

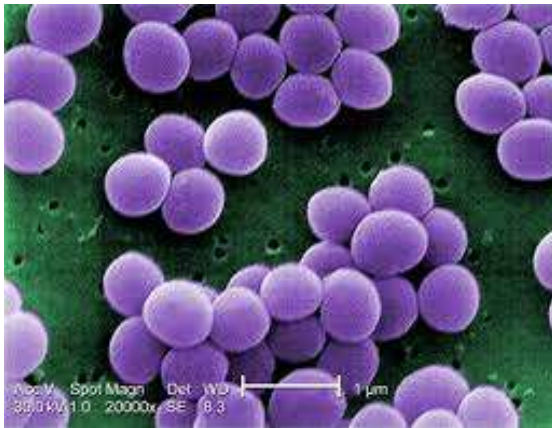
Lecture-2D: Genus Staphylococcus

Learning Objectives

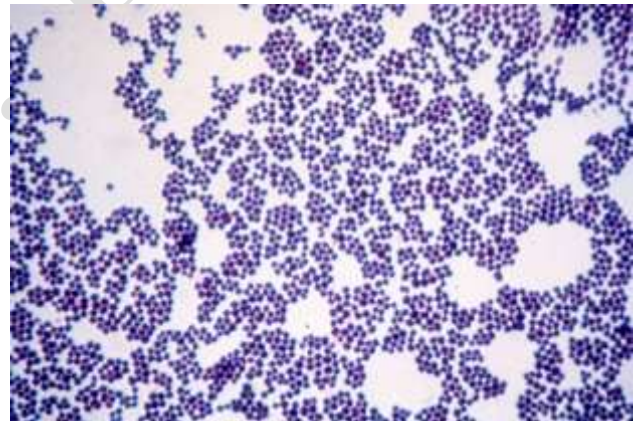
- Describe species of Staphylococcus.
- Describe **morphology** and **culture characteristics** of *Staphylococcus aureus*.
- Explain **coagulase test**.
- List and describe toxins and **enzymes of *Staphylococcus aureus***.
- Discuss **laboratory diagnosis** of infections caused by *Staphylococcus aureus*.
- Describe the following: Coagulase-negative staphylococci (CNS); micrococci.
- **Distinguish characteristics of *Staph. aureus*, *Staph. epidermidis* and *Staph. saprophyticus*.**

Staphylococcus aureus

Morphology: 1) They are spherical cocci, approximately 1 µm in diameter. 2) arrange in **grapelike clusters**. 3) Cluster formation is due to cell **division occurring in three planes, with daughter cells tending to remain in close proximity**. 4) They are **non-sporing, non-motile** and usually non- capsulate. 5) gram-positive but old organisms may be gram- negative.



Electron Microscope



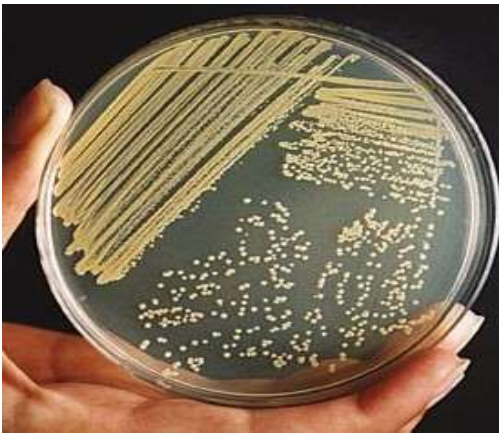
Gram Stain (blue color)

Cultural Characteristics

1) They are aerobes and facultative anaerobes. 2) Optimum temperature for growth is 37°C (range being 12-44°C). Optimum pH is 7.5. 3) They can grow readily on ordinary media.

1. Nutrient Agar: After aerobic incubation for 24 h at 37°C, 1- **Colonies** are 1-3 mm in diameter and have a smooth glistening surface, an entire edge, a soft butyrous consistency and an opaque. 2- **pigmented** appearance. 3- Most strains **produce golden-yellow** pigment (aureus). Pigmentation is characteristic of this species when grown aerobically. Pigmentation is enhanced on fatty media such as Tween agar, by prolonged incubation, and by leaving plates at room temperature. Grown anaerobically, colonies are often smaller and grayish in color.

