

Practical Bacteriology

lab 5

Gram- positive cocci:

Staphylococci

lect.

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Learning Objectives:

After this lab. You must be able to:

1- Describe staphylococci under microscope.

2-Distinguish between staphylococci and streptococci.

3- Differentiate among staphylococci species.

4-List diseases caused by each spp. of staphylococci.

5- Predict staphylococcal causative agents causing clinical cases.

Gram positive cocci

1- Staphylococcus.

2- Streptococcus.

3- Micrococcus.

The medically important genera, are: **Staphylococci** and **Streptococci**. Both of them are non motile and do not form spores. They are distinguished by two main criteria:

❖ **Microscopically:** staphylococci appear in grape-like clusters where as streptococci are in chains.

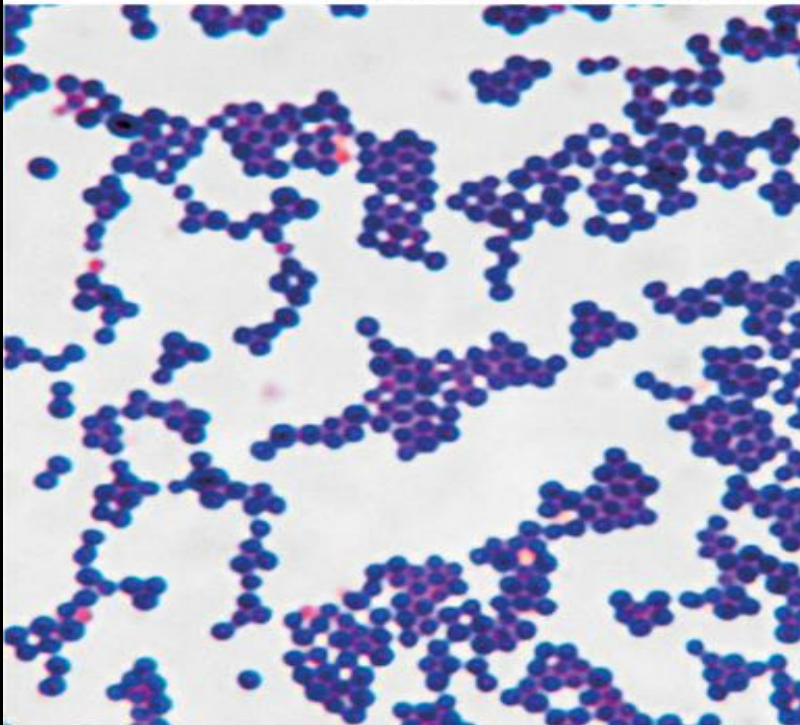
❖ **Biochemically:** staphylococci produce catalase which breaks down hydrogen peroxide, where as streptococci do not.

Difference between *Staphylococcus*, *Streptococcus* and *Micrococcus*

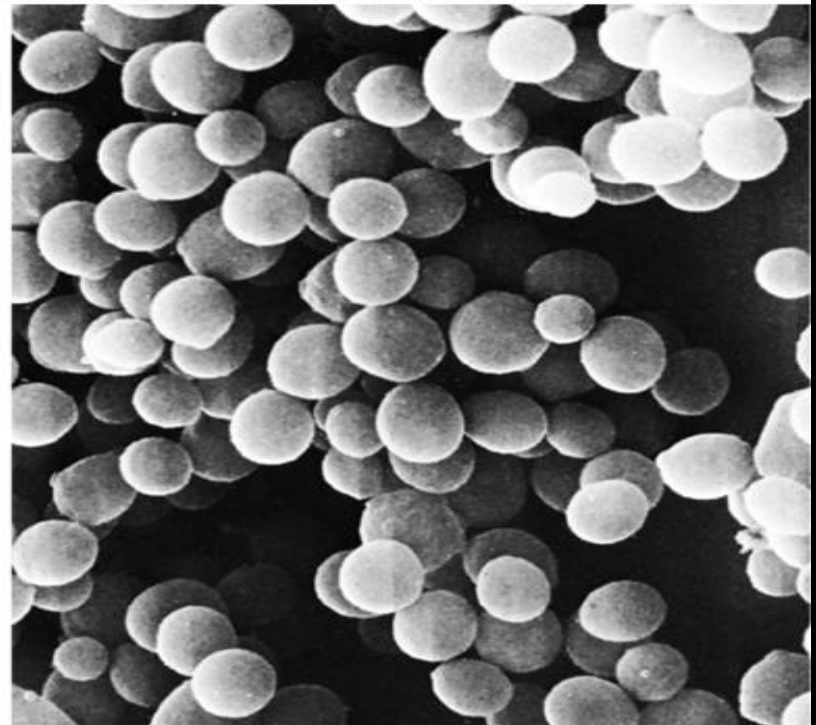
character	Staphylococci & Micrococci	Streptococci
Gram stain	Positive	Positive
Arrangement	Clusters (Micrococci in tetrads)	Chains
Size	Large 1 μm	Smaller 0.5-1 μm
Catalase	Positive	Negative

