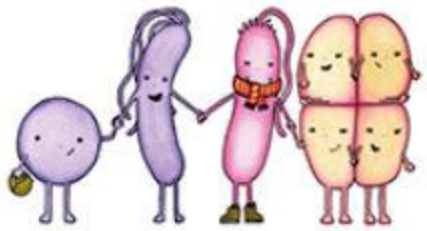

Bacterial classification and identification

By

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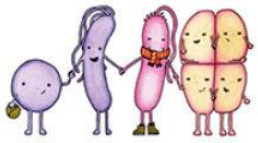


- **Taxonomy:**

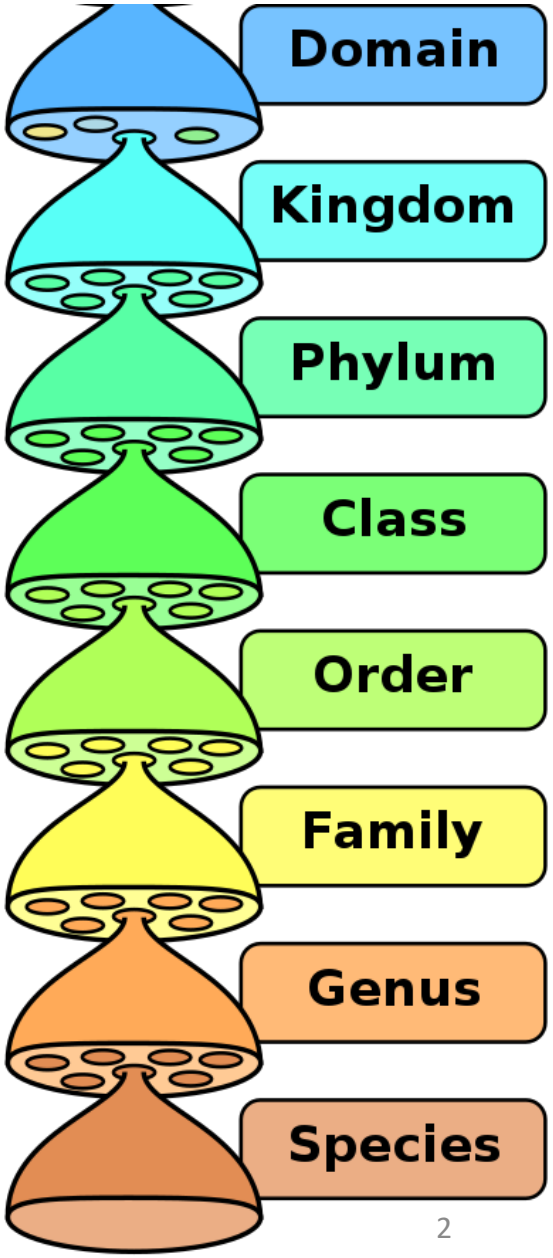
- It is the science dealing with the classification and nomenclature of living things.

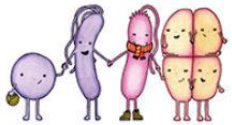
- **Bacterial classification:**

- It can be defined as the arrangement of bacteria into taxonomic groups based on similarities or relationships.

- The taxonomic groups used in classification are:

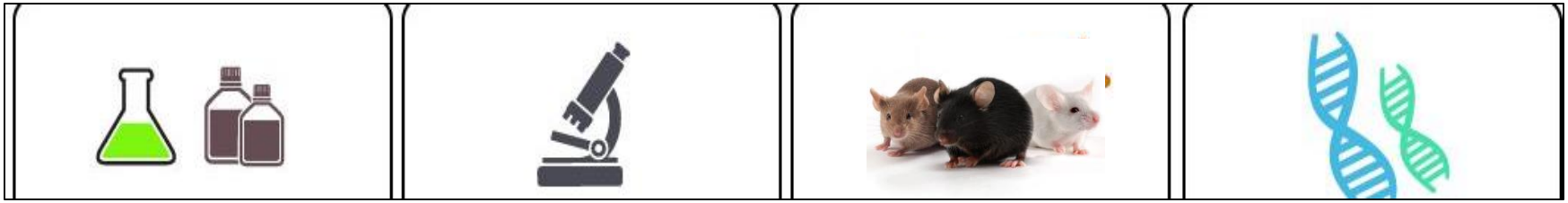
Domain, kingdom, phylum, class, order, family, genus, and species

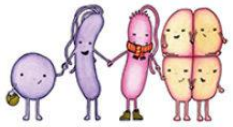




Methods of bacterial identification

1. Microscopic examination
2. Cultivation (culture)
3. Biochemical reactions
4. Serological identification
5. Animal pathogenicity
6. Molecular identification
7. Antibiotic sensitivity
8. Phage typing





1. Microscopic examination

❖ **Wet fresh unstained:** To detect organism motility.

