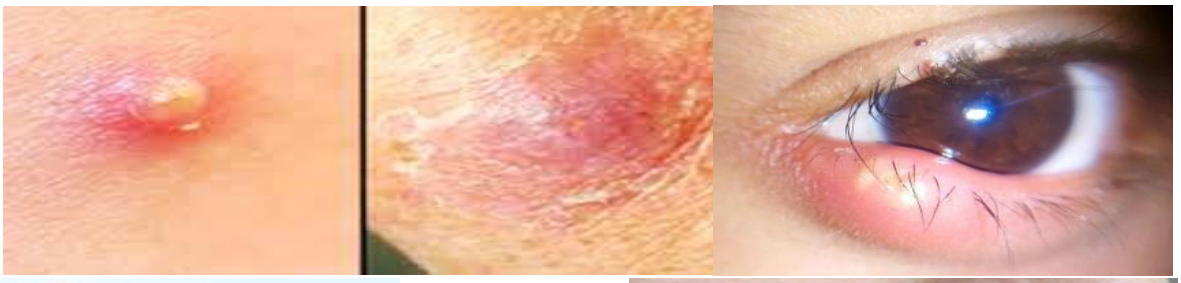
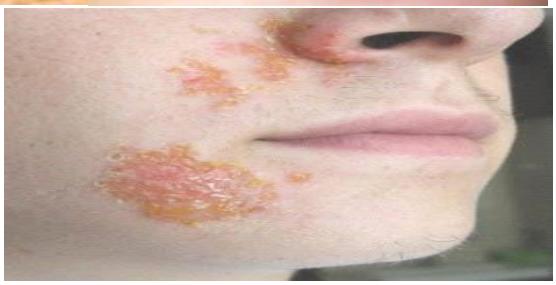
Genus Staphylococcus





Introduction:

The Staphylococcus genus includes at least 40 species but ***Staphylococcus aureus*** causes most staph infections . It is a normal human inhabitant, most commonly found in the nose, but also known to inhabit the skin and vagina. It is a common nosocomial pathogen. There are five species of staphylococci **commonly associated with clinical infections: *Staphylococcus aureus, S.epidermidis, S. haemolyticus, S. hominis* and *S. saprophyticus***.

Characteristics :

- Gram positive cocci of varying size occurring singly, in pairs and in irregular clusters.
- Non-motile, lack endospores and flagella.

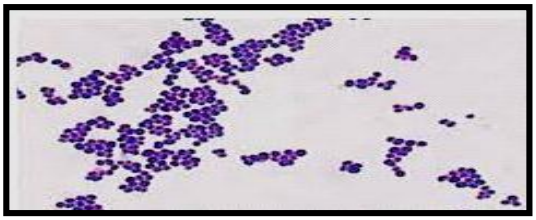
- Colonies are opaque and may be golden, white or cream and occasionally yellow or orange.
- Catalase positive and are also oxidase negative with the exception of the *S. sciuri* group (*S. sciuri*, *S. lentus* and *S. vitulinus*).
- Able to grow in 6.5% sodium chloride.
- Some species produce extracellular toxins.
- Staphylococci may be identified by the production of deoxy-ribonuclease (DNase) .

Pathogenicity:

- Skin infections (folliculitis, impetigo, wound infections, scalded skin syndrome).
- Toxic shock syndrome
- Endocarditis
- Osteomyelitis
- Pneumonia
- Food poisoning
- Infections related to prosthetic devices (prosthetic joints and heart valves; vascular shunts, catheters): Commonly associated with coagulase-negative staphylococci.