Staphylococci

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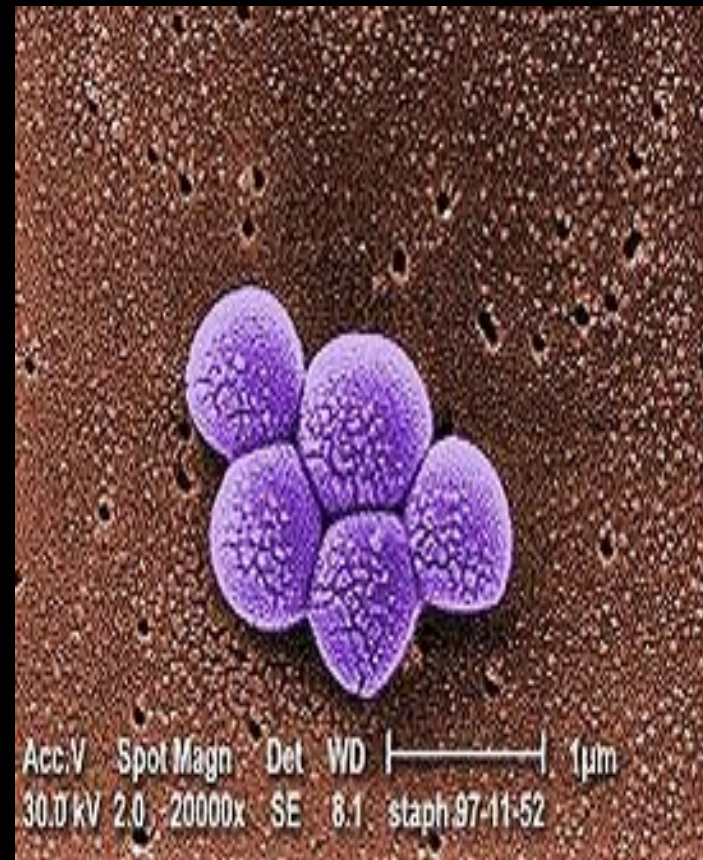
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Staphylococcus

These bacteria are widely distributed in our environment; some of them are member of normal flora particularly on skin and upper respiratory tract including anterior nares and pharyngeal surfaces, others cause wide range of pyogenic infections.

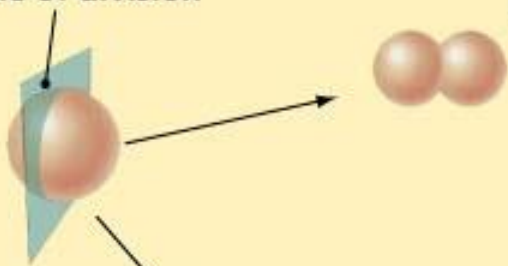


Important properties:

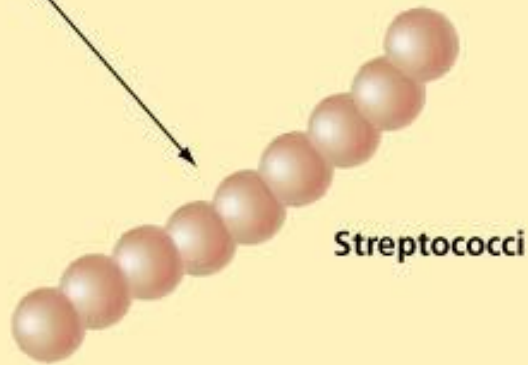
- ◆ Gram-positive cocci, arranged in grape-like clusters because cell division occurs along different planes and the daughter cells remain attached to one another.
- ◆ Non spore- forming, non motile and capsule formation is variable.
- ◆ Aerobic and facultative anaerobic, grow readily on usual culture media, has large, raised, opaque colonies with smooth entire margin.
- ◆ Salt tolerant: allows them to tolerate the salt present on human skin.
- ◆ Tolerant of desiccation: allows survival on environmental surfaces (fomites).

