

Practical Medical Bacteriology

Lab 3

Laboratory Diagnosis of Staphylococci group



2021/2022

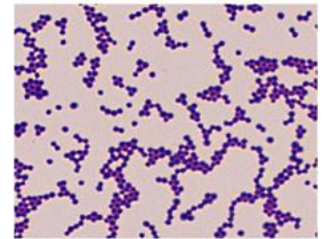
G+ Staphylococci group

- Opportunistic pathogen.
- Belongs to family Micrococaceae causing significant infections under appropriate conditions.
- The genus *Staphylococcus* has at least **40 species**, 14 to 17 species associated with human.
- The four most frequently encountered species of clinical importance are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis* , and *Staphylococcus saprophyticus*.



General characteristics

- Gram positive cocci (Greek: staphyle = bunch of grapes; kokkos = grain), that occur singly and in pairs, short chains and irregular grape-like clusters.
- Non-motile and non-spore forming and may be encapsulated, aerobic and facultative anaerobes.
- Resistant to temperature as high as 50°C, high salt concentration 7.5% of NaCl and resistant to drying.
- Catalase - positive.
- It's ecological niche is skin, but they may found as normal flora of other upper site of upper respiratory system



Classification

According to coagulase test

1. Coagulase positive (*Staphylococcus aureus*).
2. Coagulase negative (*S. epidermidis*, *S. saprophyticus* and *S. lugdunensis*)

Staphylococcus aureus

- Facultative anaerobic.
- Gram-positive arranged in grape like clusters.
- Produce large (2-4 mm), round, golden-yellow colonies, often with beta- hemolysis on blood agar
- Coagulase positive
- DNase positive



Laboratory diagnosis

1. Specimen:

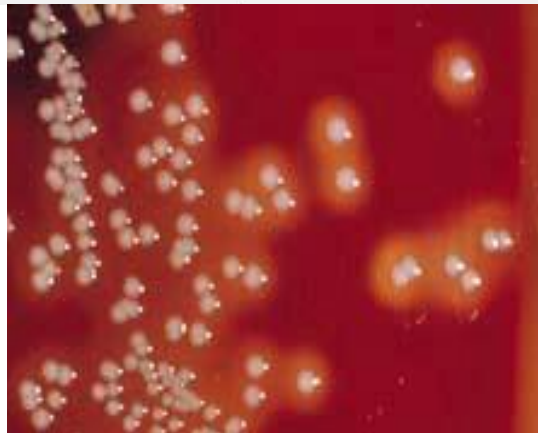
- Pus, Urine, Stool, Sputum, Blood

2. Microscopic Examination

- Gram stain: Gram-positive cocci in single, in pairs, short chains and grape-like clusters (not reliable).

3. Culture: On Blood agar (Non-Selective Media)

- Coagulase Positive Staphylococci are pigmented & Beta-hemolytic such as *Staph. aureus*
- Coagulase Negative Staphylococci are non-pigmented & non-hemolytic or hemolytic.



Laboratory diagnosis

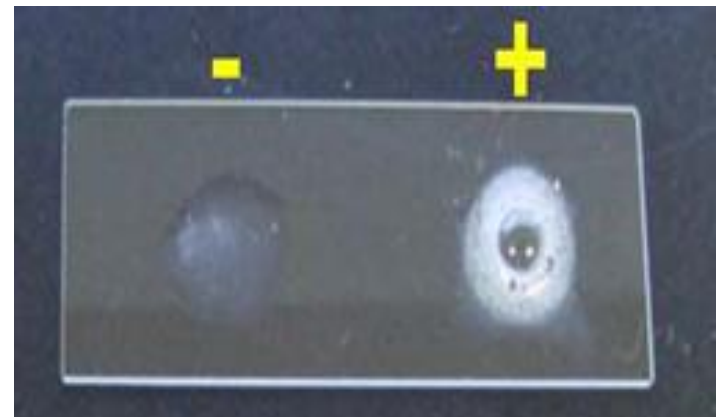
4. Biochemical (Confirmatory) tests:

A. **Catalase test:** used to distinguish Staphylococci from Enterococci and Streptococci.

➤ **Principle:** tests for enzyme catalase $2\text{H}_2\text{O}_2 = 2\text{H}_2\text{O} + \text{O}_2$

➤ **Procedure:**

- Smear a colony of the organism to a slide
- Drop H_2O_2 onto smear
- Observe the results
 - Presence of bubbles “Positive”
 - Absence of bubbles “Negative”



Laboratory diagnosis

4. Biochemical (Confirmatory) tests:

B. Coagulase test: One of the most important diagnostic tests.

➤ **Principle:** To differentiate between *S. aureus* & other *Staphylococcus* species.

➤ **Procedure**

1. Slide coagulase test:

- Put a drop of saline on slide mixed with 1 - 2 colonies
- Add equal amount of plasma,
- The positive result appears as clump during 5 - 10 seconds.



