Practical
Medical Bacteriology

Lab 4
Laboratory Diagnosis of Staphylococci group

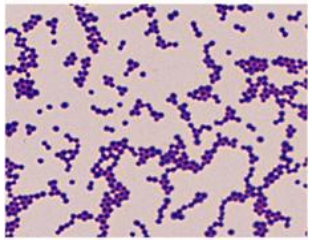
2021/2022

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)

A. Coagulase Positive Staphylococci are pigmented & Beta-hemolytic such as *Staph. aureus*

B. Coagulase Negative Staphylococci are non-pigmented & non-hemolytic or hemolytic.

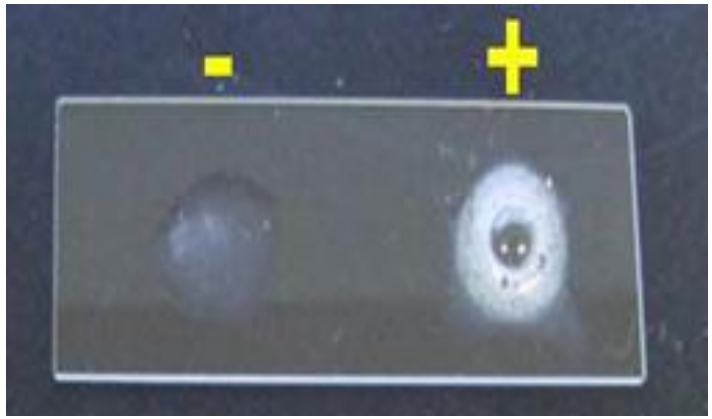
Laboratory diagnosis

4. Biochemical (Confirmatory) tests:

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

- **Principle:** tests for enzyme catalase $2H_2O_2 = 2H_2O + O_2$

- **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

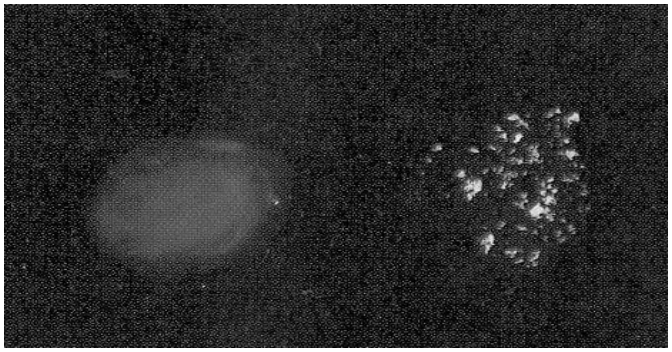
4. Biochemical (Confirmatory) tests:

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:

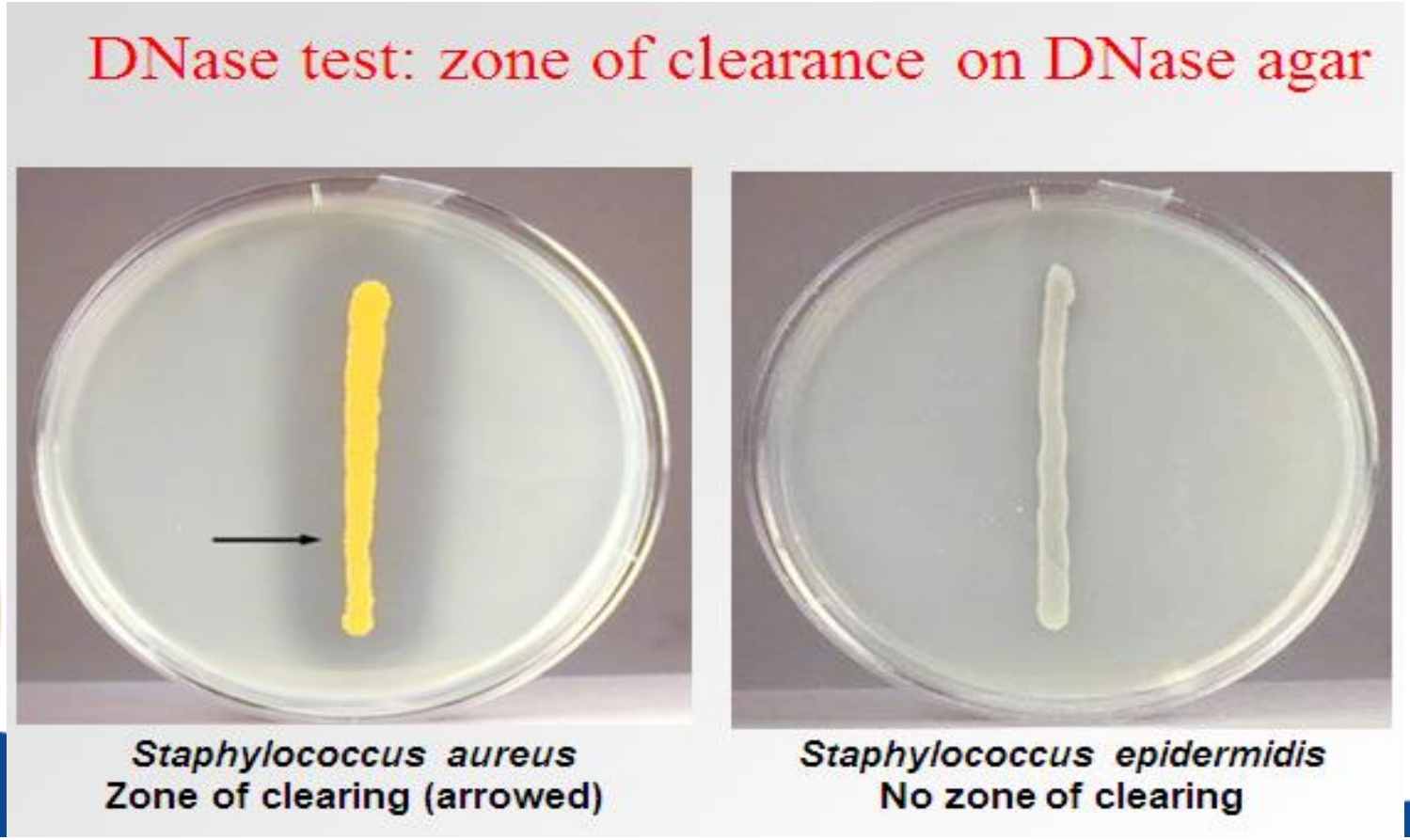
- **Principle:** Used to distinguish *S. aureus* (positive) from other *Staphylococcus* species (Negative) (second confirmatory test).