Plane of division

Diplococci

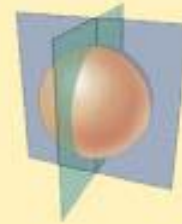


(a)



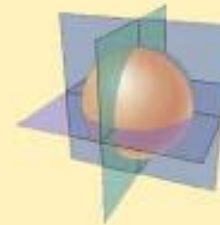
Streptococci

(b)



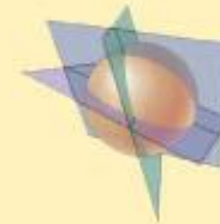
Tetrads

(c)



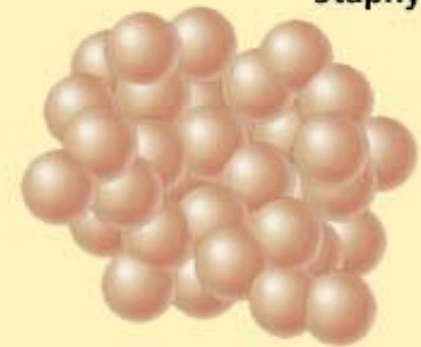
Sarcina

(d)



Staphylococci

(e)

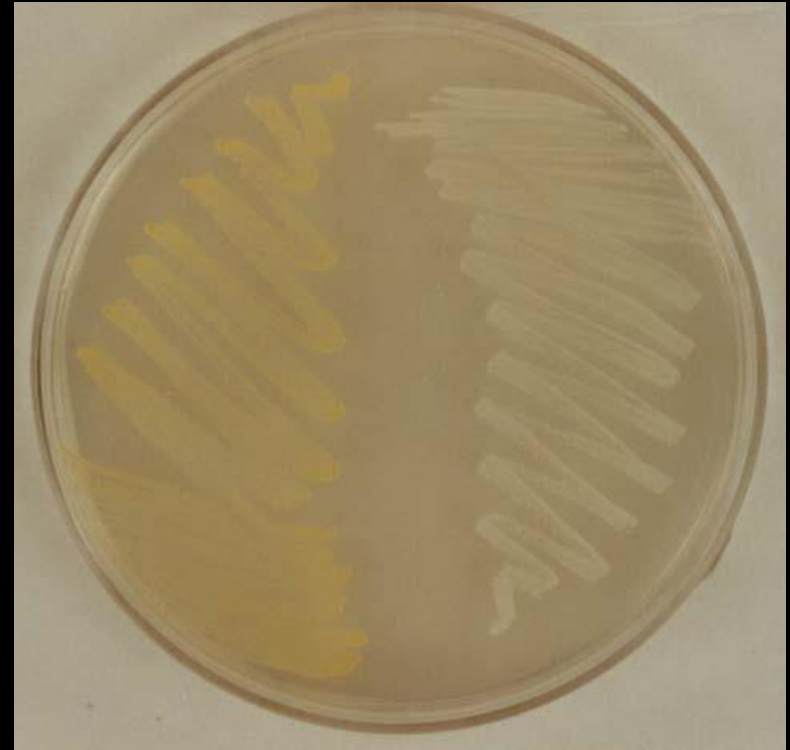


Clinically important species:

- ◆ ***Staphylococcus aureus***: most virulent species. It is responsible for a wide range of medical illnesses extending from mild localized skin infection to life threatening septicemia.
- ◆ ***Staphylococcus epidermidis***: it is normal flora of the skin and mucous membranes, it is not usually pathogenic but it may cause serious infections often associated with implanted devices (prosthetic devices, artificial joints, catheters,...etc.), if it has the opportunity
- ◆ ***Staphylococcus saprophyticus***: it is free living, common cause of UTI in young sexually active women, and non gonococcal urithritis in males.

Staphylococcus aureus – General features

- ◆ **Coagulase positive**
- ◆ β - hemolytic colonies on BA
- ◆ **Produces golden yellow pigment**
- ◆ Highly resistant bacteria: resist heat, dryness, and 10-15% NaCl solution.



