

# Microbiology



MID - Lecture 4  
Bacterial Taxonomy,  
Classification, and  
Laboratory Diagnosis (Pt.1)

# Bacterial Taxonomy

Taxon = group, Taxa = groups = classification

The science of biological classification

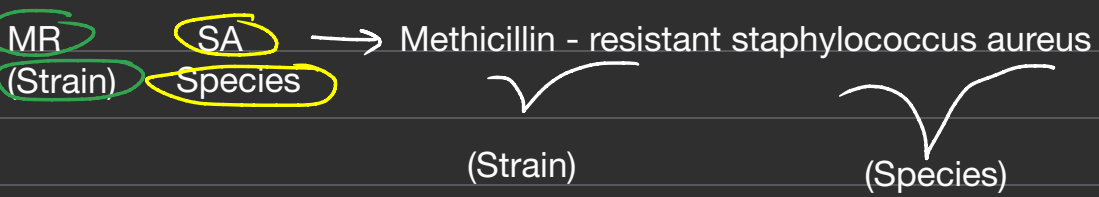
- ① classification
- ② identification
- ③ nomenclature

## Bacterial Taxonomy Rank

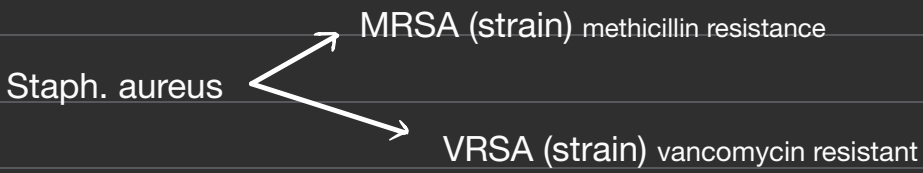
- Kingdom or Domain
- Division or Phylum
- Class
- Order
- Family
- Genus
- Species
- Strains → السلالات

↳ Individual member within a species

ex: Staph. aureus (Species), MRSA (Strain) is part of the species.



Species: A collection of strains share many stable properties.



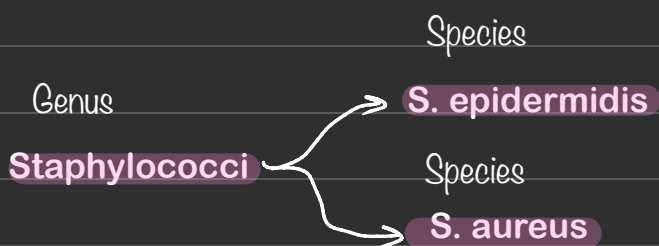
## When do we relate bacteria to a certain species ?

↳ If the DNA similarity between those bacteria is 70% or more similar

↳ If the rRNA similarity between those bacteria is more than 97% similar

\* Genus is one or more species share common properties.

↳ In order to consider one or more species under the same genus , the DNA similarity between those species must be more than 93%



**Nomenclature** : Genus + species (we may add strain).

↳ Scientific rules must be followed : ① Composed of two parts.

↳ 1st one's genus and the second is species

② Escherichia coli , genus 1st letter is capitalized and species 1st letter is lowercase

③ Italic (or underlined).

Or it could be written briefly : (E. coli)

↳ with paying attention to rules.

