

# Lab 3

Pathogenic Bacteria

- **GRAM-POSITIVE COCCI**

- The Gram-positive cocci are grouped together based on their Gram-stain reaction, thick cell wall composition, and spherical shape. Most famous organisms in this group are *staphylococcus* and *streptococcus*.

- **Genus: *Staphylococcus***
- **Characteristics:**
  - Staphylococci are Gram positive cocci, which occurs in irregular "grape-like" clusters.
  - They are non-motile (non-flagellate), non-spore forming and typically non capsulated.
  - They are able to grow in presence of high salt concentration (Halotolerant).
  - They are Catalase positive and Facultative anaerobes (survive with or without oxygen).
  - Grow on simple media (i.e. non fastidious).

- **Three species of staphylococci have medical importance:**
- *S. aureus* is a major human pathogen and frequently found as part of the normal skin flora on the skin and nasal passages. *S. aureus* has been found to be the causative agent in such ailments as pneumonia, meningitis, bacteremia, endocarditis, osteomyelitis (chronic bone infection), skin infections urinary tract infection and gastroenteritis (food poisoning).
- *S. epidermidis* is an opportunistic pathogen which is a normal resident of human skin.
- *S. saprophyticus* is a common cause of urinary tract infection and occasionally commensally found in skin.

# Laboratory diagnosis

- **Specimen collected: Depends on the type of infection:** Pus, Sputum, Urine, Blood.
- **Isolation of microorganism:** by culturing on Nutrient agar and Blood agar.
- **Identification:**

## Microscopical Examination

- **Gram Stain:** Staphylococci are gram-positive cocci, which occur in irregular "grape-like" clusters

# Culture characteristics:

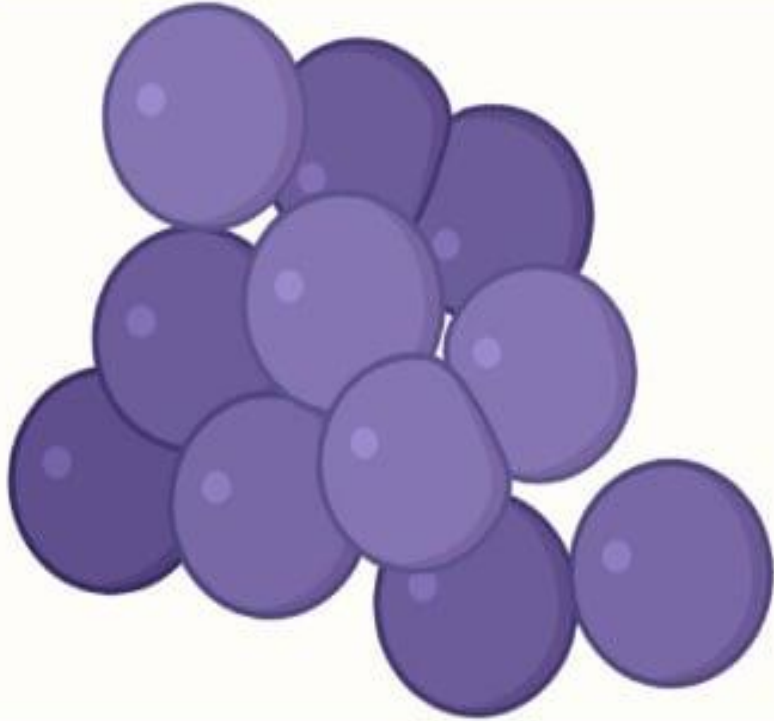
- On nonselective media:
- On Blood agar: **Colonies of coagulase-positive staphylococci i.e. *S. aureus* are pigmented and hemolytic.** However, **colonies of coagulase-negative staphylococci (e.g. *S. epidermidis*) are non-pigmented and non-hemolytic.**
- On Nutrient agar: Can grow on nutrient agar producing **golden-yellow colonies** (coagulase-positive staphylococci), or **white (or cream-colored)** (coagulase-negative staphylococci).