Staphylococci on MSA



S. aureus on blood agar

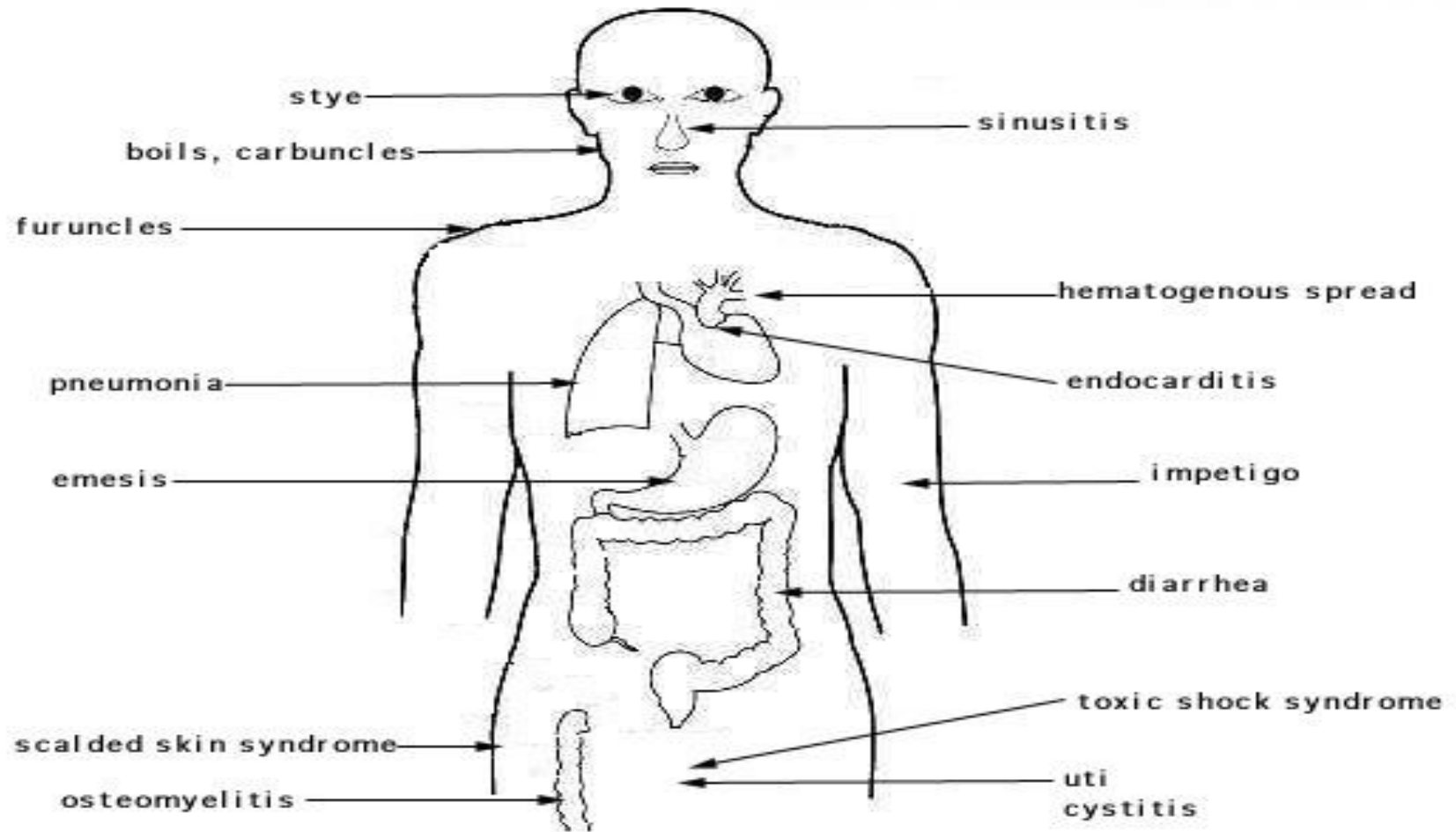


Clinical finding of S. aureus

Clinical findings can be divided to:

- **Cutaneous infections:**
 - impetigo, acne, folliculitis and furuncles (boils), mastitis.
- **Invasive infections:**
 - bacteremia, meningitis, endocarditis, and osteomyelitis, hospital-acquired pneumonia.
- **Toxin mediated infections:**
 - Staphylococcal scalded skin syndrome (SSSS),
 - Toxic Shock Syndrome (TSS),
 - Food intoxication (in 1-8hr, vomiting ,diarrhea, nausea, self limited)

Pathogenicity (Staphylococcal diseases)





