

# **Genus Staphylococci**

# **Staphylococci**

- They are **gram-positive cocci** 0.5-1.0 μm in diameter.
- are often found in the human nasal cavity (and on other mucous membranes) as well as on the skin.
- arranged in irregular grape-like clusters.
- Non motile
- The staphylococci are catalase positive.
- Tolerate relatively **high concentrations of sodium chloride** (7.5-10%). This ability is often employed in preparing media selective for staphylococci.



Three species of staphylococci commonly associated with clinical infections:

Staphylococcus aureus
S. Epidermidis
S. saprophyticus



Microscopic characteristics Gram's positive cocci, Grape-like cluster

## Human Infections caused by Staphylococcus

### \* <u>S. aureus</u> cause:

- Skin infections like impetigo (rashes ,red sores), folliculitis(infection in hair follicles), wound infection.
- Systemic infections like bacterimia (viable bacterial cells in blood), pneumonia, meningitis, deep-seated abscess.
- Toxin mediated infections like food poisoning, toxic shock syndrome.
- \* <u>S</u>. <u>epidermidis</u> cause :
- Opportunistic infections like intravenous catheter infections, CSF shunt infections.
  - Prosthetic Infection S. saprophyticus cause:
- ➢ UTIs.





# **Cultural Characteristics**

- Aerobic or facultative anaerobic
- Grow on (nutrient agar & blood agar) & can tolerate 7.5 10 % NaCl.
- The colonies are round, smooth, raised, & glistening, producing pigments
- Staphylococcus epidermides : gray to white
- Staphylococcus aureus : gray to golden yellow

OnlyStaphylococcusaureusproduceβ-haemolysisonbloodagar& fermentmannitol



Colonies on biood agar





# Specimens

Various specimens collected in staphylococcal infections	
Specimen	Condition
Pus	Supurrative lesions
Sputum	Respiratory infections
Blood	Bacterimia
Feces vomitus	Food poisoning
Urine	UTIs

## Hemolysis on blood agar

Some bacteria produce <u>hemolysins</u>, that cause red blood cells (RBC's) to burst open (hemolyse).

When these bacteria are cultured on blood agar, this hemolysis is visible as an area of clearing around the colony (zone of hemolysis).

- If the organism completely lyse the RBC's, this is termed <u>beta hemolysis</u> (β-hemolysis).
- Partial destruction of the RBC's produces a greenish color to the zone of hemolysis and is termed <u>alpha hemolysis</u> (α-hemolysis).
- **Organisms lacking hemolysins** cause no change in the color or opacity of the media and are termed **gamma hemolytic or none hemolytic**.

# **Macroscopic characteristics**



Staphylococcus aureus cause haemolysis on blood agar because of haemolysin enzyme. a zone of β – haemolysis will appear around the colony

Test	S. aureus	S. epidermidis	S. saprophyticus
Hemolysis	Usually beta	Usually none	Usually none

### **Mannitol fermentation on Mannitol Salt agar (MSA)**

Staphylococci are able to tolerate the high salt concentration found in Mannitol Salt agar and thus grow readily.

If mannitol is fermented, the acid produced turns the phenol red pH indicator from red (alkaline) to yellow (acid).



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### Mannitol Salt Agar

- Staphylococcus aureus
- Produces yellow colored colonies as it ferments Mannitol
- Fermentation causes change in color of indicator – Phenol red , from red to yellow.
- Staphylococcus epiderdimis
- Non- Mannitol fermenter, hence colorless colonies with pink colored media
- No change in color of indicator is seen.





**Positive** = acid end products turn the phenol red pH indicator from red to yellow **negative** = phenol red remains red

## **MANNITOL SALT AGAR**

#### MANNITOL SALT AGAR

NaCl 7.5%  $\rightarrow$  selective

Mannitol  $\rightarrow$  differential

Staphylococcus aureus can ferment mannitol, so the selective media used to isolate Staphylococcus aureus especially from nasal carriage is (Mannitol Salt Agar)



Test	S. aureus	S. epidermidis	S. saprophyticus
Mannitol fermentation	Positive	Negative	Usually positive



# **Coagulase Test**

#### PURPOSE

- To determine the ability of the organism to produce coagulase which clots plasma.
- To distinguish the pathogenic coagulase positive staphylococcus from the nonpathogenic coagulase negative staphylococcus.