*Staphylococcus aureus*

*Staphylococcus epidermidis*

# *Staphylococcus saprophyticus*- An Overview



**Definition, Classification, Habitat, Morphology, Cultural and Biochemical Characteristics, Virulence factors, Pathogenesis, Clinical Manifestations, Lab Diagnosis, Treatment, Prevention.**



# Staphylococci on Nutrient Agar

<b>Organism</b>	<i>S.epidermidis</i>
<b>Media</b>	Nutrient agar
<b>Appearance</b>	White colonies



# *Staphylococcus aureus*

- Colonial morphology:

- **Nutrient agar (NA):** golden yellow colonies.



- **Blood agar (BA):** beta-hemolytic, opaque colonies.



## Other *Staphylococcus* species

- *S. epidermidis (albus):*

- Blood agar (BA): white, non-hemolytic colonies, Novobiocin sensitive (S) colonies.

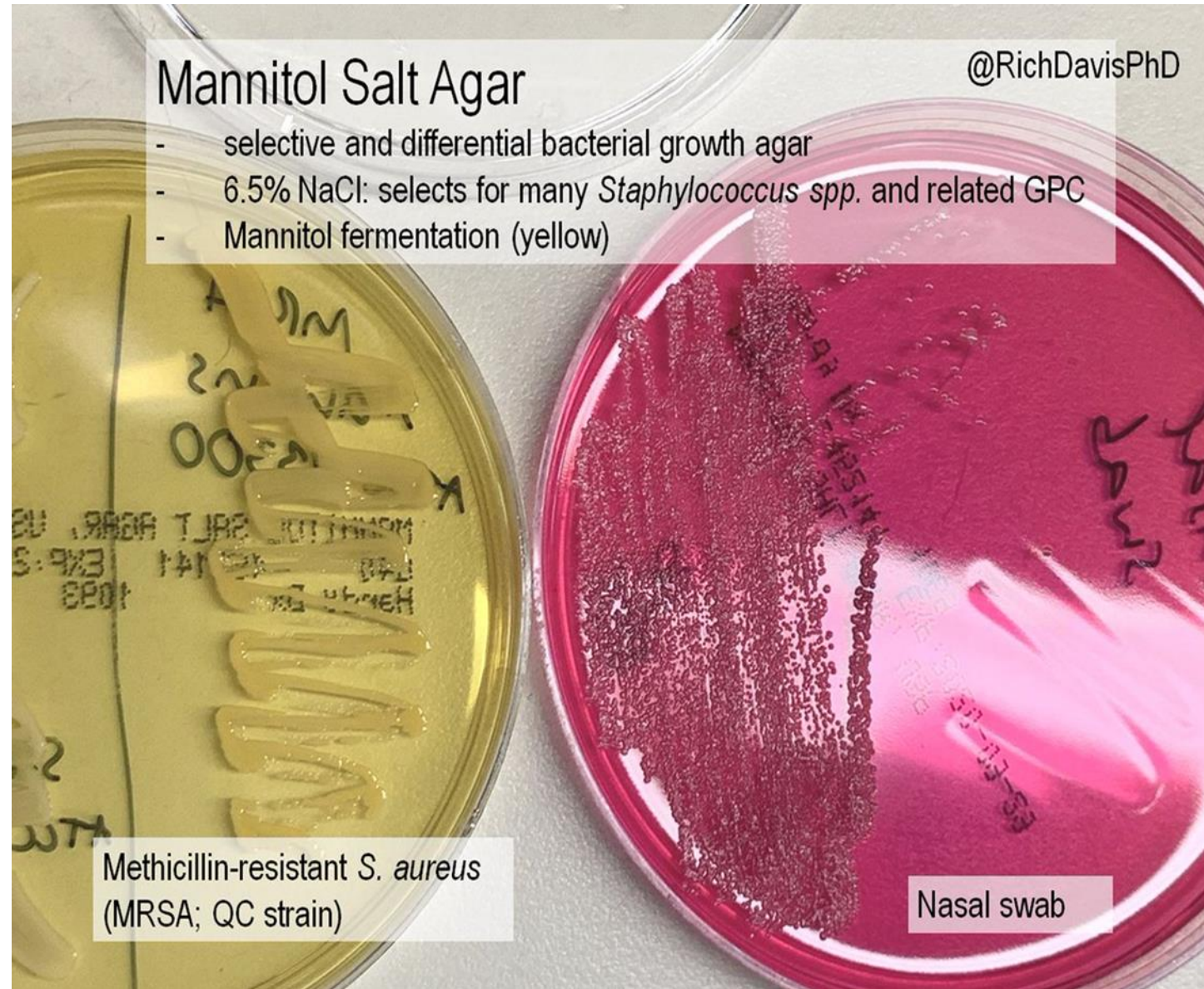
- *S. saprophyticus:*

- Blood agar (BA): bright-white to creamy, non-hemolytic, Novobiocin resistant (R) colonies.



## • On selective medium:

- Mannitol salt agar (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).
- *S. aureus* ferment mannitol and release acid which decreased in the pH. The acidic pH will cause the phenol red to turn yellow. Other *staphylococci* do not ferment mannitol and the medium remains pink.



# Biochemical reactions

- **Catalase test:**
- The catalase test is important in distinguishing *staphylococci* which are catalase-positive from streptococci (catalase-negative).
- Flood culture with drops of 3% hydrogen peroxide ( $H_2O_2$ ).
- Catalase-positive cultures bubble at once.
- The test should not be done on blood agar because blood itself will produce bubbles.
- **Coagulase production test:**
- The ability to clot plasma is widely used in differentiating *S. aureus* from other staphylococci.
- The enzyme acts by converting fibrinogen into fibrin.
- Coagulase test used to classify staphylococci into 1- Coagulase-positive staphylococci (e.g. *S. aureus*) and 2- Coagulase-negative staphylococci (*S. epidermidis*, *S. saprophyticus*).
- It is thought that coagulase-positive staphylococci may avoid host defense mechanisms by forming this fibrin clot around them.