Laboratory diagnosis

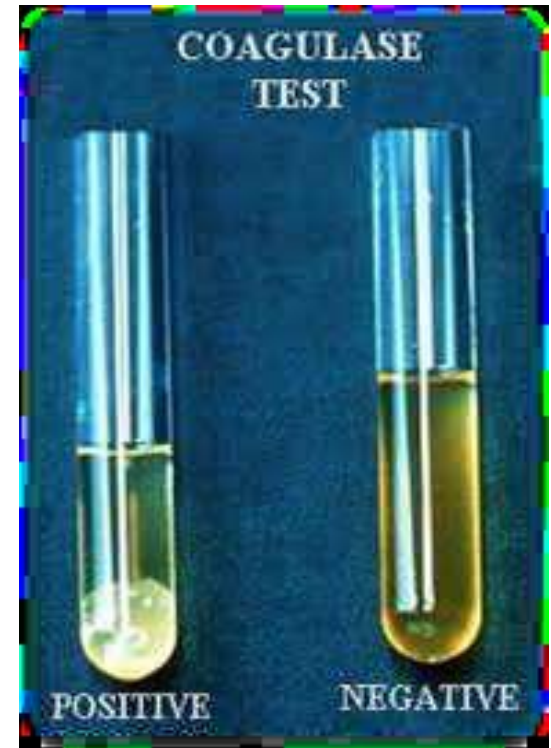
4. Biochemical (Confirmatory) tests:

B. Coagulase test:

➤ Procedure

2. Tube coagulase test:

- Inoculate 0.5 ml plasma with 0.1 ml of microorganism and incubate at 35-37 °C.
- Observe at 30 minutes for the presence of a clot if there is no clot incubate at room temperature overnight. (Why?)



Laboratory diagnosis

4. Biochemical (Confirmatory) tests:

C. DNase test:

- **Principle:** Used to distinguish *S. aureus* (positive) from other *Staphylococcus* species (Negative) (second confirmatory test).

- **Procedure**
 - Streak heavy inoculum of bacteria onto the surface of the DNase agar in a straight line.
 - Incubate at 37 °C for 24 hours
 - Add 1N HCl to the surface of the plate.
 - DNase +: zone of clearance on DNase agar

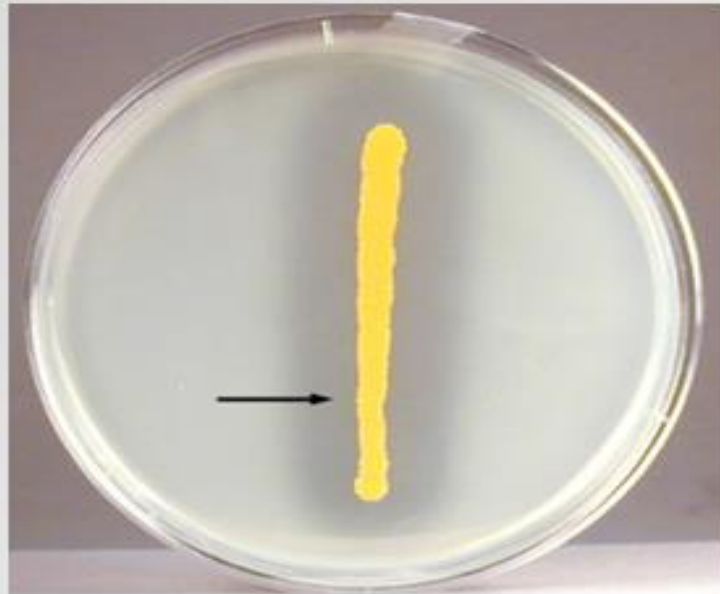


Laboratory diagnosis

4. Biochemical (Confirmatory) tests:

C. DNase test:

DNase test: zone of clearance on DNase agar



Staphylococcus aureus
Zone of clearing (arrowed)