#### **Principle:**

Coagulase is an enzyme that converts soluble fibrinogen into insoluble fibrin.

#### Two forms of coagulase:

- 1. Bound coagulase (clumping factor) -
- > Detected in the coagulase slide test
- Can directly convert fibrinogen to insoluble fibrin and causes the staphylococci to clump together

#### 2. Free coagulase

- Detected in the coagulase tube test

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



Slide Coagulase Test



S. epidermidis

## Catalase test

### **Procedure:**

- Place a drop of 3% hydrogen peroxide on a clean microscope slide.
- Place a heavy loopfull of cells from isolated colonies into the liquid (pick four to five colonies). Immediate generation of gas bubbles constitutes a positive test.
- 3. Avoid the inclusion of blood cells from blood agar plates as RBCs contain catalase.
- 4. Lack of bubbles is a negative test.

#### Catalase

 $2H_2O_2$  ----->  $2H_2O + O_2$  (bubbles)





## Novobiocin Sensitivity test

Novobiocin is an antibiotic to which Staphylococcus sp. are either resistant or sensitive. The appearance of a zone of inhibition > 16 mm indicates sensitivity.

Filter paper discs impregnated with the appropriate chemical are placed on an agar surface. The chemical diffuses through the agar. Organisms that are susceptible to the chemical will not grow on the agar containing the chemical. The size of the zone of growth inhibition determines the organisms' susceptibility to the chemical.

Test	S. aureus	S. epidermidis	S. saprophyticus
Novobiocin test	Sensitive	Sensitive	Resistant (at a concentration of 5 mg),



Staphylococcus saprophyticus Resistant (less than 16 mm)



Staphylococcus aureus Sensitive (greater than 16 mm)

#### **Production of staphyloxanthin (pigmentation)**

Staphyloxanthin is an orange pigment produced by Staphylococcus aureus that contributes to its virulence. It is the main pigment of this pathogen.

• Milk agar provides a white background to visually observe the colonies of *Staphylococcus aureus* 

#### **Procedure:**

- Streaking colonies of staphylococci on milk agar plate
- incubating overnight



- observing the golden yellow pigment of *Staphylococcus aureus* 

Test	S. aureus	S. epidermidis	S. saprophyticus
Pigment	Often creamy gold	Usually white	Usually white



### **Production of staphyloxanthin (pigmentation)**

#### Production of deoxyribonuclease (DNase) on DNase agar

DNase agar contains 0.2% DNA. To detect DNase production.

•the plate is Inoculate by making a single streak line using inoculum from an agar slant or plate and then incubated.

•After growth, the plate is flooded with hydrochloric acid (HCl).

•DNase positive cultures show a distinct clear zone around the streaked area, where the DNA in the agar was broken down by the bacterial DNase.

•DNase negative cultures appear cloudy around the growth where the acid caused the DNA in the agar to precipitate out of solution.

Test	S. aureus	S. epidermidis	S. saprophyticus
DNase production	Positive	Negative	Negative

**Positive** = clear zone around growth after adding HCl (no DNA remaining in the agar) **negative** = cloudy around growth after adding HCl (DNA remains in the agar forming a precipitate)

A Positive DNase Test



Note there is breakdown of the DNA in the agar. There is a clear zone (arrow) around the bacterial growth where there is no longer any DNA left in the agar to precipitate out of solution after the HCl was added.

### **Gelatinase test**

Nutrient gelatin is a differential medium that tests the ability of an organism to produce **gelatinase that hydrolyzes gelatin**.

 The gelatinase test can be used to differentiate between *Staphylococcus aureus* and *Staphylococcus epidermidis*.



Test	S. aureus	S. epidermidis
Gelatinase	Positive	Negative

#### Table summarized the point of **differences** between 3 imp. *Staphylococci* species

Tests	S. aureus	S. epidermidis	S.saprophyticus
Catalase	+	+	+
Coagulase	+	-	-
β – haemolysis	+	-	-
Mannitol fermentation	+	-	-
Novobiocin sensitivity (0.5 μg)	+	+	-

# **THANKS**