Abscess



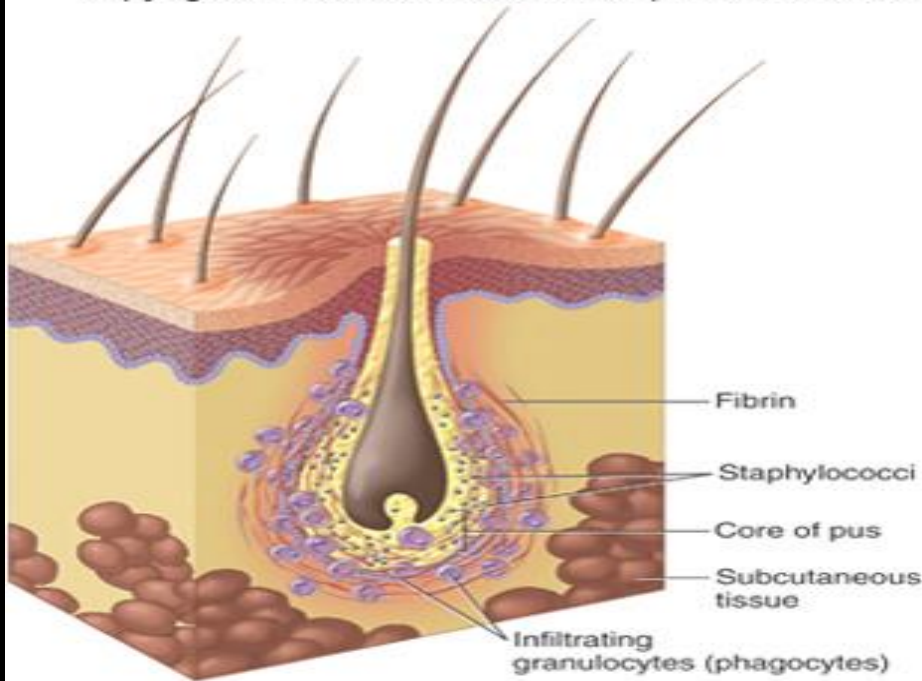
Folliculitis



Toxic shock syndrome



SSSS



(a) Sectional view of a boil or furuncle, a single pustule that develops in a hair follicle or gland and is the classic lesion of the species. The inflamed infection site becomes abscessed when masses of phagocytes, bacteria, and fluid are walled off by fibrin.