❖ **Stained sample:** To study the organism's shape, size, arrangement, and staining reaction.

❖ **Methods of staining:**

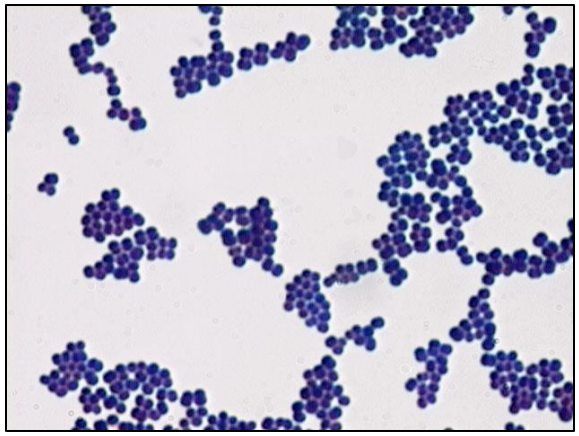
A. **Simple stains:** e.g. methylene blue.

B. **Differential stains:** first stain (has a color)-decolorizing agent- counter stain (has another color). Two common differential stains are used:

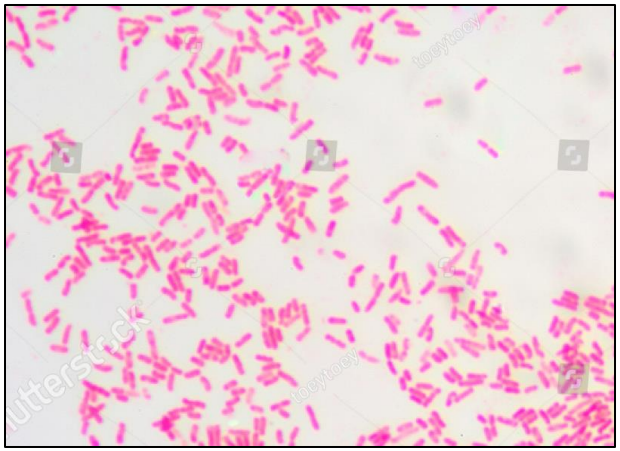
- **Gram stain:** methyl violet iodine (first stain violet in color)- alcohol (decolorizing agent)- basic fuchsine or safranine (counter stain red in color). So, the organisms can be gram-positive (violet) or gram-negative (red).
- **Ziel-Neelsen's stain:** Strong basic fuchsine (first stain red in color)- HCL or H2So4 acids (decolorizing agents)- methylene blue (counter stain blue). So, the organisms can be acid-fast which resist decolorization (red) or non-acid fast (blue).

