

*Adansonian classification :-

-The major groups are distinguished by:
Cell shape, Gram-stain reaction
and spore formation.

-Genus and species are distinguished by:
Fermentation reaction, pathogenicity
and nutritional requirements.

*Microorganisms can be examined microscopically by :-

- a-Bacterial motility : Hanging drop method
- b-Morphology and staining reactions of bacteria:

-Simple stain: methylene blue stain .

-Gram stain :

- Primary stain (crystal violet)
- Mordant (Gram's iodine mixture)
- Decolorization (ethyl alcohol)
- Secondary stain (safranin)

-Ziehl-Neelsen stain:

- Staining acid fast bacilli
- Decolorization (H_2SO_4 2% & ethyl alcohol)
- Counter stain (methylene blue)

*Types of culture media :-

a-Liquid media:

- Nutrient broth: meat extract & peptone
- Peptone water for preparation sugar media
- Growth of bacteria detected by turbidity

b-Solid media:

-Colonial appearance

-Hemolytic activity

-Pigment production

*Types of solid media :-

- 1-Simple media: Nutrient agar
- 2-Enriched media (high nutritive value): Blood agar & chocolate agar
- 3-Selective media:
Lowenstein-Jensen medium
MacConkey's agar
Mannitol salt agar
- 4-Indicator media (lact. or non-lact. ferment):
MacConkey's medium
Eosin Methylene blue agar
- 5-Anaerobic media:
Deep agar
Robertson's Cooked Meat Medium

*Pigment production :

- Endopigment
(restricted to the colonies):
Golden yellow with *Staphylococcus aureus*
White with *Staph. epidermidis*
- Exopigment
(the color diffuses in the surrounding medium):
Green exopigment with
Pseudomonas aeruginosa .

*Some correlation between morphology and disease :-

- Spiral bacteria: *Treponemes*, *Borrelia*s , *Leptospiras* tend to cause systemic diseases .
- Pathogenic Filamentous bacteria : *Actinomyces*, *No cardia*, *Mycobacteria* tend to cause chronic diseases .
- Gram positive bacteria: *Staphylococcus*, *streptococci* more likely to cause skin infections .

*Bacteria *

□With cell wall :-

- Gram +
Staphylococcus, *streptococcus*, *Clostridium*, *Bacillus*

•Gram -

- Enteric, Respiratory
Acid fast
Mycobacterium

- Wall less
Mycoplasma

□Unusual :-

- Obligate intracellular
Rickettsia, *chlamydia*

Automated bacterial identification systems :-

eg: Vitek system

-These systems identify the organism and its antibiotic sensitivity by detecting color changes or turbidity

-Results are available within 4-6 hours .

*Effect on lactose of MacConkey's agar:-

-Lactose fermenters: Rose pink colonies

eg: E.coli & Klebsiella

-Non Lactose fermenters: pale colonies

eg: salmonella & shigella

*Production of indole:- (from Eryptophan):-

-Indole is detected by Kovac's reagent.

• pink color → (+) test → E.coli

• No color → (-) test → Klebsiella

*Hydrogen sulfide (H_2S) production test:-

-Sulfide Indole Mobility (SIM) medium.

-Triple sugar iron agar (TSIA)

→ Aids in the identification & differentiation of members of Enterobacteriaceae (enterics)

→ It's especially helpful in identifying Salmonella, Francisella and Proteus species

(+) → blackening on the medium.

(-) → no blackening on the medium.

*Methyl Red reaction (MR):-

-Fermentation of glucose with production of huge amount of acid (is detected by methyl red indicator)

Red → (+) → E.coli

Yellow or orange → (-) → Klebsiella

*Voges Proskauer's Reaction (VP):-

-Production of acetyl methyl carbinol from glucose fermentation (is detected by KOH)

Pink → (+) → Klebsiella

No pink → (-) → E.coli

*Citrate utilization test :-

→ Allows the species-level identification of the members of the Enterobacteriaceae

-The citrate enzyme hydrolyses the citrate to form oxaloacetic acid & acetic acid.

-Medium used is: Simmons citrate agar

(+) → develops blue color from green growth of bacterium is occurs

(-) → No color change → no growth occurs

*Oxidase test :-

-Detection by adding few drops of colorless Oxidase Reagent

→ Colonies turn deep purple in color (+)

→ This test is used to differentiate enterobacteriaceae (-) from Pseudomonas (+)

*Catalase test :-

→ Addition of (H_2O_2) lead to production of gas bubbles (O_2 production)

→ Is used to differentiate between Staphylococci (+) and Streptococci (-)

*Coagulase test :-

-Coagulase enzyme converts fibrinogen to fibrin (plasma clot)

-Detected by slide or test tube method

-is used to differentiate staphylococcus aureus from coagulase(-) staphylococci

*Urease test :-

-Urease enzyme hydrolyze urea with production of NH_3 → alkaline pH → change color of indicator from yellow to pink

(+) → pink → Proteus vulgaris

(-) → no pink → E.coli