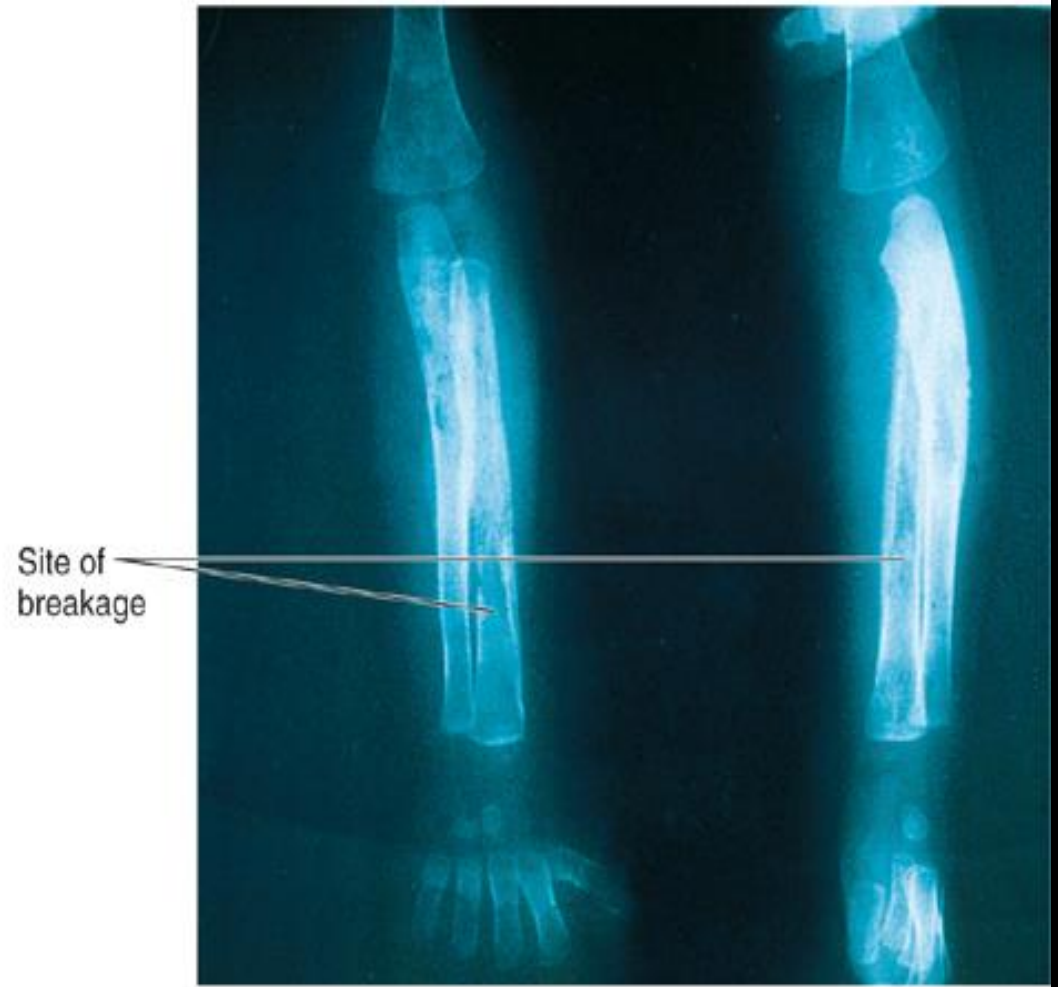
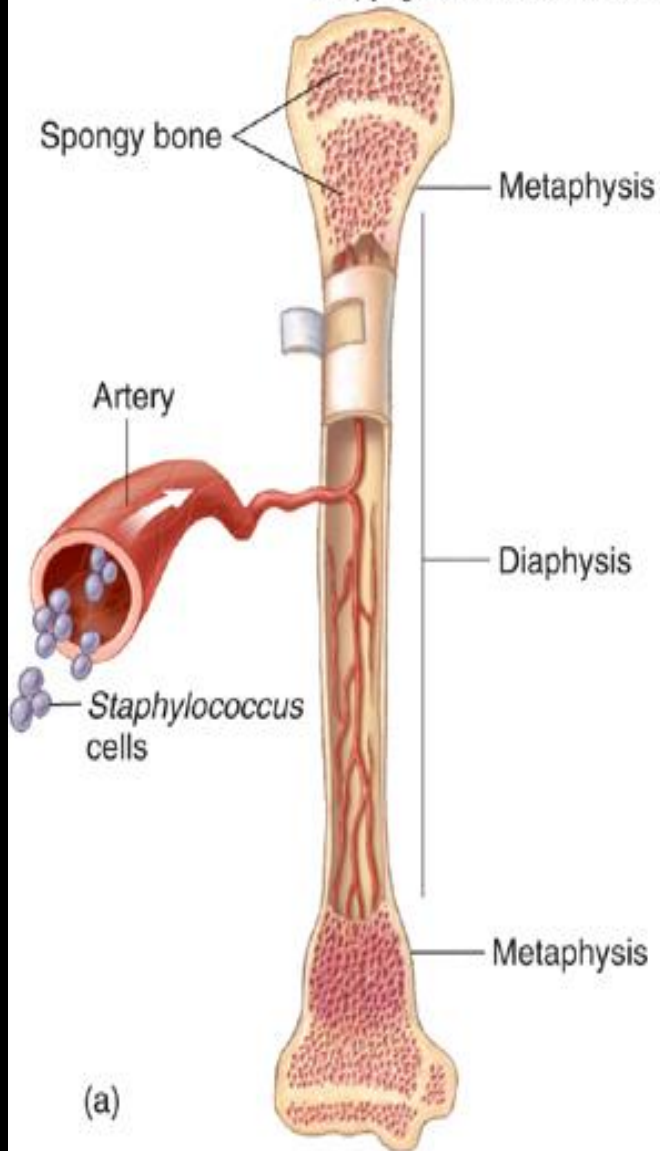
(b) A furuncle on the back of the hand. This lesion forms in a single follicle.

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(c) A carbuncle on the back of the neck. Carbuncles are massive deep lesions that result from multiple, interconnecting furuncles. Swelling and rupture into the surrounding tissues can be marked.