• Gram stain steps

1. Violet
2. Alcohol
3. Red



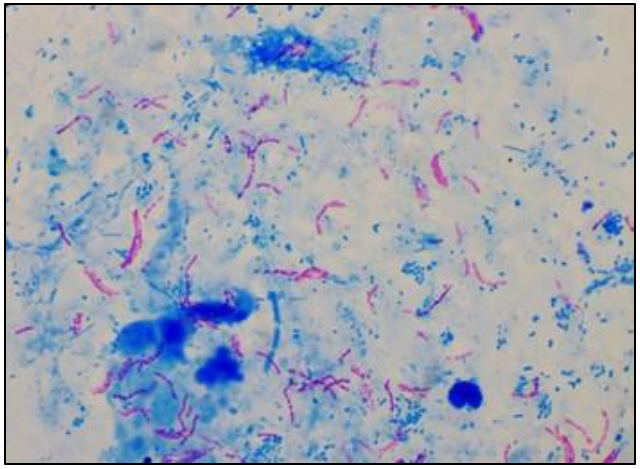
Gram -positive



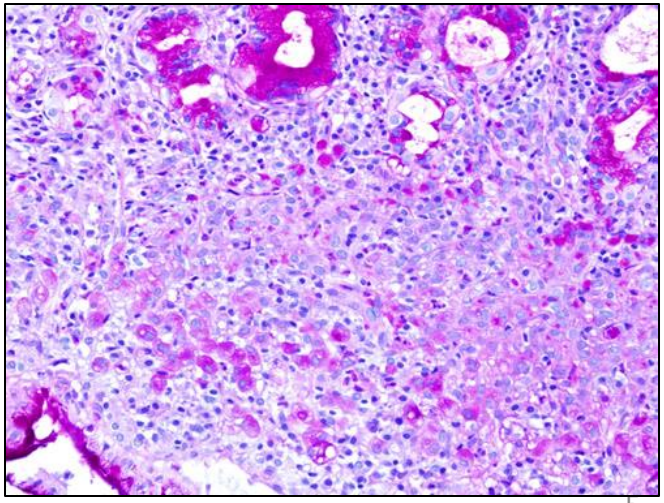
Gram -negative

• Zeil-Neelsen Stain steps

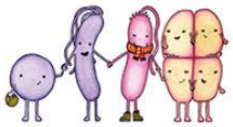
1. Red
2. Acid
3. Blue



Acid -fast



Non-acid fast



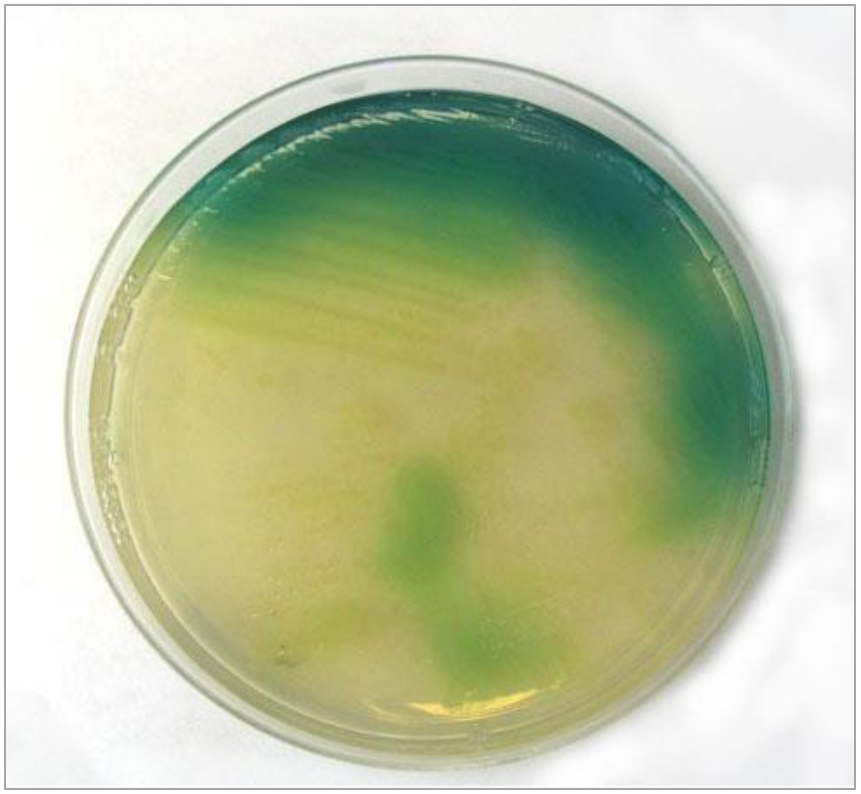
2. Cultivation

- It is a procedure in which nutritive media with known chemical composition are used to allow for bacterial growth and multiplication under controlled conditions in the laboratory.
- The culture is examined in the following ways:

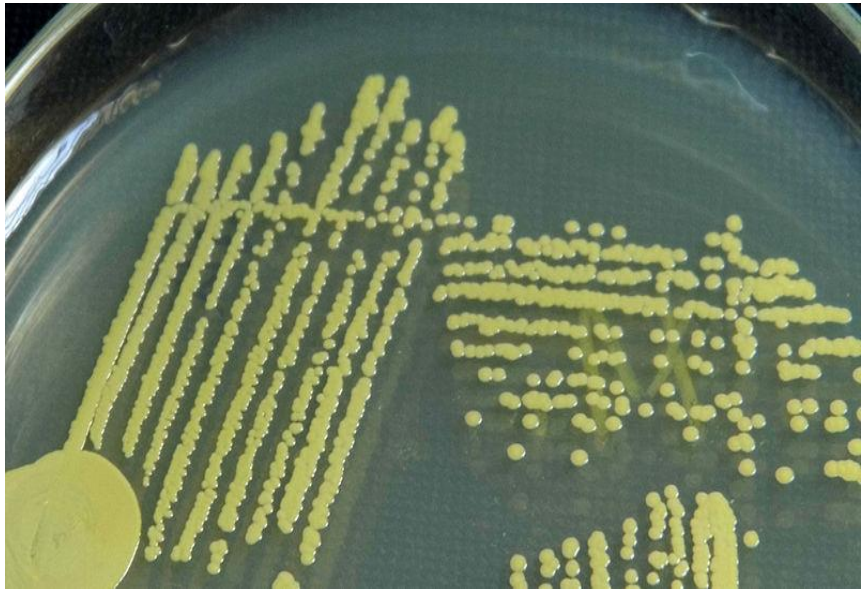
- **Naked eye appearance** of the growth (size, shape, color on solid media, pigment production etc