# Two different coagulase tests can be performed:

## 1. The slide coagulase test:

Used to detect bound coagulase or clumping factor

Add one drop of heavy bacterial suspension and one drop of plasma on a clean slide.

Mixing well and observing for clumping within 10 seconds

**Advantage:** Rapid diagnosis.

**Disadvantage:** Less accurate.

## 2. The tube coagulase test (Free):

Used to detect free coagulase.

Mix 0.1 ml of culture + 0.5 ml of plasma and incubate at 37°C for 4 h.

Observing the tube for clot formation. Any degree of clotting constitutes a positive test

**Advantage:** More accurate.

**Disadvantage:** Time consumed

**Motility test:** negative.

**Oxidase test:** All species of *Staphylococcus* are oxidase negative.

# Gelatinase test (Gelatin liquefaction test): positive.

- **Principle:**

- Gelatin is a protein derived from the animal protein collagen, has been used as a solidifying agent for a long time. The purpose of the gelatinase test is to identify bacteria that may produce an exoenzyme called gelatinase that hydrolyses (breaks down) gelatin to amino acids. These amino acids can then be transported into the cell for further metabolism. At temperature below 25°C, gelatin will remain a gel, but if the temperature rises about 25°C, the gelatin will be liquid. If you cool the liquefied gelatin, it will resolidify.

# Method:

Obtain 2 nutrient gelatin tubes. **Stab** inoculate one and leave the other uninoculated (control), then Incubate.

Every few days, gently place the two tubes in the refrigerator for 15 minutes.

After 15 minutes: **if** the gelatin remains **liquid**, the result is **positive** and you may end the test.

If the gelatin is **solid**, the result is **negative**. Continue to incubate the cultures. A **negative** result is only concluded if the gelatin remains solid through 7 days of incubation.

## Result:

+ = within 7 days of incubation, refrigerated gelatin remains liquid.

- = After 7 days of incubation, refrigerated gelatin is solid.



# Deoxyribonuclease (DNase) test:

## Principle

DNA is insoluble in acid

DNA is hydrolyzed into oligonucleotides by the action of DNase and Nucleotides soluble in acid.