# \* Classification of bacteria according to morphology (shape)

## \* Shape

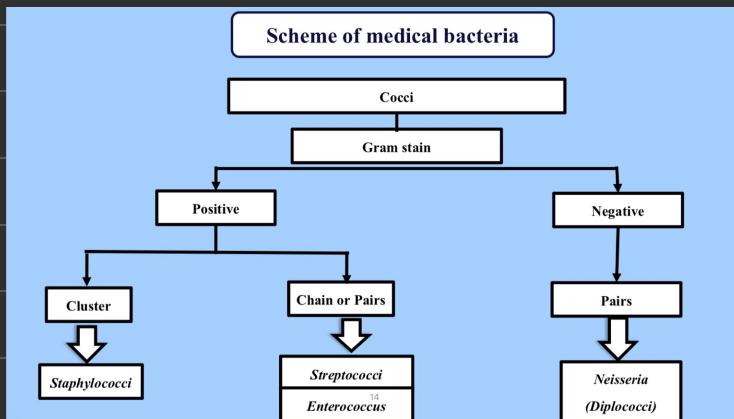
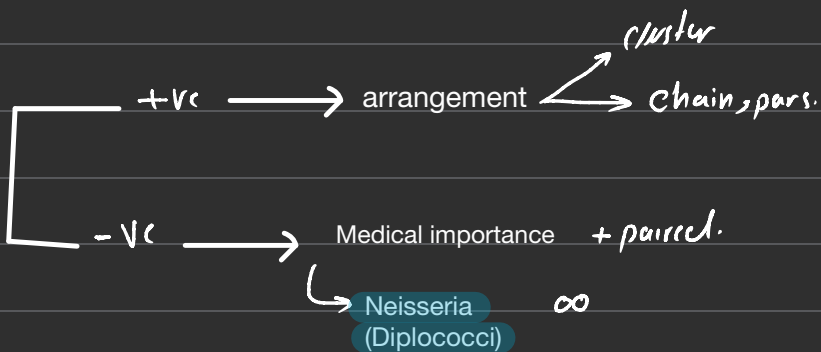
Cocci  Spherical 

Bacilli  Rods 

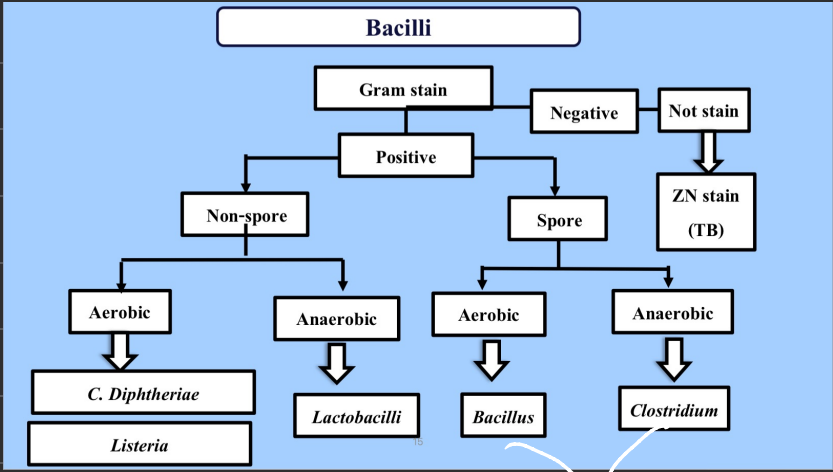
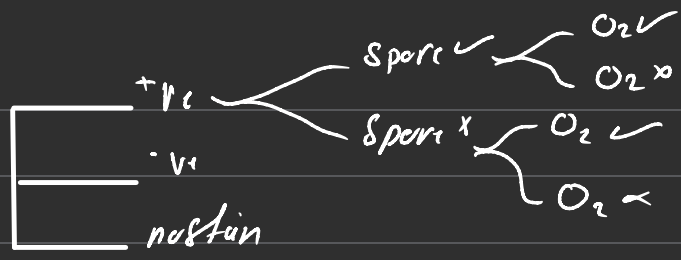
Spiral  

Miscellaneous  Multi shaped.