***Staph. Aureus* growing of different culture media:**



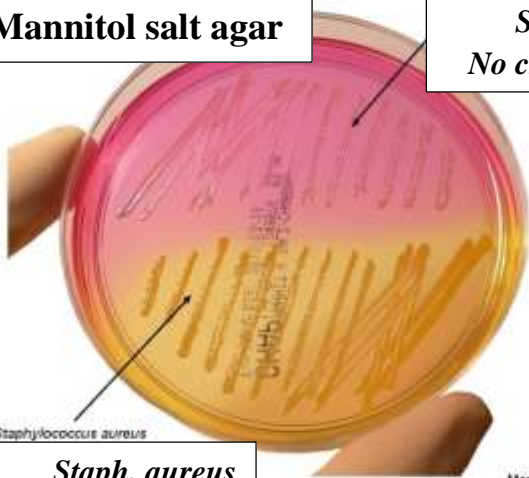
On nutrient agar (golden color)



On blood agar (beta hemolytic)

www.bacteriainphotos.com

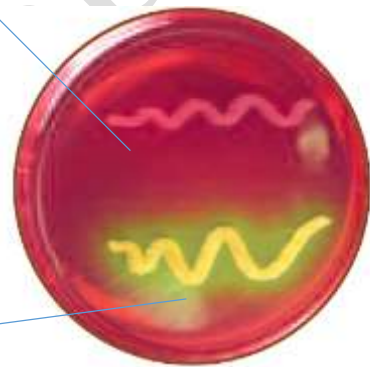
2- Mannitol salt agar



Staphylococcus aureus

Staph. aureus
(yellow colonies)

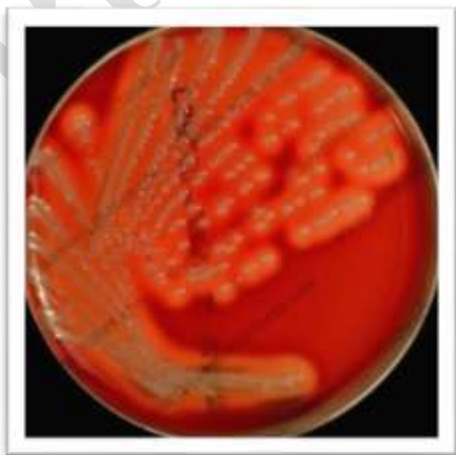
Staph. epidermidis
No change in medium



Mannitol Salt Agar

3. Blood Agar (see the figure above)

The colonies have the same appearance as on nutrient agar, but may be surrounded by a zone of β -hemolysis. Hemolysis is more likely to be present if sheep, human or rabbit blood is used instead of horse blood and if incubation is in air with 20 percent added carbon dioxide. Hemolysis is weak on horse blood agar.



Staphylococcus aureus cultivated on Columbia agar with 5% defibrinated sheep blood. Cultivation 24 hours in an aerobic atmosphere, 37°C. Colonies are surrounded by a wide zone of beta-hemolysis.

4. Milk Agar

This medium is prepared by **mixing 200 ml of sterile nutrient agar and 100 ml of fresh or sterilized milk and poured into plates**. On this medium, after overnight incubation, the colonies of *S. aureus* are **larger** than those on nutrient agar and **pigmentation** is well developed and easily recognized against the opaque white background.