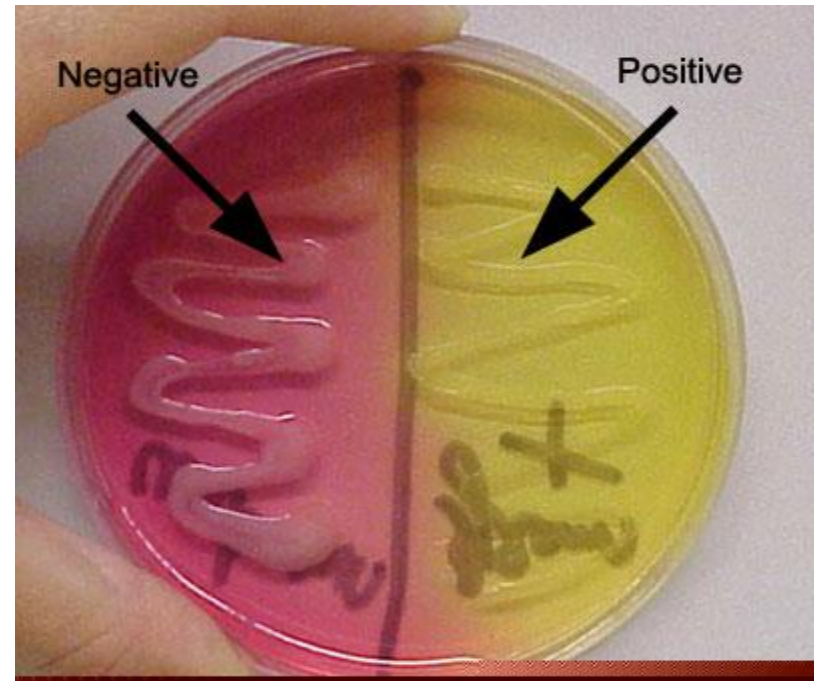
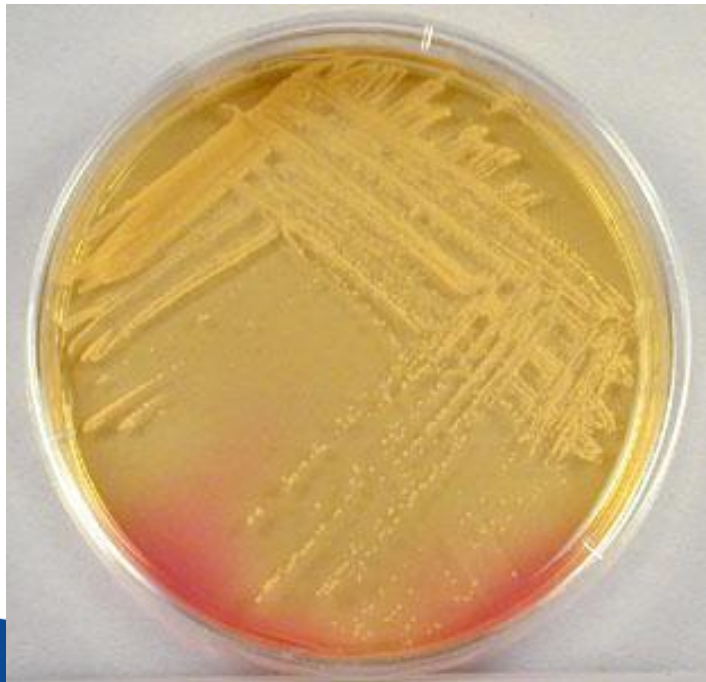
Staphylococcus epidermidis
No zone of clearing

Laboratory diagnosis

4. Biochemical (Confirmatory) tests:

D. Mannitol fermentation on MSA (7.5 % NaCl):

- *S. aureus* ferment mannitol and produce yellow-colored colonies as a result of mannitol fermentation.

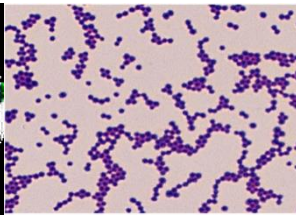


Differences *S. aureus* and other species

Tests	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. saprophyticus</i>
Catalase	+	+	+
Coagulase	+	-	-
DNase	+	-	-
Mannitol fermentation	+	-	-
Sensitivity to Novobiocin	Sensitive	Sensitive	Resistant
Blood hemolysis	+ (α, β, γ)	-	-

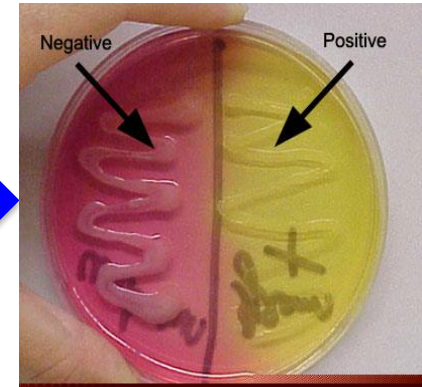


Laboratory diagnosis of *Staphylococcus aureus*

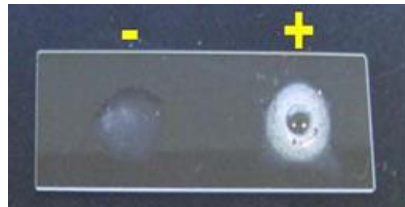


Gram stain

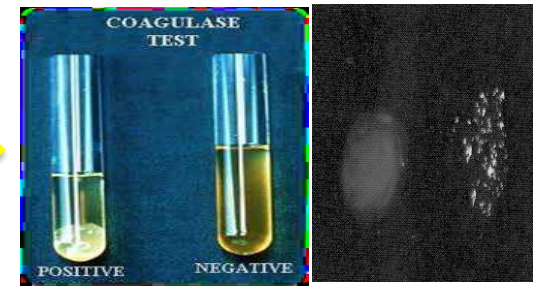
Mannitol salt agar



Catalase



Coagulase



DNase

DNase test: zone of clearance on DNase agar



Staphylococcus aureus
Zone of clearing (arrowed)



Staphylococcus epidermidis
No zone of clearing