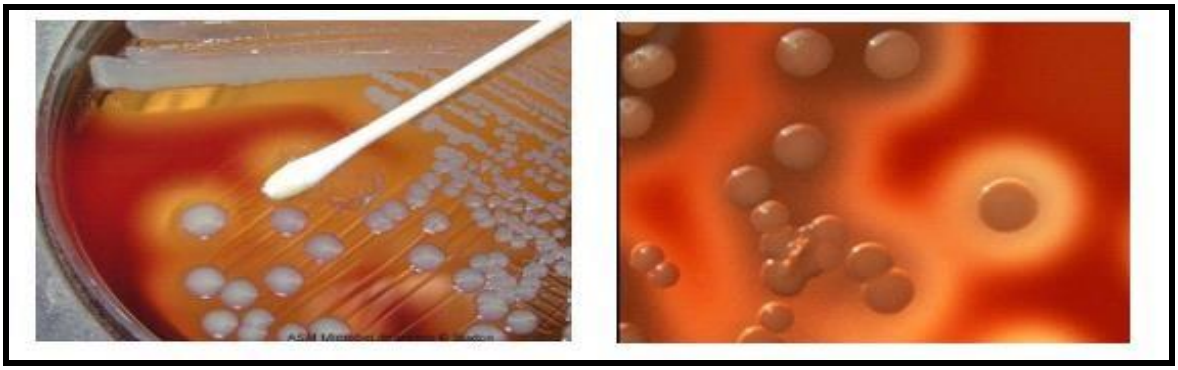
Lab work :

1. **Gram stain:**



Gram positive cocci arranged in clusters

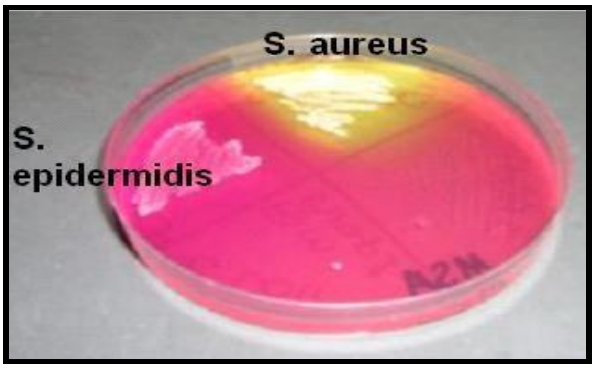
2. Blood agar: Beta hemolysis (complete hemolysis):



3. Mannitol Salt Agar (MSA):

Is a commonly used selective and differential growth medium in microbiology. It encourages the growth of a group of certain bacteria while inhibiting the growth of others. This medium is important in medical laboratories by distinguishing pathogenic microbes in a short period of time.

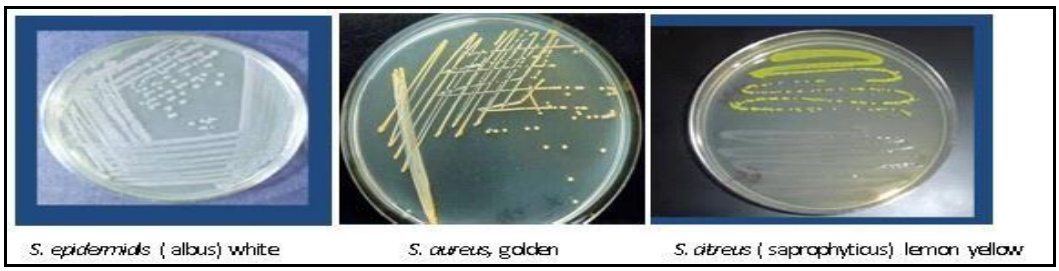
It contains a high concentration (~7.5%-10%) of salt (NaCl), making it selective for Gram positive bacterium *Staphylococci* since this level of NaCl is inhibitory to most other bacteria. It is also a differential medium for mannitol-fermenting staphylococci, containing mannitol and the indicator phenol red, a pH indicator for detecting acid produced by mannitol-fermenting *Staphylococci*.



Mannitol Salt Agar showing yellow colonies of *S. aureus*.

Staphylococcus aureus produce yellow colonies with yellow zones, whereas other *Staphylococci* produce small pink or red colonies with no colour change to the medium. If an organism can ferment mannitol, an acidic byproduct is formed that will cause the phenol red in the agar to turn yellow. It is used for the selective isolation of presumptive pathogenic *Staphylococcus.*