## \* Gram stain (Cocci)



\* Gram stain (Bacilli)



The only two types that can form spores

\* Gram -ve stain (Bacilli)

- Enterobacteriaceae
- Vibrio
- Campylobacter
- Helicobacter
- Pseudomonas
- ~ Haemophilus
- Bordetella
- Gram negative bacilli
- Brucella
- Legionella
- Gram -ve anaerobes

## \*Spiral

Treponema

•Borrelia

•Leptospira



## \*Miscellaneous group

(multi shaped )

\* No cell wall

\* Not stain by gram

• Obligate intracellular

• Mycoplasma

• Chlamydia

• Rickettsia

• Coxiella

• Actinomycetes

## Systematic Bacteriology

Morphology & Culture

Virulence factor & Pathogenesis

Diseases

Lab. diagnosis

Treatment & Prevention

# Biochemical reactions

TAKE IT EASY

## 1) Indole test

**Purpose:** detecting bacteria's ability to metabolize tryptophan / testing whether bacteria has tryptophanase

→ Extracting indol group

\* Reagent used to detect bacteria existence

→ Kovac's R + indol = red color

→ indol + v.c.

## 2) Methyl red test

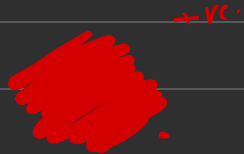
**Purpose:** detecting bacteria's ability to fermentate glucose / testing whether bacteria can transform glucose into large amount of mixed acid (lactic acid, acetic acid and succinic acid)

\* Reagent used to detect bacteria existence

→ Methyl red reagent + acids = low ph > red color

→ No acids > high ph > yellow

Incubate at 37°C for 48h



### 3) Voges-Proskauer test (V.P)

**Purpose:** detecting bacteria's ability to fermentate glucose / testing whether bacteria can transform glucose into Acitone (Acetyl methyl carbinol (Acetoin))

\* Reagent used to detect bacteria existence

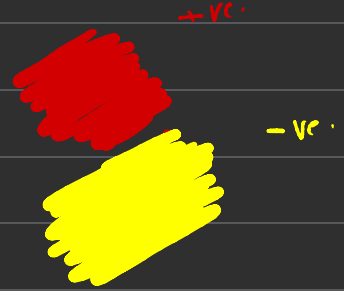
↳ alpha naphthol + 40% KOH + Acitone = Diacetyl (red color)

Incubate at 37°C for 48h

#### IMPORTANT NOTE

If methyl red is positive, the voges-proskauer should be negative and reverse is right.

A bacteria can't fermentate glucose into both lactic acid and acitone, it can follow one of the two directions, so one of the previous test (2,3) must result + and the other results -



### 4) Citrate utilization test

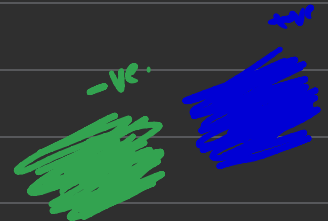
**Purpose:** detecting bacteria's ability to metabolise citrate / testing whether bacteria has citrates so it can metabolise citrate as only source of carbon

\* Reagent used to detect indol existence

↳ The indicator is bromothymol blue + sodium carbonate (alkaline) = indicator changes to blue

\* sodium carbonate source > librated co2 (from the metabolised citrate) interacts with sodium in media forming sodium carbonate which interacts with the reagent forming blue color

Incubate at 37°C for 48h





# 5) Urease test

Purpose: detecting bacteria's ability to metabolise urea / testing whether bacteria has urease so it can metabolise urea

\* Reagent used to detect bacteria existence

Phenol red indecator + ammonia (metabolised urea) = alkaline environment (pink color)  
 no amonia (acidic) = yellow color



# 6) TSI (triple sugar iron)

3 types of sugar 1% glu 1% lac 1% sucrose + ferrous sulphate

A/A  
 K/A

Media used: semi solid (gelatinous like) (the previous tests were in liquid media)

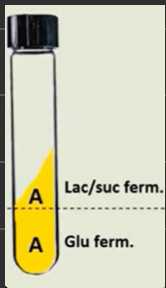
\* Reagent used to detect bacteria existence

Phenol red

① fermentation of glucose → acid formation → acid + phenol red = yellow color → then lactose, sucrose fermentation (if it was able to → forming another acid → A/A)

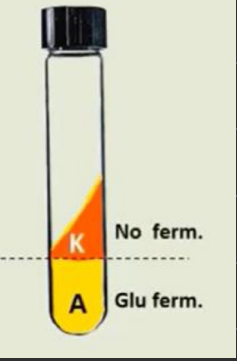
\* An indication that bacteria can produce gases - break up the medium or pushed up the tube