- **Film preparation wet and stained**

- **Source for other identification methods**.



Exopigment of *Pseudomonas* changing the color of the media



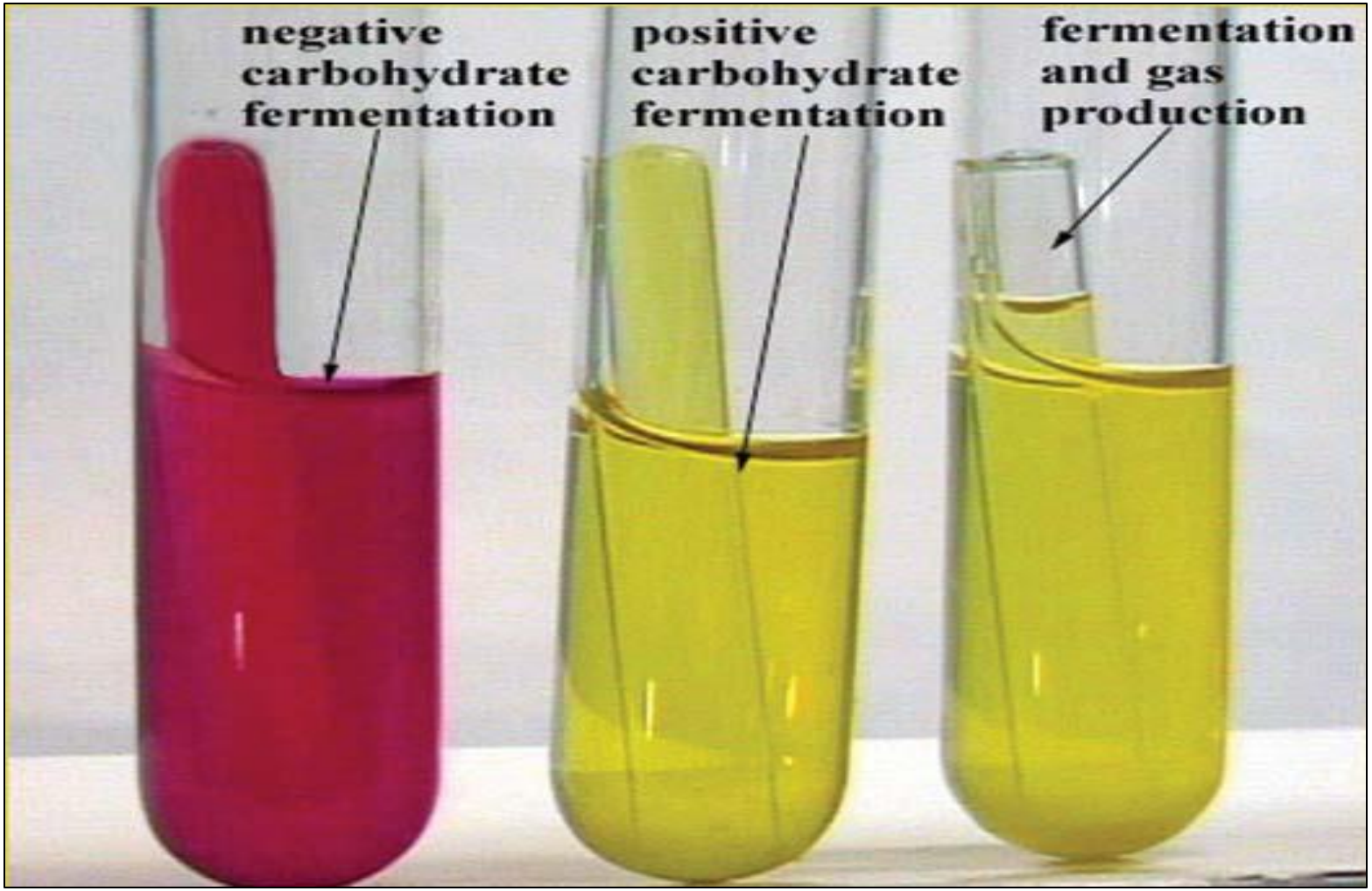
Golden yellow colonies of *Staph aureus*

