# **Staphylococcus:**

They are Gram-positive, non-motile, non-spore forming, and occasionally capsulated. They are spherical cocci which are arranged in grape-like irregular clusters due to cell divisions occur in three planes, it may also found in single, pair or short chains specially when grow in liquid media, they produce pigments that vary from white to deep yellow. Catalase positive, oxidase negative, it is aerobic and facultative anaerobic.

Some staphylococci are part of the normal flora, they are present on skin and in the mucous membranes, but others can cause suppuration, abscess formation, pneumonia and meningitis. The most common type of food poisoning is caused by staphylococcal enterotoxin. Staphylococci rapidly develop resistance to many antimicrobial agents and cause difficult therapeutic problems.

There are three important species of staphylococcus: Staphylococcus aureus, Staphylococcus epidermidis (albus), and Staphylococcus saprophyticus (citrus).

### Differences between Staphylococcal species

Character	Spp.	S. aureus	S. epidermidis	S. saprophyticus
1-colony colour		golden yellow	white	lemon yellow
2- coagulase		+	-	-
3- fermentation of		+	-	-
mannitol				
4-Beta haemolysis		+	-	-

### Morphology and Culture characters:

Staphylococcal cells are spherical a bout 1µm in diameter arranged in irregular clusters. Single cocci, pairs , tetrads, and chains are also seen in liquid cultures. Micrococcus species often resemble Staphylococci, they are found free-living in the environment and form regular packets of four or eight cocci. Colonies on solid media are round, smooth, raised, and glistening. S. aureus (coagulase positive) usually forms gray to deep golden yellow colonies. Coagulase negative Staphylococcus like S. epidermidis colonies usually are gray to white, though some may be slightly pigmented, usually cream or yellow like S.saprophyticus colonies on primary isolation.

S.aureus produced Alpha-toxin that causes wide zone of clear (beta – type) hemolysis on blood agar. in rabbits it causes local necrosis and death.

The other two species lack alpha toxin (do not exhibit hemolysis and are coagulase – negative, also *Staphylococcus aureus* grows with NaCl in concentration 7.5-10% that inhibit most types of other bacteria.

## Pathogenecity of Staphylococcus aureus:

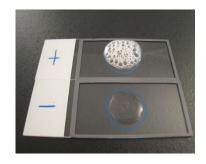
- **1-** The ability of lysis RBCs (hemolysis) by producing hemolysin enzyme.
- **2-** The ability to coagulate plasma by the production of the enzyme coagulase which catalyses the transformation of fibringen to fibrin.
- 3- Other enzymes produced by staphylococci include **hyaluronidase** (spreading factor); **staphylokinase** (cause fibrinolysis); **proteinases**; **lipases**; **gelatinase** and  $\beta$ -lactamase.
- **4-** Ability to produce different types of toxins.

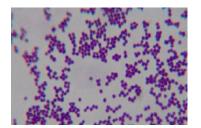
### **Biochemical tests:-**

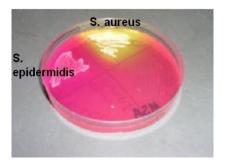
- 1- catalase positive
- 2-oxidase negative
- 3- coagulase positive (slide and tube method)
- 4- gelatinase positive
- 5- fermentation of mannitol.
- 6- beta-hemolysis.

# **Specimens:-**

Pus, blood, urine, surface swab, tracheal aspirate, or spinal fluid for culture, depending upon the localization of the process.









# Lab. Diagnosis

**1- Gram stain** (G+ cocci, grape, tetrad, pairs and singles some time in the form of short chains at least 5 cells).

- **2- Blood agar (Enriched media):**  $\beta$  hemolytic (complete hemolysis) in *S. aureus* only.
- **3- Mannitol salt agar (differential media)** it contains mannitol 7.5 % sodium chloride and phenol red indicator. NaCl inhibits organisms other than *Staphylococci* if the mannitol is fermented to produce acid the phenol red in the medium changes color from red to yellow.
  - **4- Staphylococcus medium (110):** (Selective media)

It contains NaCl and mannitol, but it lacks phenol red, no color change and takes place as mannitol is fermented.

**5- DNA ase agar:** Contain (Mannitol, DNA, Bromothymol blue as indicator, it's specific for *S.aureus* (Coagulase + ve) that able to hydrolyze DNA.

**6-** Catalase test:- To test for present of enzyme Catalase to differentiate between genera.

#### Method:-

A drop of hydrogen peroxide solution (3 % hydrogen peroxide -  $H_2O_2$ ) is placed on slide, and a small amount of the bacterial growth is placed in the solution. The formation of bubbles (The release of oxygen) indicates a positive test.

**Result:-** Positive test immediate bubbling by  $(O_2)$  formed or gas Liberation.

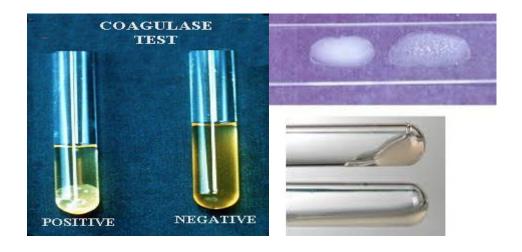
#### Mechanism:

$$H_2O_2 + H_2O_2$$
 bacterium  $2H_2O + O_2$  Catalase water gas

**7-** Coagulase test: The test is an excellent definitive test for confirming identification of S.aureus (detect clumping factor present in S.aureus and absent from most Staphylococci).

The Two method for identification Staph-coagulase as positive

- A) Slide coagulase test.- This test detects clumping factor(bound coagulase) which is cell wall component that causes the organisms to clump when mixed with plasma.
- Emulsify a Staphylococal colony in a drop of water on a microscope slide with a minimum of spreading. If the isolate does not form a smooth, milky suspension, does not proceed Add large drop of plasma (rabbit or human plasma) and mix well.
  - \* Positive result agglutination of suspension during 10sec.
- **B)**Tube coagulase test:- This test detects an extracellular enzyme called coagulase. It activates a coagulase reacting factor(CRF) normaly present in plasma, causing the plasma to clot by conversion of fibrinogen to fibrin.
- In sterile glass tube add (0.5 ml) of undiluted plasma (human or rabbit plasma).
  - Add 0.5 ml of an (18-24 hr.) pure broth of *Staphylococcus*.
  - Rotate tube gently and incubation at 37 C  $^{\rm o}$  for 4 hr .
  - observe every 30 min for a clot forming.



- **8-Novobiocin sensitivity test:** Its resistance to novobiocin distinguishes *S.saprophyticus* from other clinically important staphylococci. Streak- inoculate the cultures under test on to a plate of the medium, incubate aerobically at 37 C ° for 24 hr. and examine for the presence (resistant) or absence (sensitive) of growth.
- 1- Practical Microbiology for dental students by Bharti Arora. D.R.Arora.
- 2- Jawetz Medical Microbiology 26th Edition.
- 3-Koneman, s Color Atlas and Text book of Diagnostic Microbiology.