ارتفاع بالوسط ←



Glucose fermenter  
 Lactose fermenter

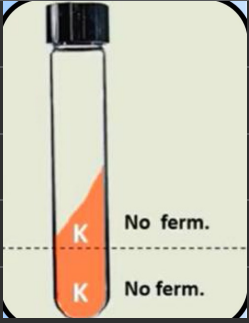
② fermentation of glucose → acid formation → acid + phenol red = yellow color → no lactose fermentation (not able to) → science it's water media there is other components like proteins → utilizing protein → alkaline media → phenol red → revert to red k/A



K/A  
Glucose fermenter  
Non-lactose fermenter

③ Alkaline over Alkaline (K/K)

If the organism can not use the glucose in the medium. The color of the medium remains red.



No sugar fermenter

## 7) H<sub>2</sub>S production

Purpose: detecting bacteria's ability to produce H<sub>2</sub>S / testing whether bacteria can reduce sulfur ( existed in the media )

① bacteria reduces sulfur existed in the cultural media → H<sub>2</sub>S production → H<sub>2</sub>S interacts with iron in the media → forming ferric sulfide → making the media black

## 8) Phenylalanine deaminase

Purpose: detecting bacteria's ability to convert phenylalanine into phenyl pyruvic acid + NH<sub>3</sub>, if the bacteria has Phenylalanine deaminase



Reagent used to detect bacteria existence

↳ Ferric chloride + phenyl pyruvic acid + NH<sub>3</sub> = green color

Distinguishes Proteus from **Salmonella & Shigella**

## 8) Ornithine decarboxylase

↳ Existed in the media

Providencia rettgeri & Morganella morganii (+ve)

Purpose: testing if bacteria has the ornithine decarboxylase / can metabolise ornithine ( source of carbon)

① there is carbon → energy formation → purple color

↳ Morganella morganii is usually ornithine decarboxylase positive, meaning the medium will turn purple due to alkaline end products

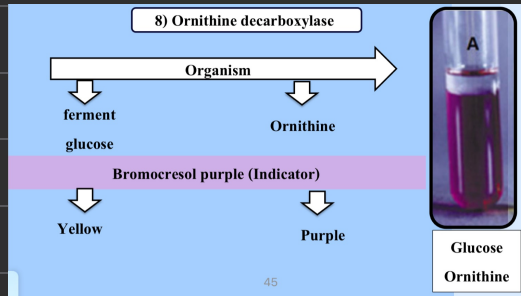
② no Carbon → yellow color

Providencia rettgeri is typically ornithine decarboxylase negative, so the medium will remain yellow.

## 8) Ornithine decarboxylase

Reagent used to detect bacteria existence

Bromocresol purple (Indicator)



## 9) The analytical profile index (API)

commercial stripe contains multiple tubes for various biochemical reactions.

(Biochemical tests for identification)

this device has catalog, we'll use it to read changes (red+ yellow -) and seeing which bacteria follows this pattern



Ex:(not for memorising)

API 20E & API 20NE (Enterobacteria)

API 20 STREP (Streptococci) etc.

For example, if we have a Streptococcus bacteria and want to further identify which Species it belongs to, we use this type of test. Based on the color changes, we can determine which species we are examining.

- Most important 3 tests :
- ① oxidase test
  - ② catalase test
  - ③ coagulative test

## 1) oxidase test

Purpose: Differentiating between Pseudomonas (is not a type of Enterobacteriaceae) and all Enterobacteriaceae members

\* This bacteria (Pseudomonas) has oxidase enzyme so we use Oxidase reagent (Colonies) to detect enzymes present

- Reagent used to detect bacteria existence

↳ oxidase reagent (colonies) (colourless)

a purple color - indicates the presence of Pseudomonas bacteria.  
no color is present -> the bacteria is member of Enterobacteriaceae.