3. Biochemical reactions

- It is a method used in bacterial identification based on the changes they produce in different substrates as a result of their metabolic activity.
- The most common tests used in the laboratory are:

A. Action on sugar:

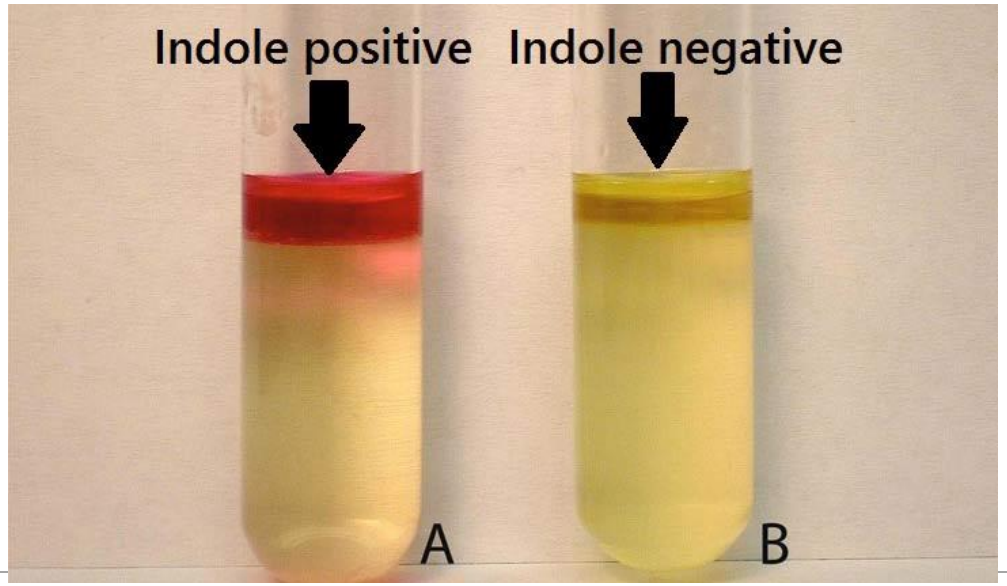
- Bacteria are cultured **on peptone water media containing sugar** to be tested for sugar fermentation. If the bacteria ferment sugar with the production of acid only or acid and gas or don't ferment sugars. The medium contains a PH indicator which changes its color upon acid production and a small inverted tube is introduced into the medium to collect any gases liberated above the fluid level. A set of standard sugars is commonly used (glucose, lactose, maltose, mannite, sucrose etc. Any other sugar can be tested as required.

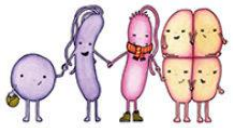

negative
carbohydrate
fermentation
positive
carbohydrate
fermentation
fermentation
and gas
production

3. Biochemical reactions

B. Indole production:

- Indole is produced from the amino acid tryptophane present in peptone. For testing, if the bacteria produce indole or not, a few drops of **Ehrlich's reagent** are added to the peptone water culture of the suspected organism, if indole is present, a purple color is obtained.