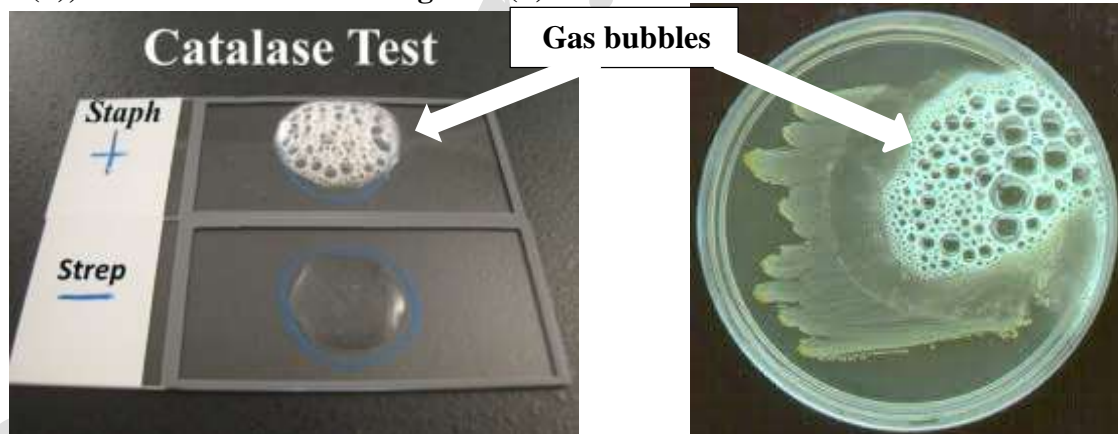
5. Selective Salt Media

Selective medium may be useful for the isolation and enumeration of staphylococci from materials, such as feces, food and dust, likely to contain a predominance of other kinds of bacteria. Therefore, **7 to 10 percent of sodium chloride** may be added to nutrient agar (salt agar) or milk agar (salt milk agar). **Mannitol salt agar containing 1 percent mannitol, 7.5 percent NaCl, and phenol red in nutrient agar is the selective medium for *S. aureus*.**

Biochemical Reactions:

1. **Sugar fermentation:** *S. aureus* ferments a range of sugars (**glucose, maltose, lactose, sucrose, including mannitol**) producing **acid** but no **gas**. Sugar fermentation is of no diagnostic value except for **mannitol**, which is usually fermented anaerobically by *Staph. aureus* but not by other species.

2. **Catalase:** Catalase positive (unlike streptococci). **By mixing a drop of 3% hydrogen peroxide (H₂O₂) with a colony of the test bacteria on slide or on plate. Producing air bubbles = positive (+), without air bubbles = negative (-)**



3. **Phosphatase test:** They also produce phosphatase. This is a useful screening procedure for **differentiating *Staph. aureus* from *Staph. epidermidis* in mixed cultures**, as the former gives prompt phosphatase reaction, while the latter is usually negative or only weakly positive.

4. **Deoxyribonuclease (DNAase) test:** It produces a deoxyribonuclease (DNAase), and a heat-stable nuclease (thermonuclease, TNAase).



DNA hydrolysis by DNase enzyme

5. Penicillinase: This is a secreted form of beta-lactamase producing staphylococci. It disrupts the beta-lactam ring of the penicillin molecule, thereby inactivating the antibiotic.

6. Hyaluronidase ("Spreading Factor"): This protein breaks down proteoglycans in connective tissue (**hyaluronic acid**).

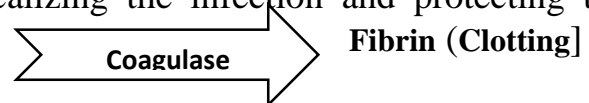
7. Staphylokinase: This protein lyses formed fibrin clots.

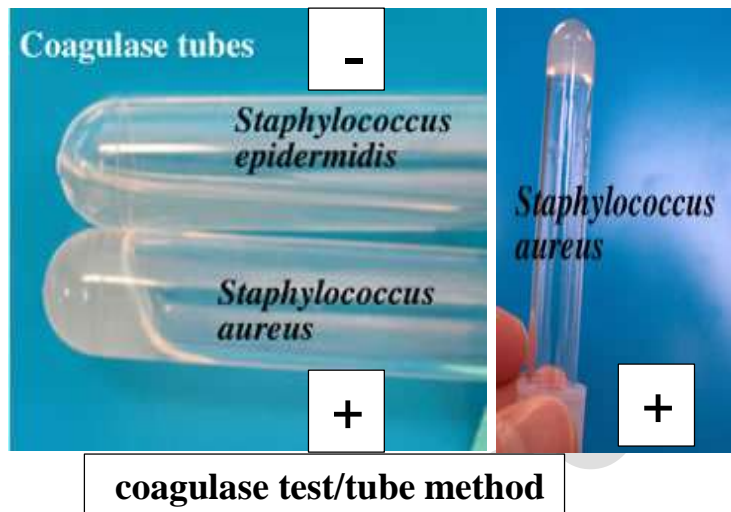
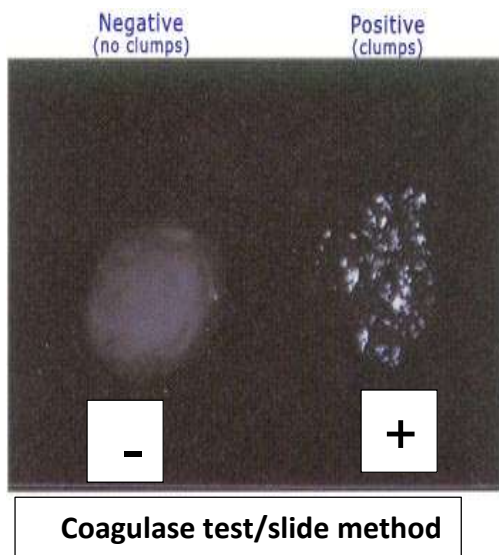
8. Lipase: This enzyme degrades fats and oils, which often accumulate on the surface of our body. This degradation facilitates *Staphylococcus aureus*' colonization of sebaceous glands.