- **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:

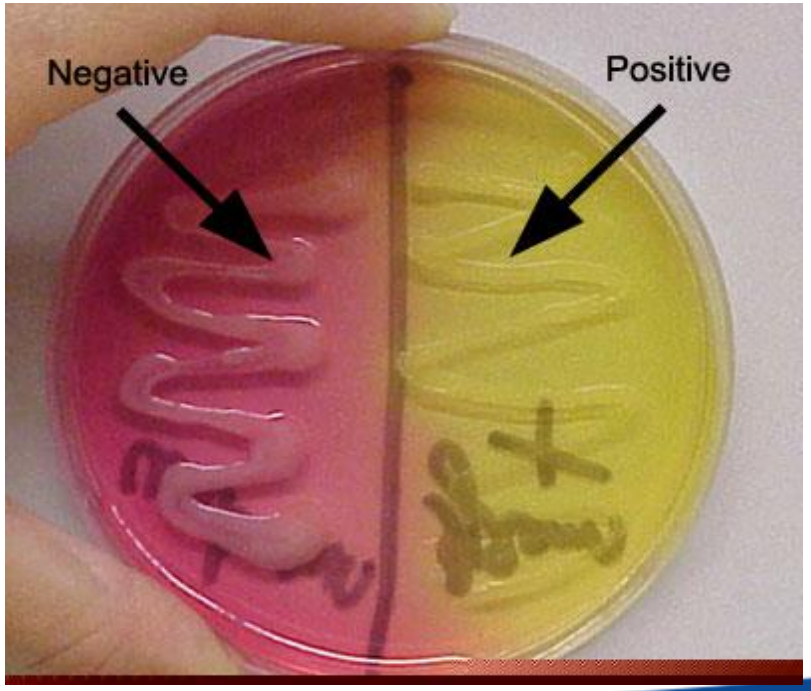


Laboratory diagnosis

4. Biochemical (Confirmatory) tests:

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

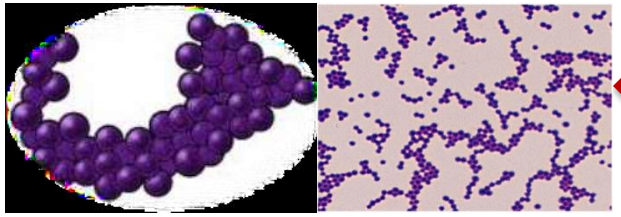
- *S. aureus* ferment mannitol and produce yellow-colored colonies as a result of mannitol fermentation.



Differences *S. aureus* and other species

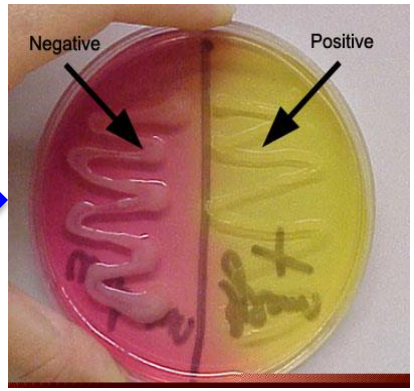
Tests	*S. aureus*	*S. epidermidis*	*S. saprophyticus*
Catalase	+	+	+
Coagulase	+	-	-
DNase	+	-	-
Mannitol fermentation	+	-	-
Sensitivity to Novobiocin	Sensitive	Sensitive	Resistant
Blood hemolysis	+ (α,β,γ)	-	-

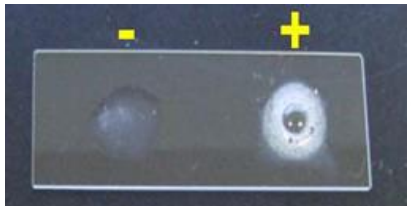
Laboratory diagnosis of *Staphylococcus aureus*



Gram stain

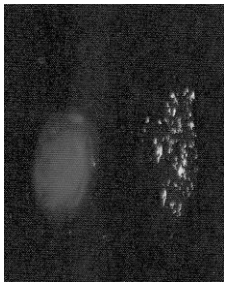
Mannitol salt agar





Catalase

Coagulase



DNase