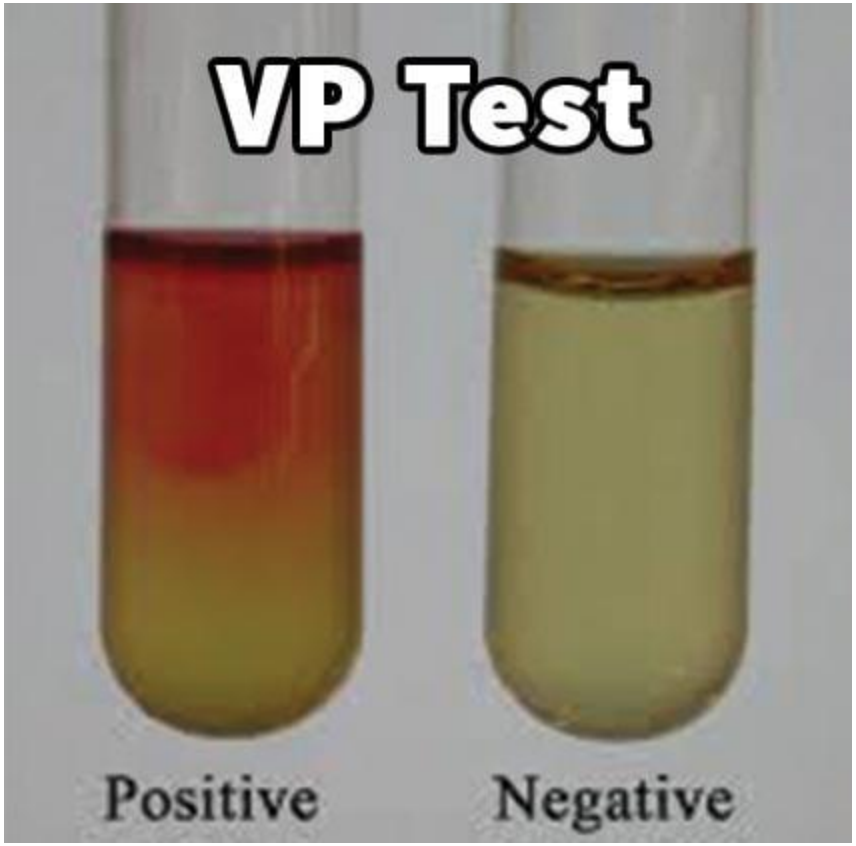




3. Biochemical reactions

C. Voges Proskauer's (V.P) test:

- Coliform bacteria **of non-animal origin** produce acetyl methyl carbinol as a side product of glucose utilization. When grown on **glucose phosphate peptone medium** and the addition of **concentrated KOH**, a pink color will appear. Coliform bacteria **of animal or human origin** give negative tests. This test is of value in the diagnosis of fecal water pollution. A negative test in drinking water is indicative of fecal pollution.



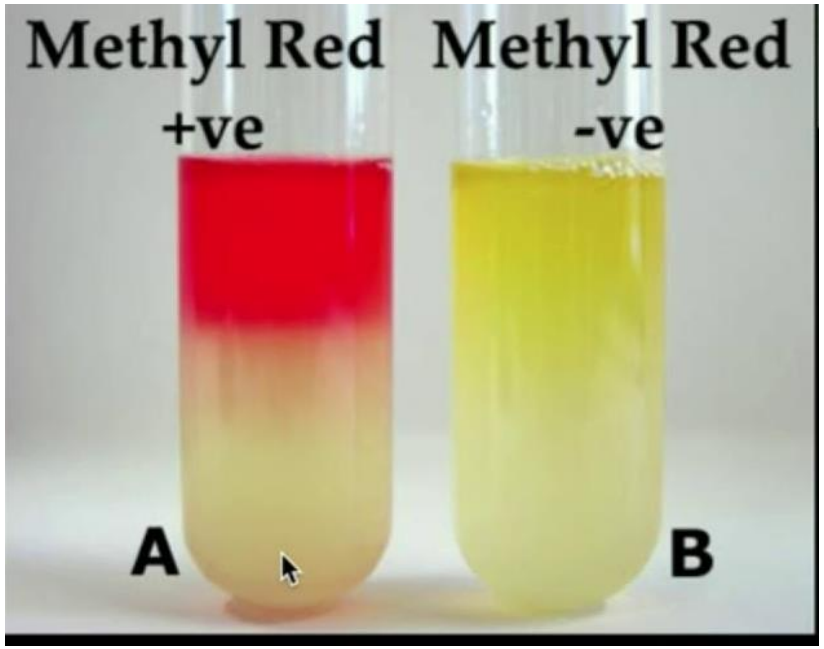
Non-animal origin **Animal or human**

3. Biochemical reactions

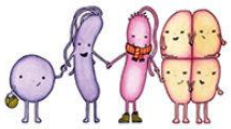
D. Methyl red test (M.R.):

- Coliform bacteria **of non-animal origin** ferment glucose with less amount of acid so, the PH is above 4. Coliform bacteria of **animal origin** ferment glucose with the production of a large amount of acid lowering PH below 4. The test is done by growing the organism on **glucose phosphate medium** containing **methyl red indicator**. The indicator is red below 4 and yellow above 4.