9. Protease: destroys tissue protein.

10. Coagulase:

S. aureus produces an extracellular enzyme called coagulase which brings about clotting of **human or rabbit plasma**. It acts along with a '**coagulase reacting factor**' (**CRF**) present in plasma, binding to prothrombin and converting fibrinogen to fibrin. **Coagulase does not clot plasma of guinea pigs and some other species because they lack CRF.** CRF is similar to pro-thrombin but is probably not identical with it. ***Staph. aureus* strains usually secrete both coagulase and clumping factor.** Coagulase test is the standard criterion for the identification of *Staph. aureus* isolates. The role of coagulase in the pathogenesis of disease may cause the formation of a fibrin layer around a staphylococcal abscess, thus localizing the infection and protecting the organisms from phagocytosis. [Fibrinogen





Clumping Factor (Bound Coagulase): The component on the cell wall of *S. aureus* that results in the clumping of whole staphylococci in the presence of plasma is referred to as the clumping factor (also called bound coagulase). This factor reacts directly with fibrinogen in plasma, converts it to insoluble fibrin, causing the staphylococci to clump or aggregate.

Slide coagulase test: It can be detected by emulsifying a few colonies of the bacteria in a drop of normal saline on a clean glass slide and mixing it with a drop of rabbit plasma. Prompt clumping of the organisms indicates the presence of clumping factor (bound coagulase). Since this factor is detected by performing the test on a slide, therefore, the test is known as slide coagulase test.

Comparison between bound coagulase and free coagulase

Bound coagulase	Free coagulase
1. Heat stable.	1. Heat labile.
2. Constituent of cell wall.	2. Secreted free into the medium.
3. It does not require the CRF for its action.	3. Requires the CRF for its action.
4. Clot plasma of guinea pigs and some other species.	4. Does not clot plasma of guinea pigs and some other species because they lack CRF.
5. Only one type of clumping factor has been identified.	5. Eight antigenic types (A-H). Most human strains form coagulase type A.
6. Detected by slide method test.	6. Detected by tube method test.

Laboratory Diagnosis:

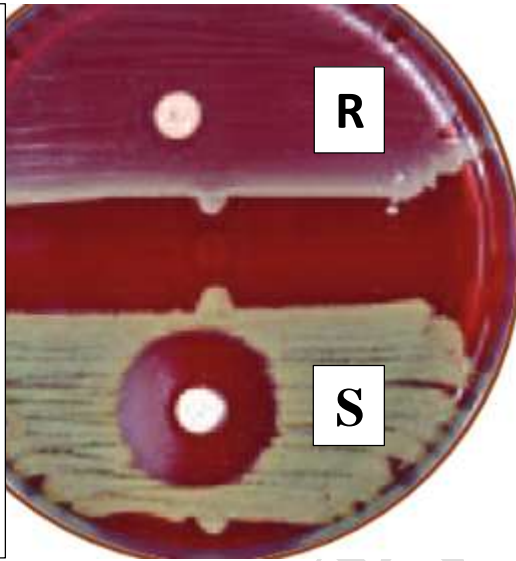
- 1. Specimens:** The specimens to be collected **depend on the type of lesion**, for example; **Pus** from **suppurate lesions**; **sputum** from respiratory infections; **food remains** and **vomit** from cases of **food poisoning**; **nasal** and **perineal swabs** from suspected carriers. Swabs of the perineum, pieces of hair and umbilical stump may be necessary in special situations.
- 2. Direct Microscopy:** Direct microscopy with **Gram stained smears** is useful in the case of pus, **where cocci in clusters** may be seen. This is of **no value** for specimens like sputum where mixed bacterial flora is normally present.
- 3. Culture:** The specimens are cultured on a **blood agar plate**. Staphylococcal colonies appear after overnight incubation. **Specimens**, where staphylococci are expected to be outnumbered by other bacteria (e.g. wound swab and feces), **are inoculated on selective media like Ludlam's or salt-milk agar or Robertson's cooked meat** medium containing 10 percent NaCl. The inoculated media are incubated at 37°C for 18-24 hours. **On blood agar plate, look for hemolysis around the colonies**. The plates are inspected for **golden-yellow or white colonies**. Smears are examined from the culture and **coagulase test** done when staphylococci are isolated.
- 4. Identification:** **Relatively simple biochemical tests (e.g. positive reactions for coagulase, heat-stable nuclease, alkaline phosphatase, and mannitol fermentation) can be used to differentiate *S. aureus* and the other staphylococci.**
- 5. Coagulase Test:** Coagulase test is done by two methods—slide and tube coagulase test. (Previously mentioned).
- 6. Antibiotic Sensitivity Tests:** As a guide to treatment, antibiotic sensitivity tests should be performed appropriate to the clinical situation. This is important as staphylococci readily develop resistance to drugs.

Characteristics distinguishing three species of the genus <i>Staphylococcus</i>			
<i>S. aprophyticus</i>	<i>S. epidermidis</i>	<i>S. aureus</i>	<i>Characteristic</i>
-	+	+	Anaerobic growth
-	-	+	Mannitol fermentation
-	-	+	Coagulase
-	-	+	DNAase
-	-/weak+	+	Phosphatase
-	-	+	α -Toxin
-	-	+	Protein A in cell wall
Resistant	Sensitive	Sensitive	Novobiosin sensitivity

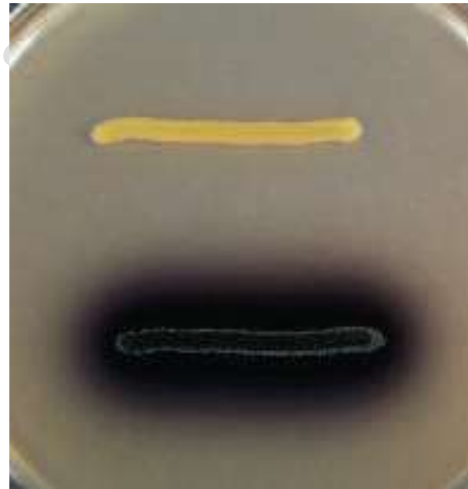
Bacitracin susceptibility on a sheep blood agar plate:

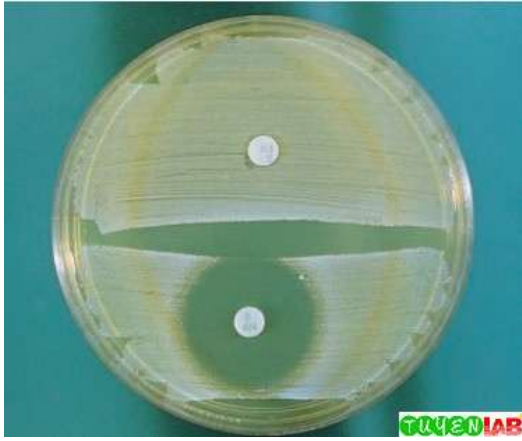
Staph. aureus (Resistant = R).

Micrococcus luteus (Susceptible = S).



Bile susceptibility test (BST): This plate (Bile Esculin Agar-BEA) was inoculated with *Staphylococcus aureus*/**top (negative result)** and *Enterococcus faecium*/**bottom (positive result)**. The **darkening** of the medium around *E. faecium* indicates a **positive result**.





Novobiocin susceptibility test (NST) is used to differentiate between *Staph. saprophyticus* (resistant/top) from other coagulase negative staphylococci.

Prof. Dr. Habeeb S. N.

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