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(b)

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Effects of staphylococcal toxins on skin



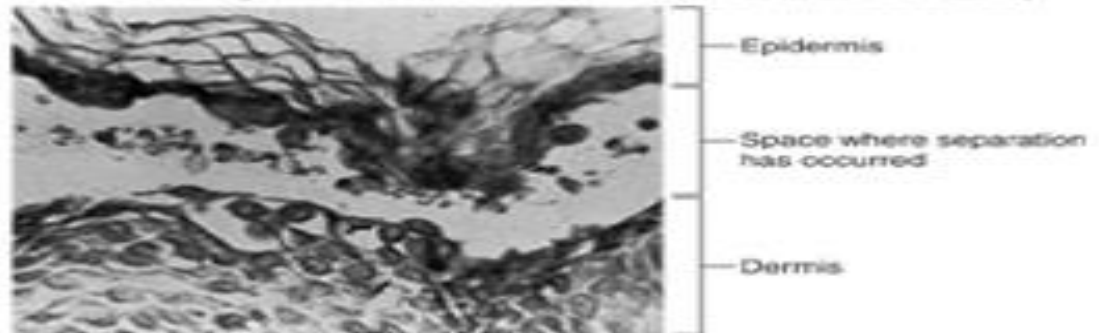
(a)

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(b)

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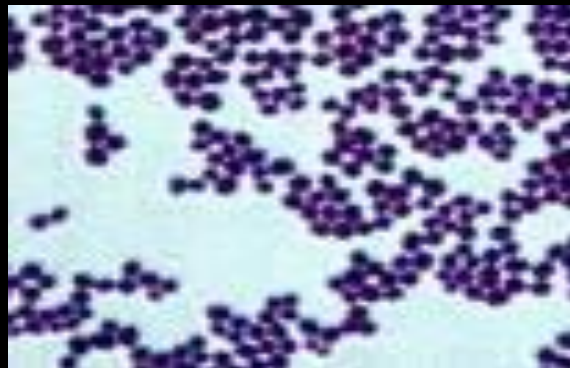
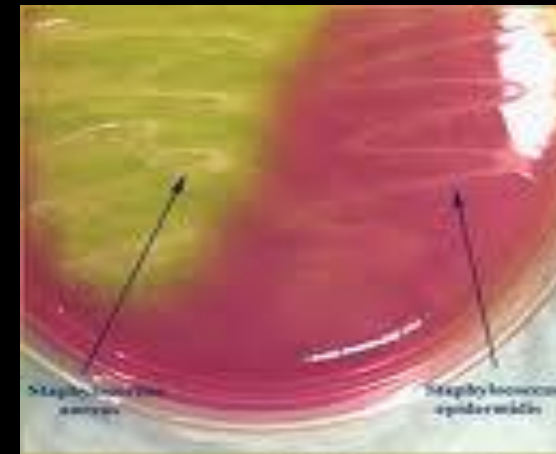


(c)

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Lab diagnosis – *S. aureus*

- ◆ **Specimens:** wound swab, pus, sputum, blood, urine CSF.
- ◆ **Culture**
 - BA : **beta hemolysis**
 - NA : golden yellow pigment
 - MSA: **ferment mannitol** (yellow colonies).
- ◆ **Microscopy:** Gram stain - **GPC** in clusters



◆ Biochemical tests:

- **Catalase:** +ve (differentiate staphylococci from streptococci).
- **Coagulase:** +ve (differentiate *S. aureus* from other staphylococci).
 - **Slide method:** for detection of clumping factor (bound)
 - **Tube method:** for detection of plasma coagulase (free)
- **Novobiocin disc:** -ve (differentiate *S. saprophyticus* which is resistant, whereas, other are sensitive).

Catalase test:

- ◆ **Principle:** Catalase is an enzyme, which is produced by microorganisms that live in oxygenated environments to **neutralize toxic forms of H_2O_2** . Catalase mediates the breakdown of hydrogen peroxide H_2O_2 into oxygen and water.
- ◆ **Procedure:**
 - 1) Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile wooden stick
 - 2) Place a drop of 3% H_2O_2 on to the slide and mix.
 - 3) A positive result is the **rapid evolution of oxygen** (within 5-10 sec.) as evidenced by **bubbling**.
 - 4) A negative result is **no bubbles** or only a few scattered bubbles.
 - 5) Dispose of your slide in the biohazard glass disposal container.