So, fecal water pollution is suspected when V.P test is negative and M.R test is positive.



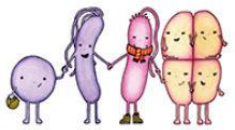
Animal origin **Non-Animal origin**



3. Biochemical reactions

E. Urease test:

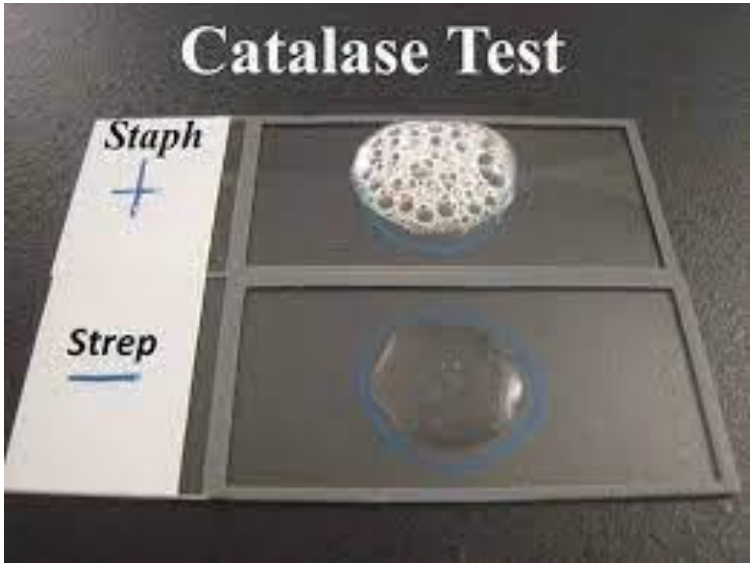
- Some bacteria e.g. *Proteus* and *Helicobacter pylori* produce urease enzyme which can be detected by growing the organism on **a medium containing urea** and **phenol red indicator**. Urease splits urea with the release of ammonia that changes the medium PH to alkalinity so the medium changes into a deep red color.

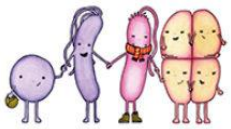


3. Biochemical reactions

F. Catalase test:

- Some organisms as *Staphylococci* produce catalase enzyme which can be detected by immersing the bacterial colonies in a few drops of **hydrogen peroxide. A rapid effervescence** indicates oxygen production and a positive test.

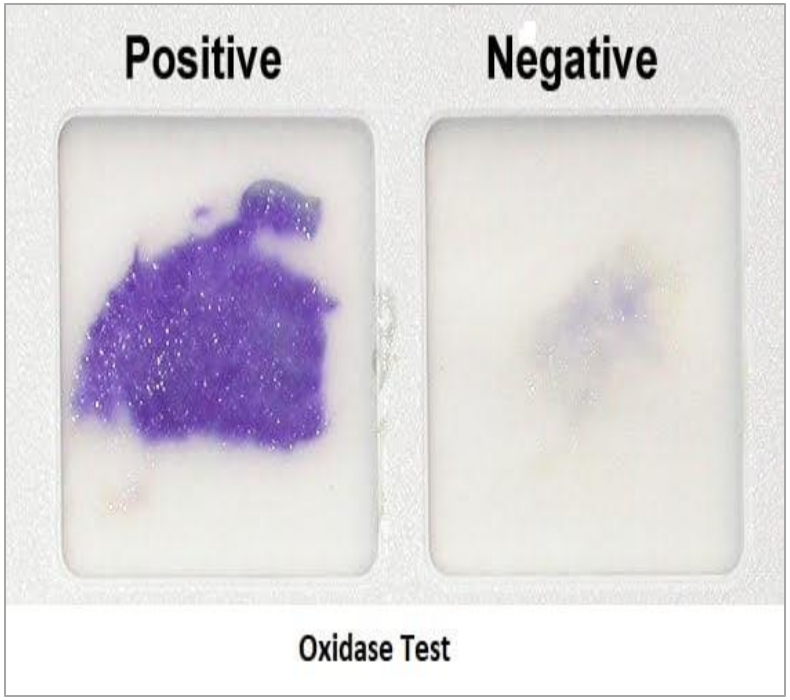




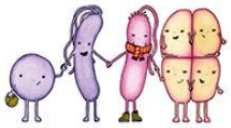
3. Biochemical reactions

G. Oxidase test:

- Some bacteria e.g. *Pseudomonas* produce oxidase enzyme. This can be detected by smearing bacterial colonies on a filter paper impregnated with **oxidase reagent.** An immediate development of a deep purple color indicates a positive test.