## Procedure & result:

Inoculate DNA agar with the tested organism and Incubate at 37°C for 24-48 h.

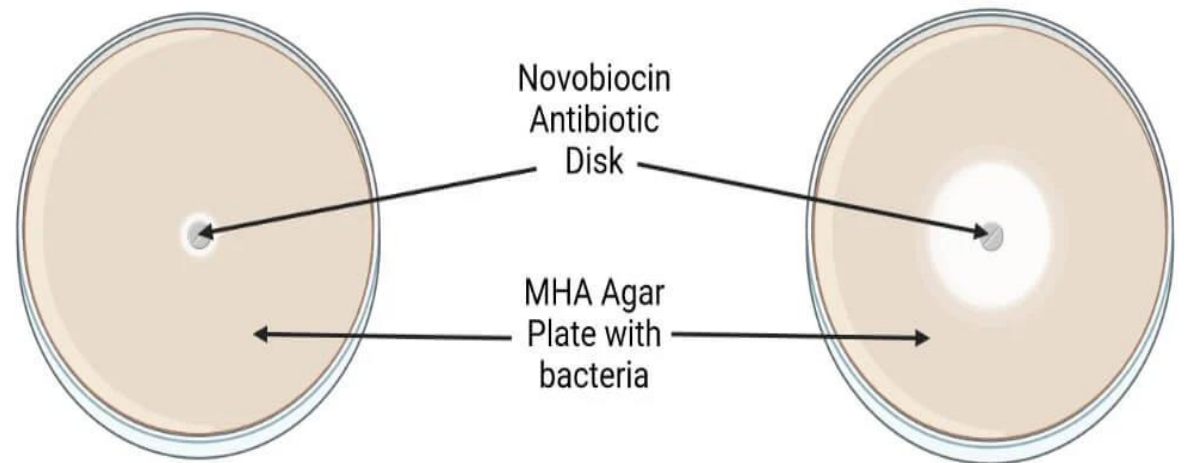
Observe DNase activity by adding 1N HCl to the agar surface, a zone of clearing indicates a positive test. The zone represents the absence of DNA. (*S. aureus* is positive and other is negative)

The medium around colonies not producing DNase remains opaque, which is a reflection of the precipitation of DNA by the added acid.

# Novobiocin Resistance:

- A simple disk diffusion test for estimating novobiocin susceptibility.
- Inoculated **Mueller-Hinton agar**, add novobiocin disk on plate and incubate at 37°C overnight.
- Novobiocin Resistance staphylococci (*S. saprophyticus*) and Novobiocin Sensitive (*S. aureus*).