4. Colony pigment: Nutrient agar

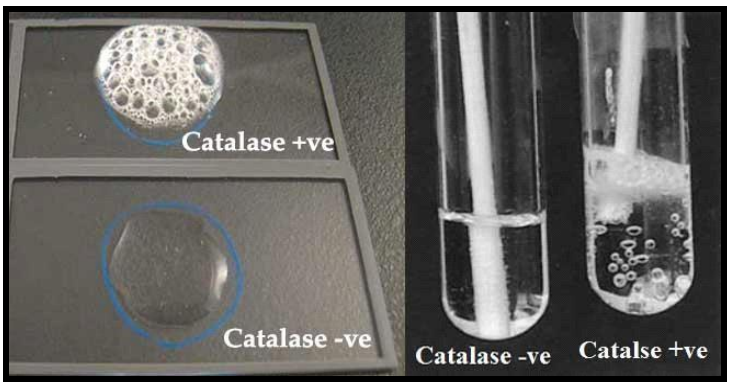


Aureus means gold, so most Staphylococcus *aureus* colonies are golden yellow. However, there are **variants**, which can be almost white. Beta-hemolysis on sheep blood agar is characteristic, as is a positive coagulase test. If the coagulase test is positive and colony morphology and gram stain is typical, then the isolate can be called *Staphylococcus aureus* without any further testing. Many Staphylococcus species are coagulase negative, and these are often lumped together by the laboratory as " Coagulase Negative Staph, (CNS)".

5. Catalase test:

Catalase test is performed by adding 3% hydrogen peroxide to a colony on agar medium. Staphylococci contain catalase, and break down peroxide, produces O_2 and bubble, so they are catalase positive, what distinguish them from streptococci.

$$2\,H_2O_2 \longrightarrow 2\,H_2O + O_2(gas)$$



6. Coagulase Test:

Coagulase test: The purpose of this test is to determine the ability of the organism to produce coagulase which clots plasma and to distinguish pathogenic coagulase + staphylococcus from Coagulase Negative Staphylococci (CNS) . There are two forms of coagulase:

- **Bound (clumping factor)** which is detected in the coagulase **slide test**, can directly convert fibrinogen to insoluble fibrin and causes the staphylococci to clump together.
- **Free coagulase** detected by **tube test**. staphylothrombin catalyzes the conversion of fibrinogen to insoluble firin resulting in clotting of plasma. The enzyme produces **coagulation** of blood, allowing the organism to "wall" its infection off from the host's protective mechanisms rather effectively.

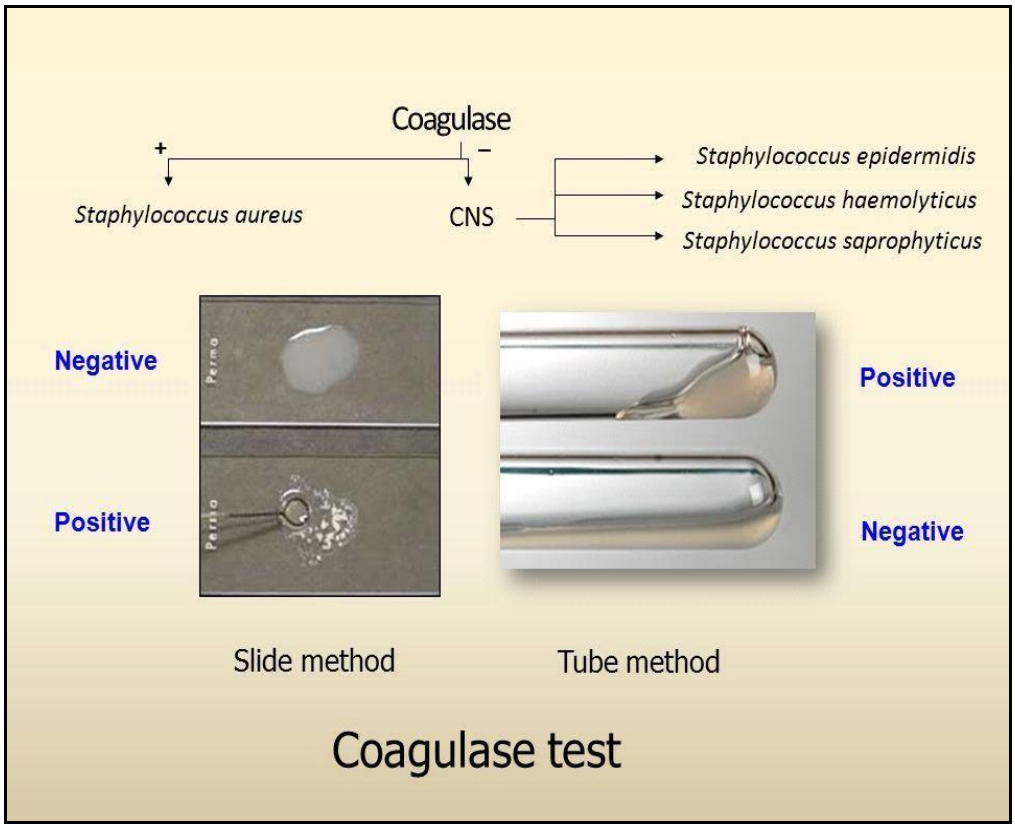
There are two methods for Coagulase test to be carried out:

1- Slide method.(slide Coagulase test for bound clumping factor only)

2- Tube method.(tube Coagulase test for free and bound clumping factor)

Slide Test:

- Make a 1 inch diameter circle on a clean glass slide using a wax pencil.
- Place two drops citrated rabbit plasma into the circle, using a wooden pick or a clean loop.
- Add a HEAVY inoculum and emulsify it in the plasma (should be milkylooking).
- Fibrin threads form between the cells, causing them to agglutinate, or clump.
- There will a visible clumping of cells within 10-15 seconds



Tube Test:

- Inoculate a tube containing ½ ml of rabbit plasma with the bacterial inoculum.
- Place at 37º C and check at ½ hour or at next lab period (some strains will give a +ve reaction in a few hours, other strains take longer) by tipping the slide at an angle.
- Any degree of coagulation is considered a positive test for the free coagulase enzyme

7. DNAase test:

DNase agar is a differential medium that tests the ability of an organism to produce an exoenzyme, called **deoxyribonuclease** or **DNase**, that hydrolyzes DNA.
Deoxyribonuclease allows the organisms that produce it to break down DNA into smaller fragments. It is used to differentiate ***S.aureus*** (DNase +ve) from other Staphylococci that do not produce such enzyme. The DNase test is particularly useful when plasma is not available to performed a **coagulase test** or when the results of a coagulase test are difficult to interpret.

DNase agar contains nutrients for the bacteria, DNA, and mostly methyl green as an indicator.

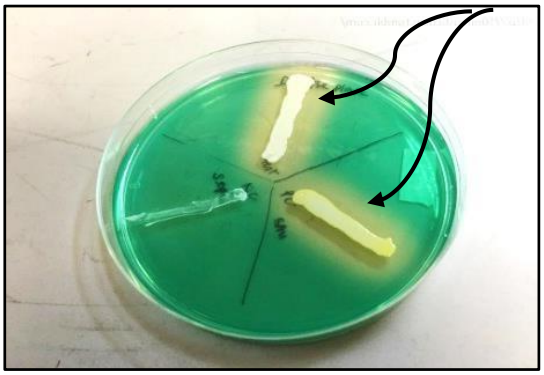
Deoxyribonuclease allows the organisms that produce it to break down DNA into smaller fragments. When the DNA is broken down, it no longer binds to the methyl green, and a clear halo will appear around the areas where the DNase-producing organism has grown.

In DNase agar without indicator (methyl green), the hydrolysis of DNA is observed by a clearing of the agar after addition of HCL (oligonucleotides dissolves in acid but DNA salts are insoluble). The acid precipitates unhydrolyzed DNA making the medium opaque. Therefore, DNase producing colonies hydrolyze DNA and produce a clear zone around the growth.

Procedure:

- Make a single line down the center of the plate (if running 2 ***Staphyococci*** species, you can divide the plate in half and run each organism on a side).
- Incubate at 30º C or 37º C.
- If using the Methyl green agar, place the agar plate on a **white background** for good contrast. Look for yellowish/ tan zones around the growth areas.

No reagent is added.



positive

8. Medium without methyl green:

Flood the plate with HCl and wait for 1 minute, a clear zone (large or small is +) around the growth area. Be sure that the plate is sitting on the black table top: