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((Microbiology))

stage 2

Fourth lecture

Laboratory diagnosis of bacteria

By

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Method of bacterial identification

1. Microscopic examination.
2. Cultural appearance.
3. Biochemical reactions.
4. Serological identification.
5. Animal inoculation.
6. Bacteriophage typing.
7. Molecular methods.

1-Microscopic examination

-Gram stain: gram positive: streptococci and staphylococci

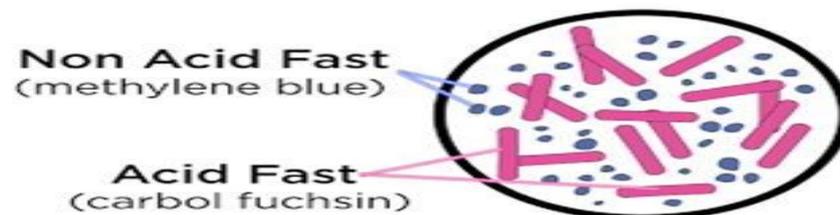
gram negative: salmonella , pneumonia



-Acid fast stain:

acid fast : (Mycobacterium gordonae)

non acid fast : (staphylococcus epidermidis)



2-Cultural appearance

Pigment production:

-Endopigment (restricted to the colonies):

1-Golden yellow with Staphylococcus aureus.

2- White with Staph. epidermidis.



-**Exopigment** (the color diffuses in the surrounding medium):

Green exopigment with *Pseudomonas aeruginosa*



Hemolysis on blood agar:

Blood Agar: is a general purpose enriched medium often used to grow fastidious organisms and to differentiate bacteria based on their hemolytic properties. "blood agar" is usually prepared from Tryptic Soy Agar or Columbia Agar base with 5% Sheep blood, Rabbit, horse or human blood may be used for growth of microorganisms. Many species of bacteria produce toxic by-products that are capable of destroying red blood cells.

Type of Hemolysis on blood agar:

1- **Complete (beta) hemolysis:** *Staphylococcus aureus* and *Streptococcus pyogenes*.

2- **Partial (alpha) hemolysis:** *Streptococcus viridans* and pneumococci.

3- **No (gamma) hemolysis:** Enterococci.





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Effect on lactose of MacConkey's agar:

MacConkey agar (MAC) is a bacterial culture medium named after bacteriologist Alfred T. MacConkey (1861-1931). MacConkey agar is a selective and differentiating agar that only grows gram-negative bacterial species; it can further differentiate the gram-negative organisms based on their lactose metabolism. The fermentation of lactose produces organic acids, particularly lactic acid, which decreases the pH of the agar. MAC contains a pH indicator that turns pink under acidic conditions. Therefore, lactose-fermenting-gram-negatives (lactose-fermenters) will form pink colonies, while non-lactose fermenters will form off-white opaque colonies.

Type of Lactose fermenters:

1-Lactose fermenters:

-Appear as rose pink colonie, example: E. coli & klebsiella.

2-Non Lactose fermenters:

-Appear as pale colonies, example: salmonella & shigella.



Lactose non fermentor

Lactose Fermeter

Macconkey Agar