Lecture 12-13: Methods for identification of etiological agents of infectious disease

1- *Staphylococcus.*

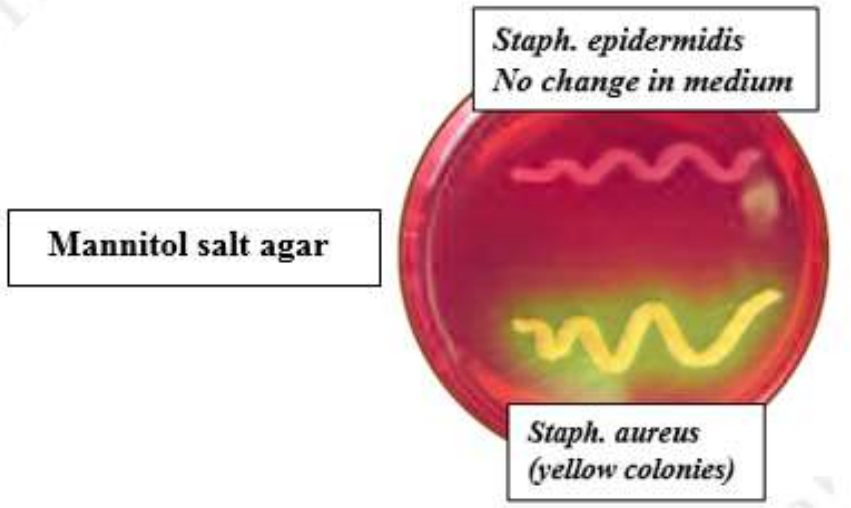
Morphology: They are Gram positive, Cocci, Grapelike clusters (Cluster formation is due to cell division occurring in three planes, with daughter cells tending to remain in close, non-sporing, nonmotile and usually non- capsulate.

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.

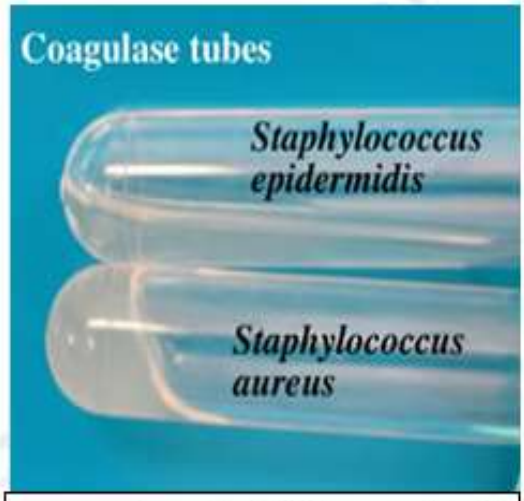
3. Culture: Specimens are inoculated on a blood agar plat, on selective media such Mannitol salt-agar. After incubation of blood agar, look for hemolysis around the colonies, The golden-yellow colonies on nutrient agar. The isolate is examined from the coagulase test.

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.

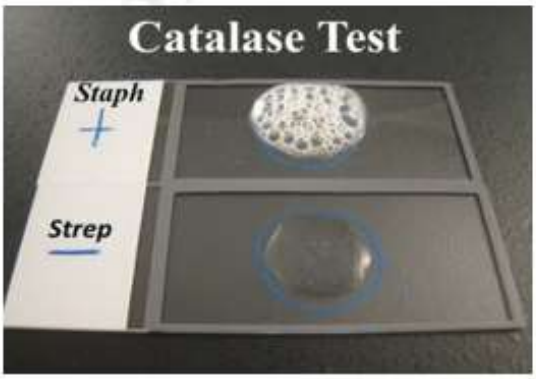
Coagulase test.

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.

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2- *Streptococcus:*

Morphology and General characteristics:

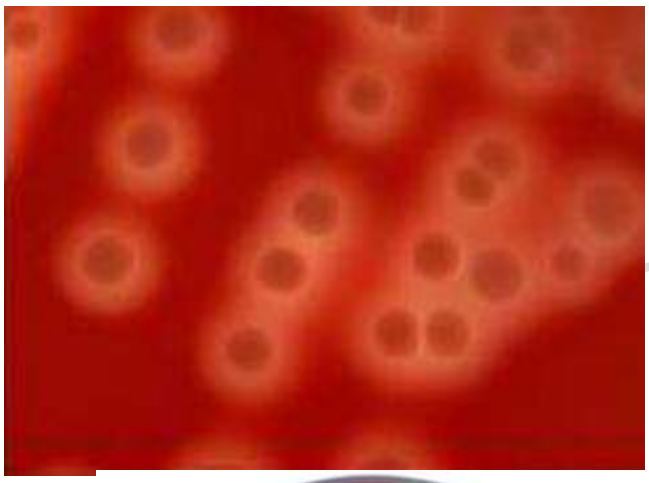
Gram positive cocci arranged in chains, non-motile and non-sporing. They require media enriched with blood for growth. They are human pathogens causing pyogenic infection. They are responsible for non-suppurative lesions (acute rheumatic fever and glomerulonephritis). Group A streptococci have a hyaluronic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

Cultural characters:

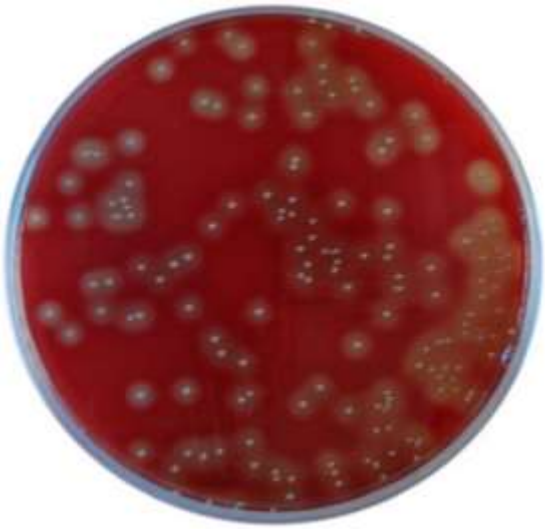
Streptococcus pyogenes is aerobic and facultative anaerobes with optimum temperature of growth being 37°C. It grows in enriched media with whole blood or serum.

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.

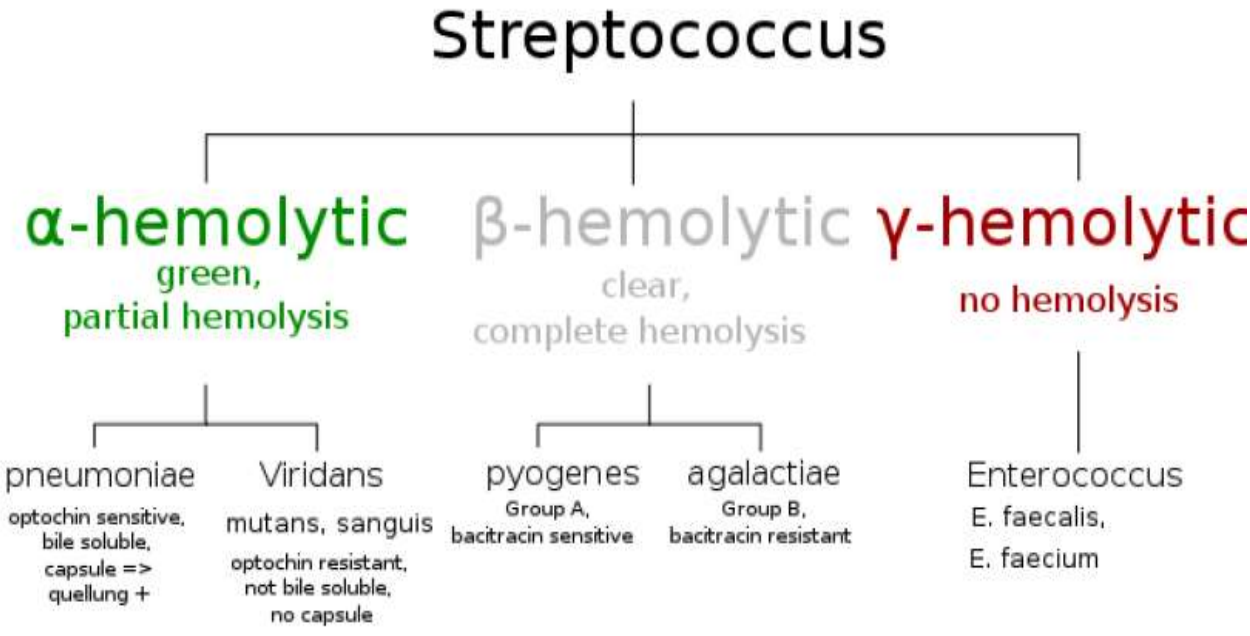


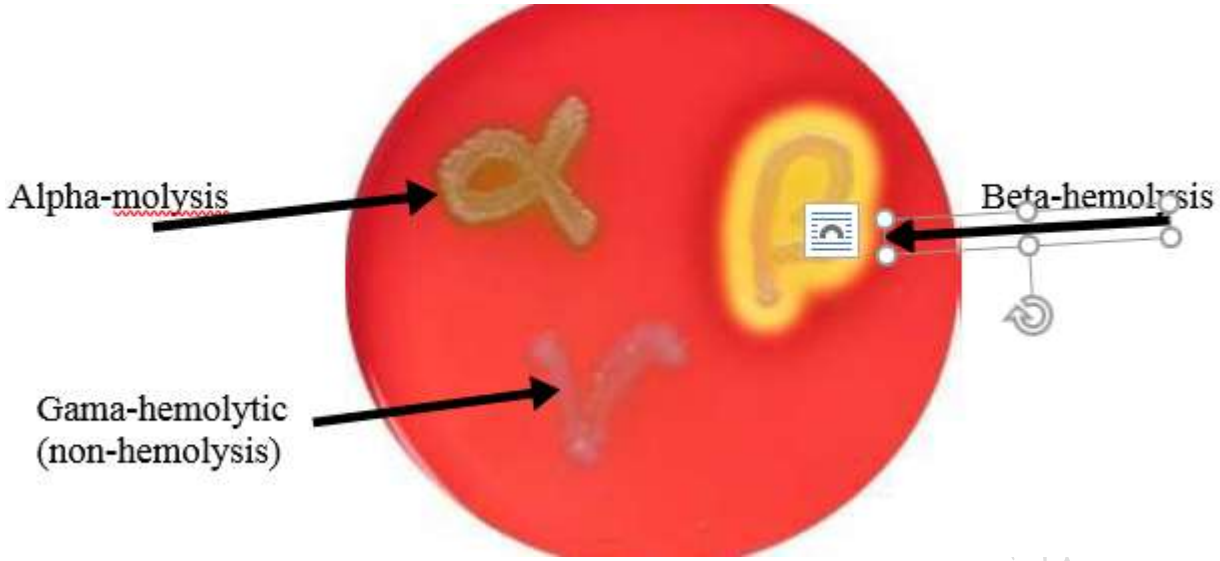
***Streptococcus pyogenes* growth of blood agar medium, Beta-hemolysis.**



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

Streptococcal classification





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.

4- *Streptococcus pneumoniae*

Morphology:

1-gram-positive cocci in pairs (diplococci), slightly elongated cocci, with one end rounded, non-motile and non-sporing, All freshly isolated strains are capsulated and the capsule encloses each pair.

Cultural Characteristics

1-They are aerobes and facultative anaerobes.

2- It grows best in air or hydrogen with 5-10 percent CO2.

Biochemical Reactions

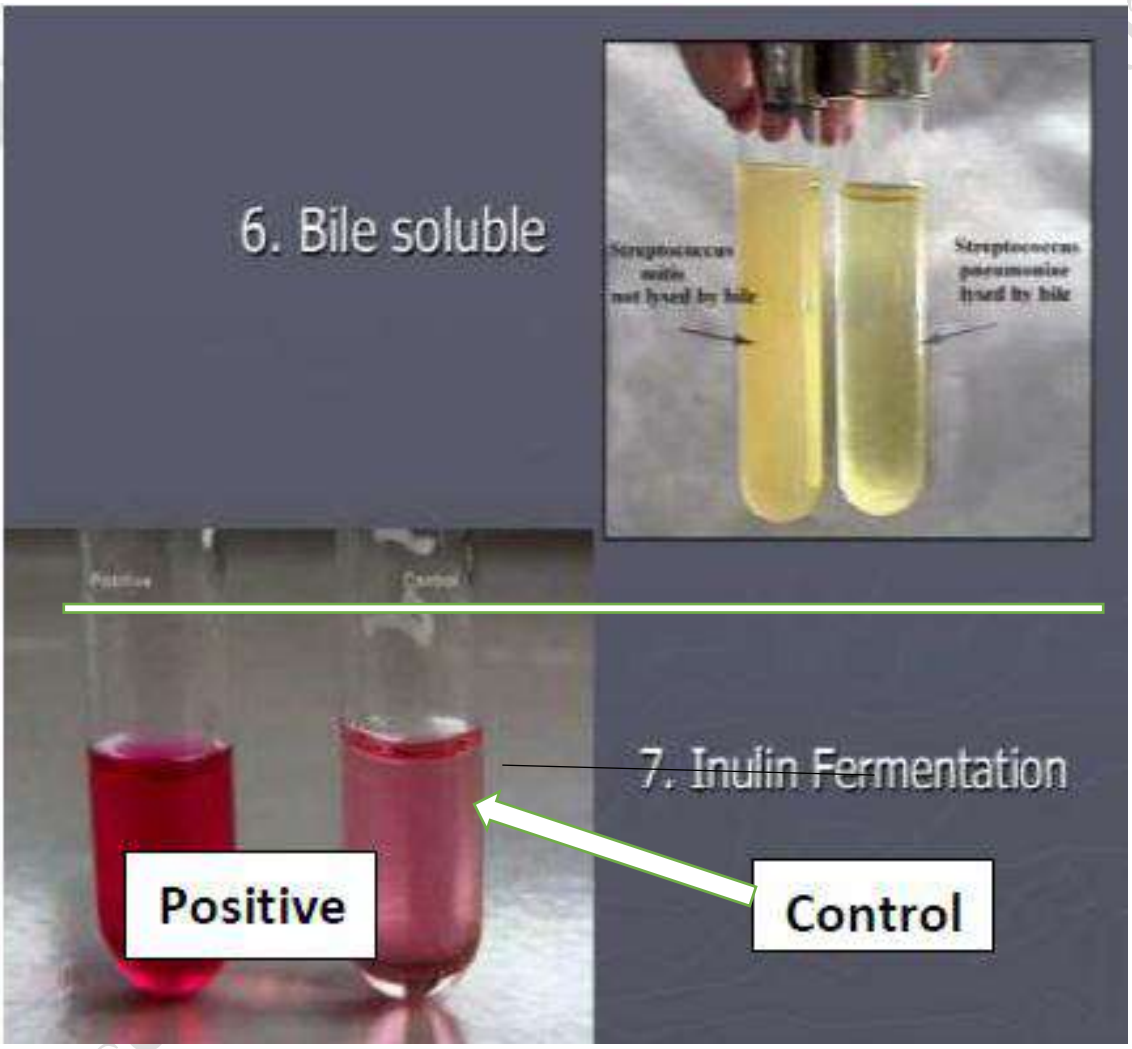
1. Inulin Fermentation: Pneumococci ferment inulin with the production of acid without gas. Fermentation of inulin by pneumococci is a useful test for differentiating them from streptococci as the latter do not ferment it.

2. Bile Solubility Test:

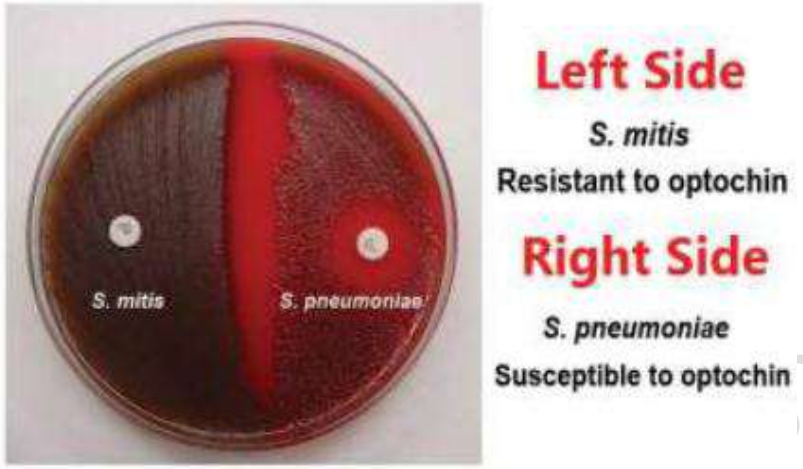
1- Grow the isolate to be tested for 18 hours at 37°C in 5 ml serum, digest broth or infusion broth.

2- While still warm, add 0.5 ml of 10 percent, bile salt (sodium deoxycholate solution) and re-incubate at 37°C. Pneumococci are lysed within 15 minutes and the initially turbid culture becomes clear and transparent. Pneumococci are soluble in bile; viridans and other streptococci are not.

3-



3- Optochin Sensitivity:



Laboratory Diagnosis

1. Specimens: Sputum, lung aspirate, pleural fluid, cerebrospinal fluid (CSF) or blood are collected according to the site of lesion. Sputum specimens must be mucus from the lungs rather than samples of saliva.

2. Microscopy and Antigen Detection

Gram stain of sputum specimens is a rapid way to diagnose pneumococcal disease. If the smears are gram-positive lancet-shaped diplococci, a presumptive diagnosis of pneumococcal pneumonia may be made. A centrifuged deposit of the CSF should be examined immediately in a Gram film in case of meningitis and presumptive diagnosis may be made by finding gram-positive diplococci.

3. Culture:

Specimen is inoculated on plates of blood agar and heated blood agar (chocolate agar) and incubated in air with 5-10% CO2 for 18-24 hours.

5- *Pseudomonas aeruginosa*

It is gram negative, motile and rod shaped. It occurs as single bacteria, in pairs, and occasionally in short chains.

Specimens: Specimens depend on the site of infection including skin lesions, pus, urine, blood, spinal fluid, sputum, and other material should be obtained by different procedures.

Culture: Pseudomonads grow readily on most culture media. It does not ferment lactose and is easily differentiated from the lactose- fermenting bacteria.

P. aeruginosa is an obligate aerobe but can grow anaerobically if nitrate is available, that grows readily on many types of culture media, sometimes producing **a sweet or grapelike** or corn taco–like odor. Some strains **hemolyze blood**.

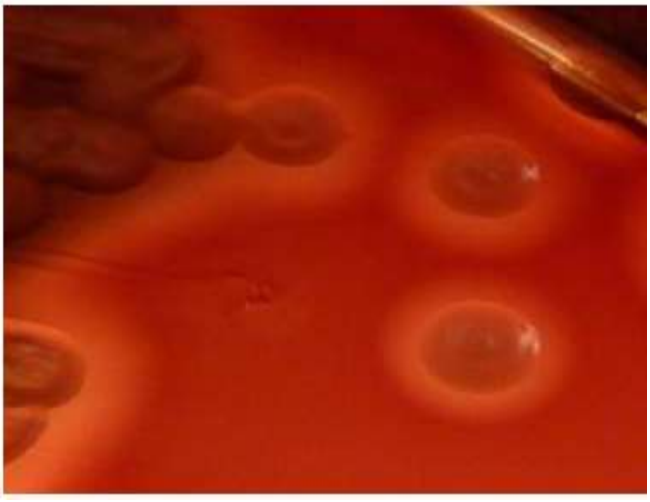
P aeruginosa forms smooth round colonies with a **fluorescent greenish color**. It often produces the; 1- non-fluorescent **bluish pigment pyocyanin**, which diffuses into the agar. Many strains produce the 2- **fluorescent pigment pyoverdin**, which gives a greenish color to the agar. Other strains produce the 3- **dark red pigment pyorubinor**, or the 4- **black pigment pyomelanin**.

Pseudomonas aeruginosa

on Citrimide medium

On MacConkey agar plates (**as shown below**) it produces non-lactose fermenting colonies (**compared with *E. coli* or *Klebsiella***) and the pigments are often poorly observed.

On **blood agar** plates, it is surrounded by a zone of hemolysis (**as show below**), while in broth culture it forms a dense turbidity with a surface **pellicle**.



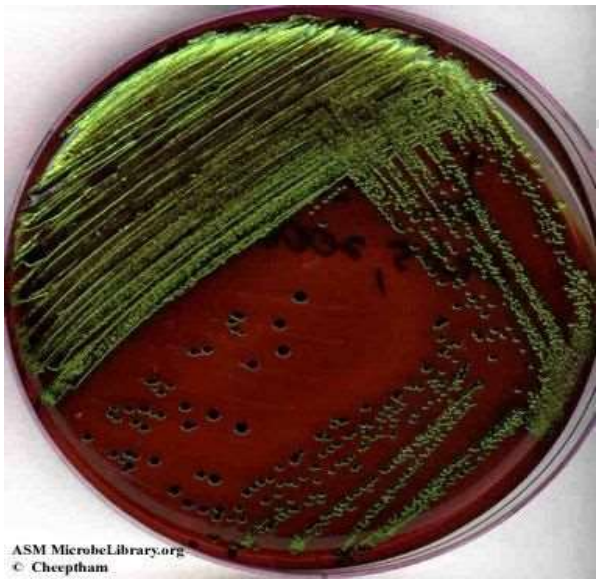
6- Family of *Enterobacterceae*

Gram-negative rods related to the enteric tract include a large number of genera.

Diseases Caused by Members of the Enterobacteriaceae

Escherichia: Urinary tract infection, traveler's diarrhea, neonatal meningitis. Produces green metallic sheen colonies on Eosin Methylene blue (EMB).

***E. coli* on EMB agar**



Shigella: The causative agent of Dysentery, non-motile. Lactose (-) on MacConkey.

Slmonella: The causative agent of typhoid fever and enterocolitis.

Klebsiella: Causes Pneumonia, urinary tract infection. It produces heavy mucoid colonies.

Proteus: commonly causes urinary tract infection. Swarming growth on culture media.

Blood agar: Swarming effect over plate as Proteus is active in motility

Yersinia: The causative agent of Plague, enterocolitis, mesenteric adenitis.

Diagnosis:

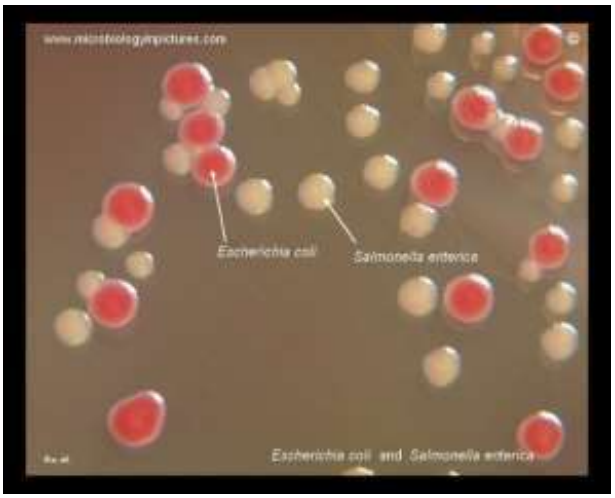
Culture Media

Specimens have suspended in broth and cultured on ordinary as well as differential media (**MacConkey agar**, EMB agar) to permit separation of non-lactose fermenting gram-negative rods from other enteric bacteria. If salmonella infection has suspected, the specimen has also placed in an enrichment medium (**selenite broth**) for 18 hours before has plated on differential media (**Hektoen enteric or Shigella- Salmonella agar**).

Identification of *Enterobacteriaceae* on MacConkey agar:

MacConkey agar is inoculated with tested organism using streak plate technique. Incubate the plate in incubator at 37 C for 24 hrs., then read the results as the following:

- LF organism appears as **pink colonies** (e.g. ***E. coli*** and ***Klebsiella***)
- NLF organism appears as **colorless colonies** (***Salmonella*** *and* ***Shigella***).



7- *Neisseria meningitides*: Family: *Neisseriaceae*: Genus: *Neisseria.*

N. meningitides is aerobic, gram-negative cocci typically arranged in **pairs** (**diplococci**) with adjacent sides flattened together (**resembling coffee beans**).

Specimens collection

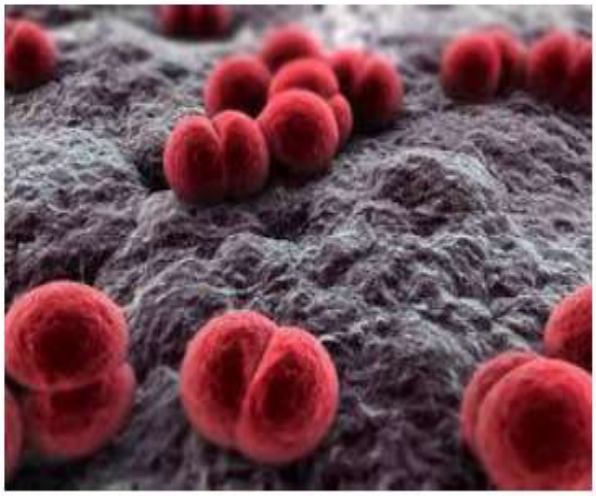
- Nasopharyngeal swabs; body fluids (**joint** fluid or **CSF**) should be stored at 37°C because it was sensitive to cold.
- Any volume (greater than 1 ml) of clear body fluid should be centrifuged at room temperature at 1500xg for 15 min. the sediment should be vortexed and inoculated onto appropriate media.

Diagnosis:

a) Direct detection methods

1- By Gram stain

As indicated above, *N. meningitides* is **Gram negative diplococci** with adjacent sides flattened. They are often referred to as **(Kidney bean)** shaped diplococci.



N. meningitides by electron microscope

2- Antigen detection

The detection of *Neisseria meningitids* **capsular polysaccharides antigen** in body fluids is no longer recommended.

b) Cultivation

The culture media used for Neisseria **5% sheep blood** agar and **chocolate agars**. Colonies of *N. meningitidis* are grey and unpigmented on a blood agar and appear round, smooth, moist, shiny, and convex, with a clearly defined edge. *N. meningitidis* appear as large, colorless-to-grey, opaque colonies on a chocolate agar.

N. gonorrhoeae, *N. meningitidis*, and *M. catarrhalis* grow best under conditions of increased CO2 (3% to 7%).

Colonial appearance (morphology)

N. meningitides colonies are **medium**, **smooth**, **round**, **moist**, **gray to white**; **encapsulated strains are mucoid**; may be greenish cast in agar underneath colonies.
