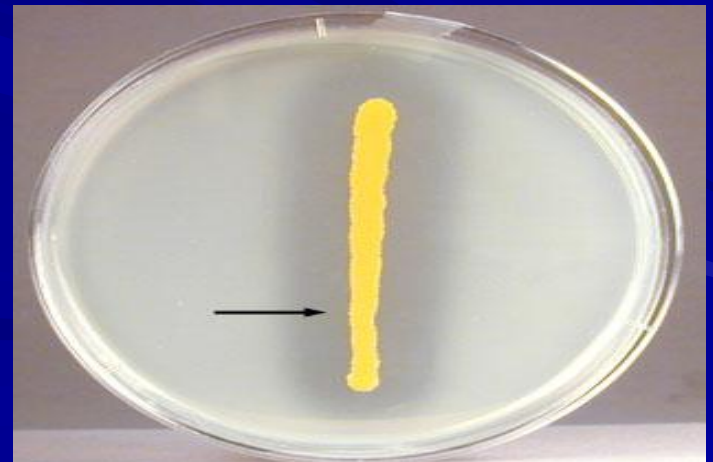
Negative  
*S. epidermidis*  
*S. saprophyticus*



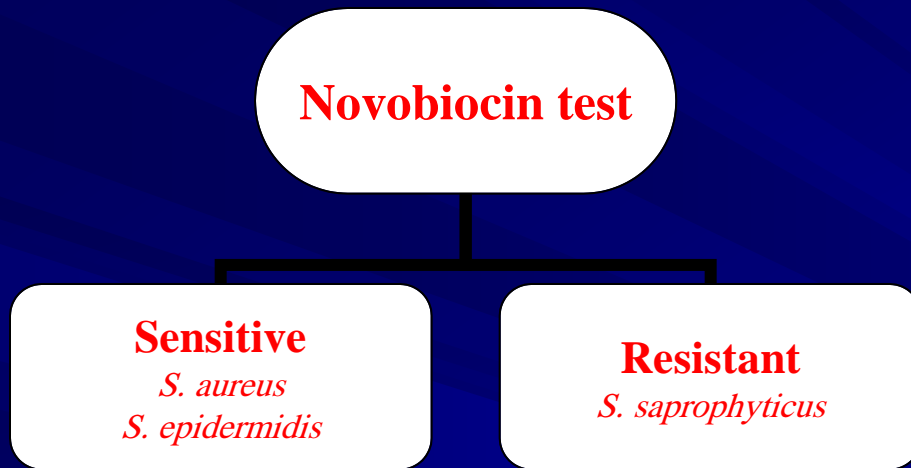
# DNase Test

## ■ Procedure & result:

- Inoculate DNA agar with tested organism in circular motion
- Incubate at 37C for 24-48h
- Observe DNase activity by adding 1N HCl to the agar surface, a zone of clearing indicates a positive test
- The zone represents the absence of DNA
- The medium around colonies not producing DNase remains opaque, which is a reflection of the precipitation of DNA by the added acid.



# Novobiocin Sensitivity



- A simple disk diffusion test for estimating novobiocin susceptibility used to distinguish *S. saprophyticus* from other clinically species
- Inoculated overnight culture on Mueller-Hinton agar
- Add novobiocin disk on inoculated plate
- Incubate at 37°C overnight
- Novobiocin resistance is intrinsic to *S. saprophyticus* but uncommon in other clinically important species.

# Preparation of Smear and Staining

## ■ Preparation of smear

- Solid culture
- Liquid culture
- Distribute culture in slide
- Air dry
- Heat fix
- Ready to stain

## ■ Gram Stain

- Primary Dye (C.V.)
- Mordant (iodine)
- Decolorizer (Alcohol)
- Counterstain (Safranin)
- All applied for 1 min
- After each step wash with water
- Blot dry
- Add one drop of immersion oil
- Examine under oil immersion lens

# Practical Work

- Gram stain
- Catalase test
- Mannitol fermentation on MSA
- Coagulase Test by Tube and Slide Method
- DNAase Test