Oxidase Test



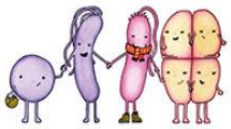
4. Serological tests

- **Serologic tests** are used to determine if a person has antibodies against a specific pathogen, or to detect antigens associated with a pathogen in a person's sample.
- **Types:**

- ELISA

- RIA

- Immunofluorescence



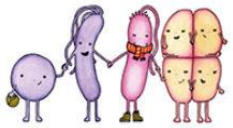
5. Animal Pathogenicity

- The use of laboratory animals is mainly in research for the following reasons:

- Distinguish between **pathogenic and non-pathogenic strains of bacteria.**

- To determine **toxin production**.

- For growing organisms that **do not grow in culture** like *Lepra* bacilli and some viruses.

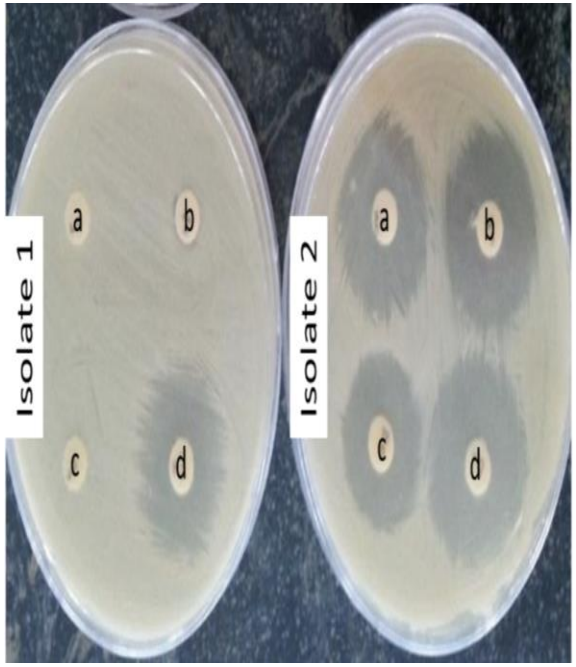


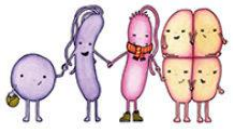
6. Molecular identification

- Detection of microbial nucleic acid.

7. Antibiotic sensitivity

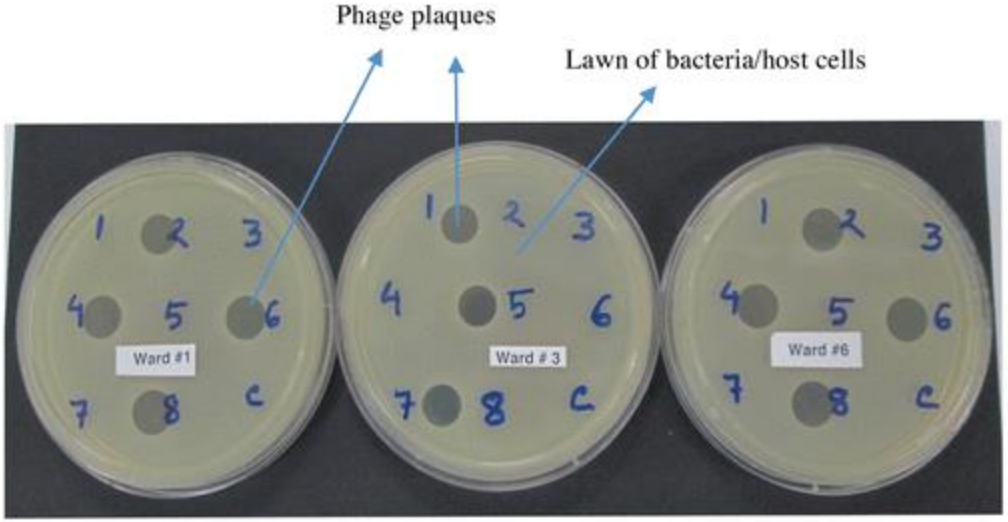
- The susceptibility of bacteria isolated from pathological samples to different types of antibiotics to determine the effective drug to be used in treatment.





8. Phage typing

- Determining the susceptibility of a bacterial isolate to the lytic action of a bacteriophage or a series of phages.




Thank
You