## Novobiocin Susceptibility Test Results

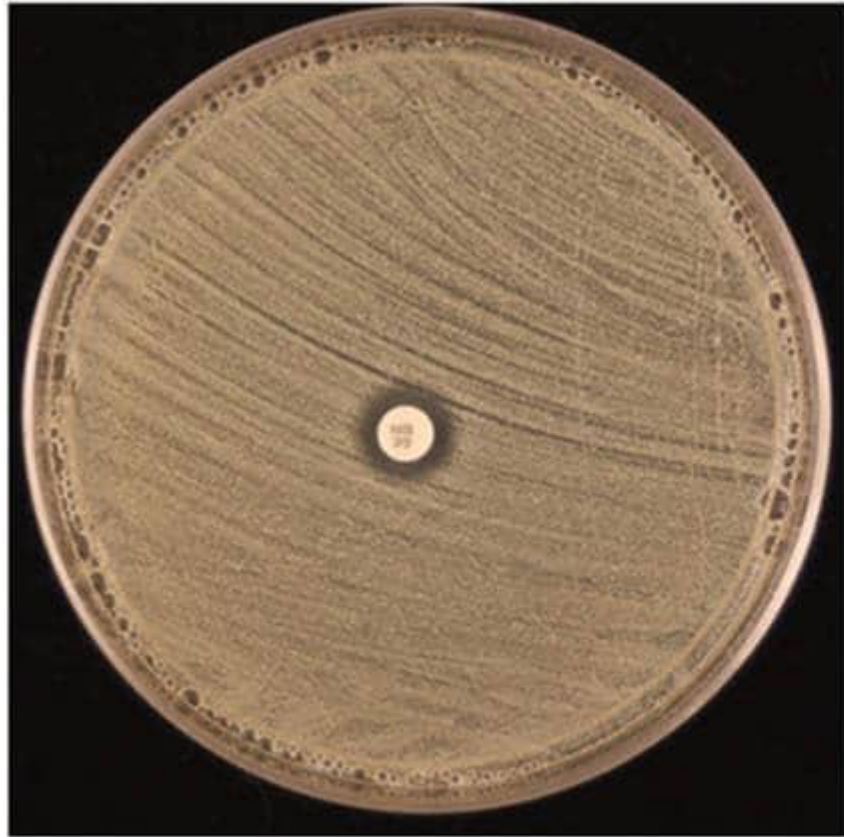


Zone size of  $\leq 16$  mm  
Resistant to novobiocin

*Staphylococcus saprophyticus*,  
*S. kloosii*, *S. cohnii*

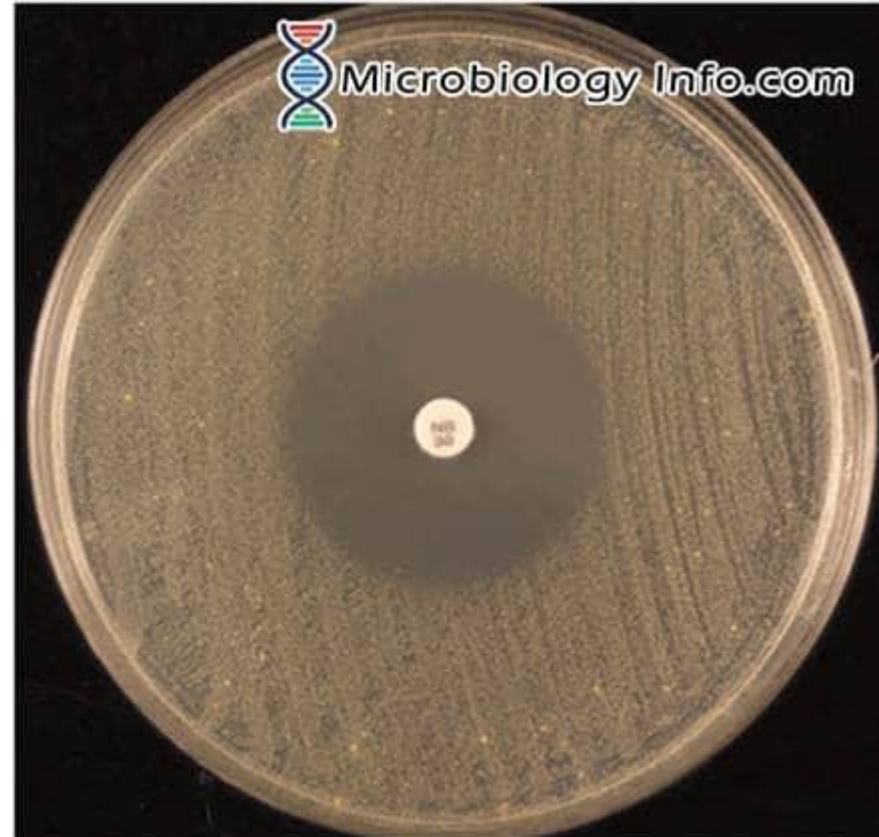
Zone size of  $> 16$  mm  
Sensitive to novobiocin

*Staphylococcus aureus*, *S. epidermidis*,  
*S. haemolyticus*, *S. hominis*, *S. capitis*



***Staphylococcus saprophyticus***  
**Resistant (less than 16 mm)**

**Z-O-O-B-O-Z**



***Staphylococcus aureus***  
**Sensitive (greater than 16 mm)**