Al-Mustaqbal University
Dept. Medical Lab. Techniques
Diagnostic Microbiology 25-2026
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Lecture-13: Methods for identification of etiological agents of infectious disease

1- *Staphylococcus.*

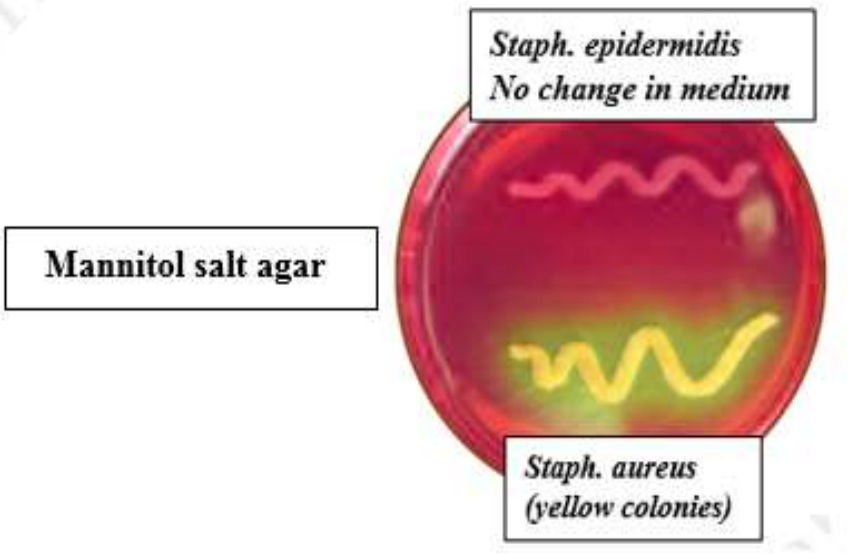
Morphology: They are Gram positive, Cocci, Grapelike clusters (Cluster formation is due to cell division occurring in three planes, with daughter cells tending to remain in close, non-sporing, nonmotile and usually non- capsulate.

Cultural Characteristics: They are aerobes and facultative anaerobes, Optimum temperature for growth is 37°C, pH is 7.5, can grow readily on ordinary media.

1. On Nutrient Agar: Colonies are soft and smooth surface, entire edge, most strains produce golden-yellow pigment (*Staph. aureus*). Pigmentation is enhanced on fatty media such as Tween agar.

2. Blood Agar. Colonies may be surrounded by a zone of β-hemolysis on blood agar of sheep, rabbit or human blood.

3. Selective Salt Media. Mannitol salt agar containing 1% mannitol, 7.5% NaCl, and phenol red in nutrient agar is the selective medium for *S aureus*.



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.

2. Direct Microscopy: Gram stained smears is useful in the case of pus, where cocci in clusters may be seen.

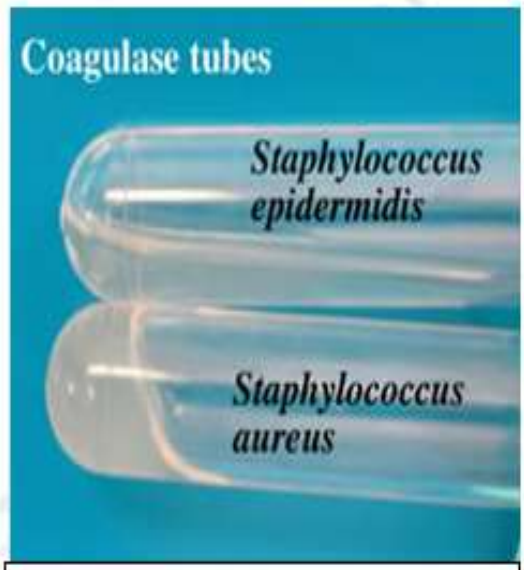
3. Culture: Specimens are inoculated on a blood agar plat, on selective media such Mannitol salt-agar. After incubation of blood agar, look for hemolysis around the colonies, The golden-yellow colonies on nutrient agar. The isolate is examined from the coagulase test.

4. Identification: 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: this test is done by two methods, slide method and tube method.

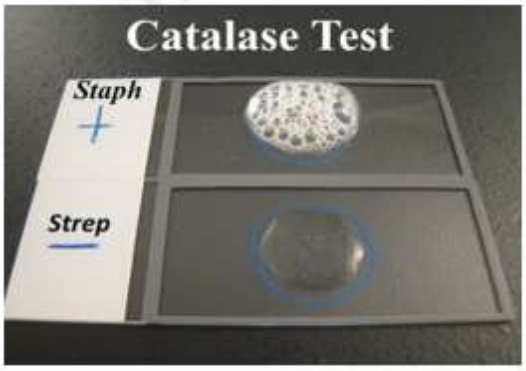
Coagulase test.

Coagulase test/slide method



coagulase test/tube method

6- Catalase test: By mixing a drop of 3% hydrogen peroxide (H2O2) with a colony of bacteria on slide or on plate. Producing air bubbles = (+), without air bubbles= (-)



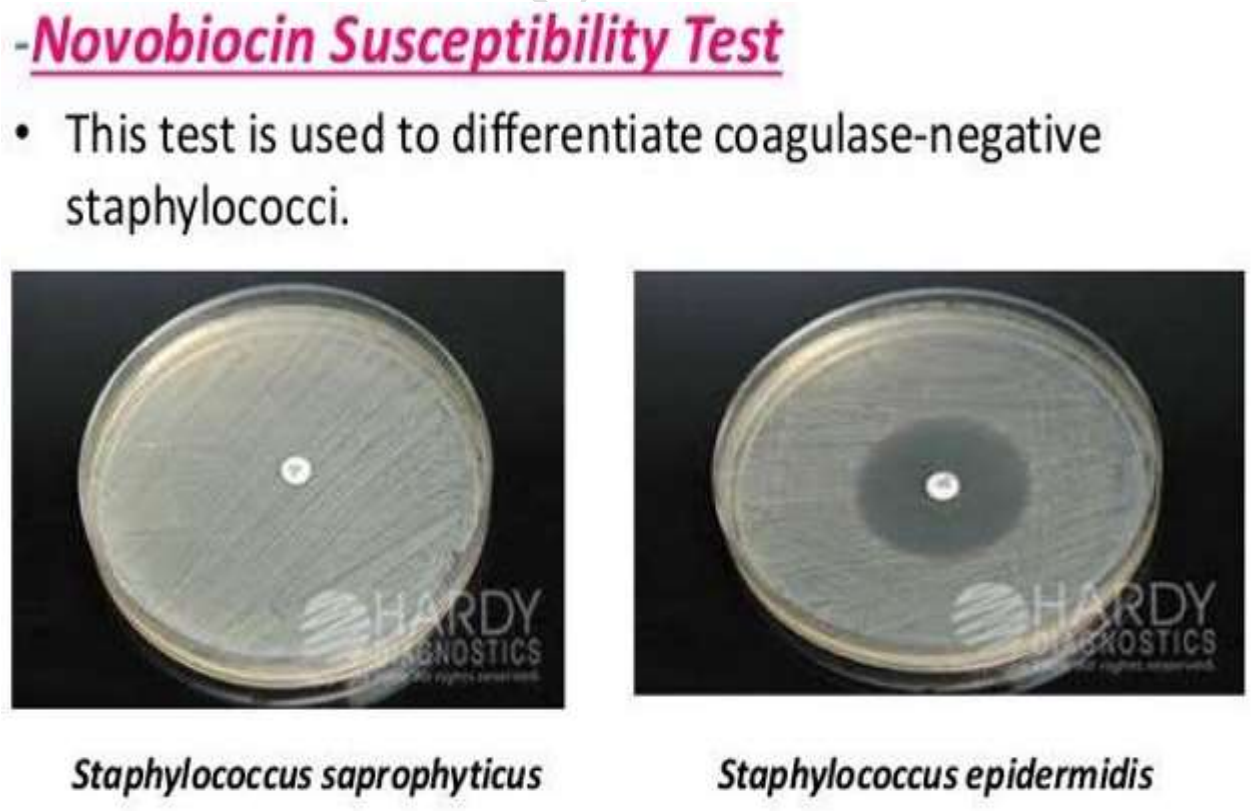
Catalase test on slide

Gas bubbles

Catalase test on plate

7- 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.

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



Staphylococcus saprophyticus

Staphylococcus epidermidis

6. Antibiotic Sensitivity Tests: As a guide for treatment, antibiotic sensitivity tests should be performed appropriate to the clinical situation. This is important as staphylococci readily develop resistance to drugs.

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2- *Streptococcus:*

Morphology and General characteristics:

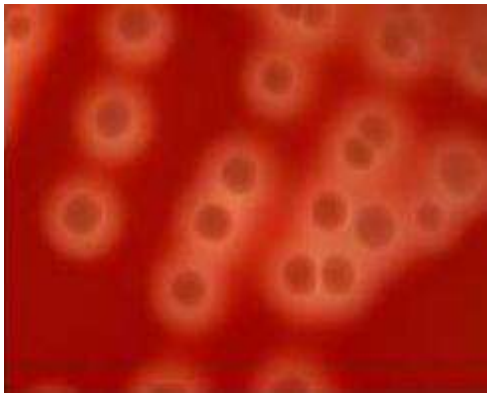
Gram positive cocci arranged in chains, non-motile and non-sporing. They require media enriched with blood for growth. They are human pathogens causing pyogenic infection. They are responsible for non-suppurative lesions (acute rheumatic fever and glomerulonephritis). Group A streptococci have a hyaluronic acid capsule.

Cultural characters:

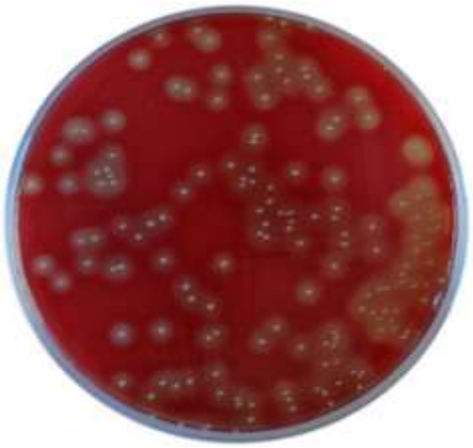
Streptococcus pyogenes is aerobic and facultative anaerobes with optimum temperature of growth being 37°C. It grows in enriched media with whole blood or serum.

a. Fluid media: Serum broth, 24 hours after culture shows granular growth with powdery deposits.

b. Blood agar: After 24 hours' incubation colony is small (pin point colonies), circular, transparent, low convex with area of hemolysis. Strains with capsules produce mucoid colonies.

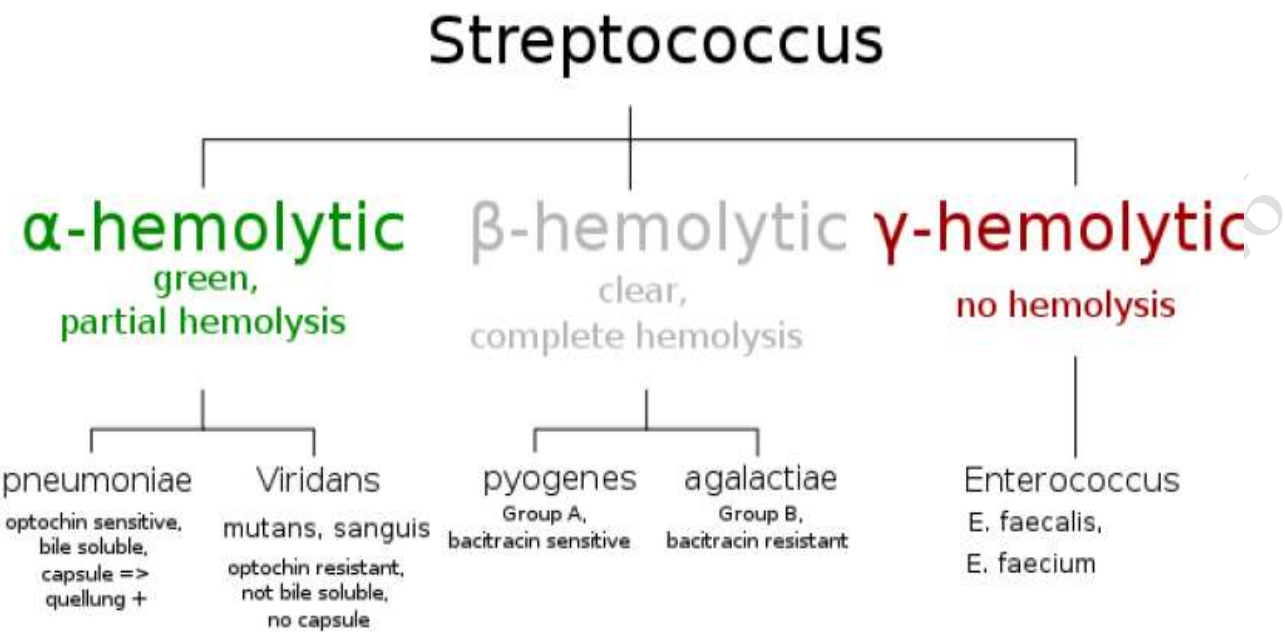


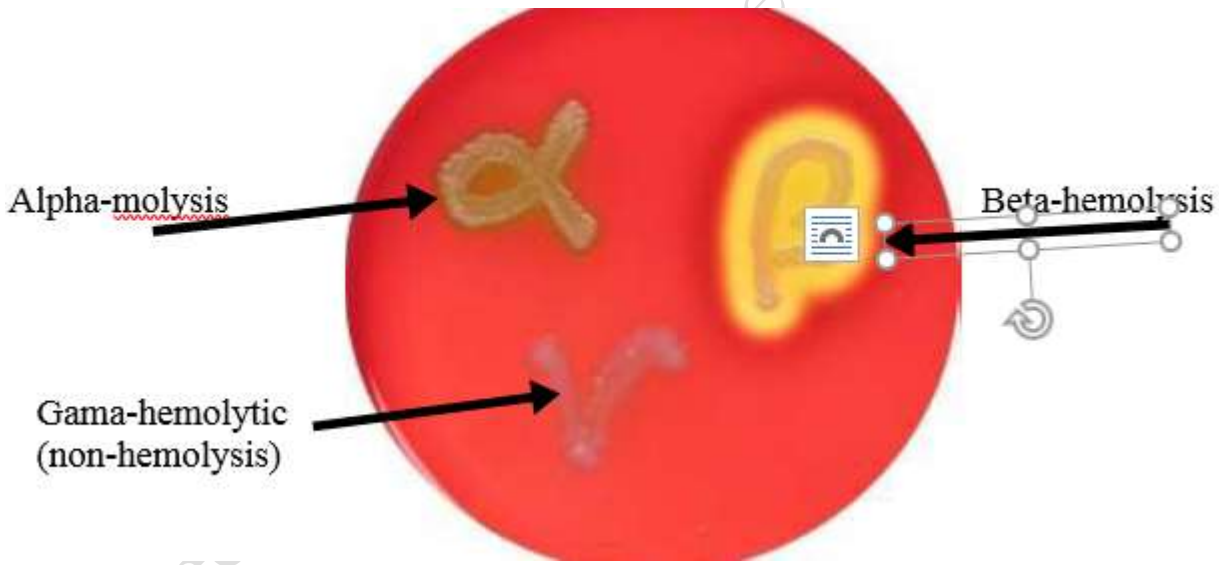
***Streptococcus pyogenes* growth on blood agar medium, (See the Beta-hemolysis).**



Columbia Agar Base with 5% Defibrinated Horse Blood. <u>It is selective medium for the isolation of *Streptococcus spp*.</u> from clinical samples. **It is made selective by the addition of Colistin and Oxolinic Acid**.

Classification of Streptococcal





3- *Enterococcus:*

The enterococci (enteric cocciǁ) were previously classified as group D streptococci. This group consists of gram-positive cocci, non-motile and non-capsulated, that are natural inhabitants of the intestinal tracts of humans and animals. They grow in the presence of 6.5 percent NaCl, 40% bile at 45°C. It survives heating at 60°C for 30 min, a feature distinguishing it from streptococci. On MacConkey medium they produce deep pink colonies. Enterococci are PYR test positive. They do not hydrolyze hippurate.

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