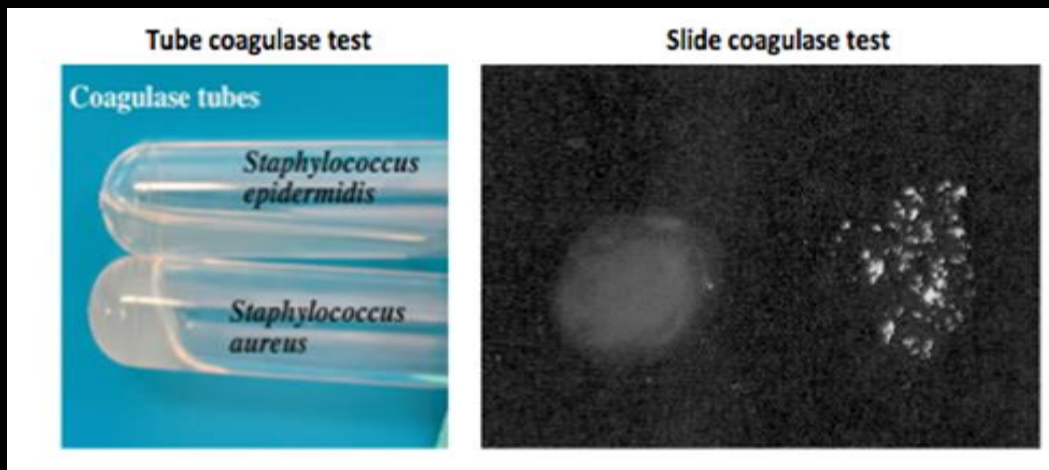
Coagulase test

- ◆ **Principle:** coagulase is an enzyme found in *S. aureus* which has the property of clotting human or rabbit plasma by converting fibrinogen to fibrin.
- ◆ **Procedure:**
 - A. **Slide method: to detect bound coagulase**
 1. Pick up few colonies of bacteria from agar culture and emulsify in two drops of saline placed on a slide.
 2. Add a drop of undiluted human or rabbit plasma and mixed gently.
 3. Clumping after 5-10 sec. indicates the presence of bound coagulase.

Coagulase test

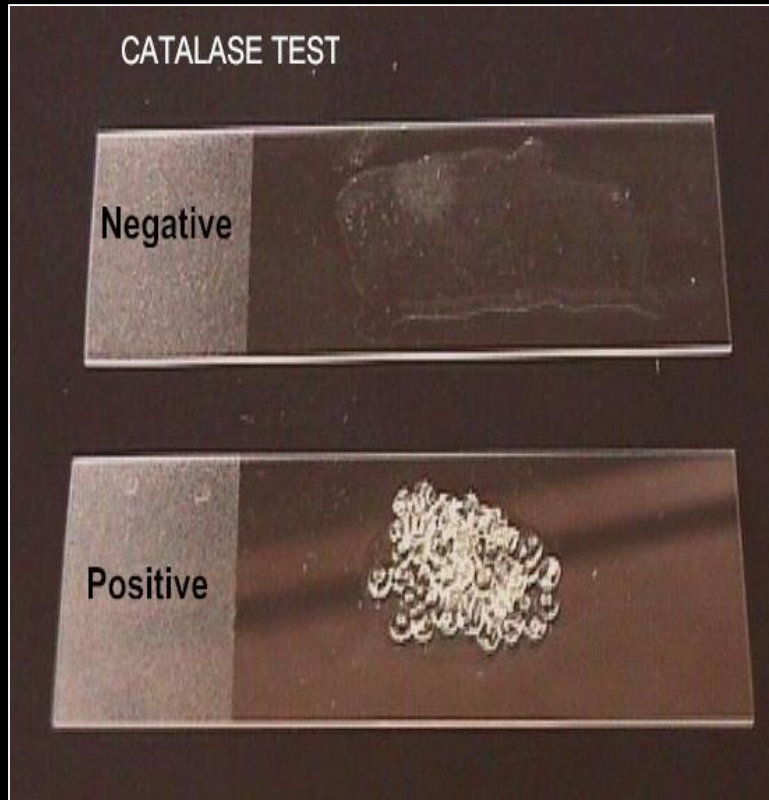
B. Tube method: to detect free coagulase

1. Put 0.5 ml of citrated or heparinized plasma (diluted 1:5 with saline) in a narrow test tube
2. Add 0.1 ml of an overnight broth culture or agar culture suspension to the plasma
3. Incubate the tube in a water bath at 37 °C for 3-6 hrs
4. Clotting indicate positive result



Catalase positive

Rapid Staph test



A photograph of a white lighthouse with a red lantern room, situated on a dark, rocky cliff. The scene is captured at sunset, with the sky transitioning from blue to orange and yellow. The lighthouse is illuminated from the side, and the foreground is dominated by dark, jagged rocks. In the background, the ocean is visible with some distant islands or headlands under a hazy sky.

THANKS FOR YOUR ATTENTION