## 2) Catalase test

Purpose: Differentiating between Staphylococci (catalase +ve) and Streptococci (catalase -ve). Staph has the catalase, Strept doesn't

- Reagent used to detect bacteria existence

↳ hydrogen peroxide

Bubbles are formed → catalase +ve → staph

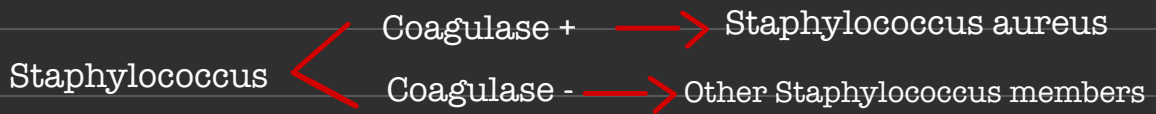
No bubbles → catalase -ve → strept

Characteristic	Staphylococci	Streptococci
Catalase Test	Catalase positive	Catalase negative
Morphology	Forms clusters	Forms chains
Gram Staining	Gram-positive	Gram-positive
Shape	Cocci (spherical bacteria)	Cocci (spherical bacteria)

### 3) Coagulase test

~~Purpose~~ Staph has too many type , this test is used to differentiate between Staphylococcus aureus from coagulase-negative staphylococcus family members (Staphylococcus family )

Staphylococcus aureus is always (the only one in the family ) coagulase positive



If it has Coagulase enzyme ; it will converts fibrinogen(in plasma) to fibrin (plasma clot)  
Detected by slide or test tube method

We can conclude that Staphylococcus aureus is:

1. gram-positive.
2. cocci-shaped, forms clusters.
3. catalase-positive, and coagulase-positive.

# - From modified slices -

Test	What it tests for ?	Principle ( How it works)	Indication
1. Indole test	bacterium's possession of Tryptophanase	By adding tryptophane, that would give indol when broken which react with kouac's R	<b>red</b> color indicates a positive result.
2. Methyl red test	Whether bacteria can ferment glucose and produce a <u>mixture of acids</u>	By sensing the pH changes in the medium	<b>red</b> , indicating an MR-positive result. <b>yellow</b> , indicating an MR-negative result.
3. Voges-Proskauer test (V.P)	the ability of bacteria to ferment glucose, producing acetoin	Glucose → acetoin + alpha-alphanaphthol + 40% KOH → diacetyl (reacts with peptone)	<b>red</b> color, indicating a V.P-positive result.
4. Citrate utilization test	Presence of citrase (citrate lyase)	In a medium which has citrate as the only carbon source. If citrase is present, citrate will be metabolized, releasing CO <sub>2</sub> which combines with Na <sup>+</sup> forming Na <sub>2</sub> CO <sub>3</sub> .	Positive Test → High pH → <b>Blue</b> . Negative Test → Neutral pH → <b>Green</b> (indicator used is <b>Bromothymol blue</b> ).
5. Urease test	If the bacteria possess the urease enzyme	If the bacteria break down urea by urease enzyme, ammonia (which is alkaline) is produced, which react with phenol red indicator	<b>pink</b> indicate a positive urease test <b>yellow</b> indicate a negative urease test.
6. TSI test	<ol style="list-style-type: none"> <li>Whether the bacteria is a glucose fermenter</li> <li>Whether the bacteria is a lactose/sucrose fermenter</li> <li>Whether the bacteria can produce H<sub>2</sub>S (use sulfur as e<sup>-</sup> acceptor)</li> </ol>	<p>1 &amp; 2: by observing the medium change of pH due to fermentation</p> <p>3: by observing the reaction between H<sub>2</sub>S and iron (producing Fe<sub>2</sub>S<sub>3</sub>)</p>	<p>1 &amp; 2: we have two compartments in the medium: <b>yellow</b> (A) → (+); <b>red</b> (K) → (-)</p> <p>3: the black color of ferric sulfide is observed.</p>

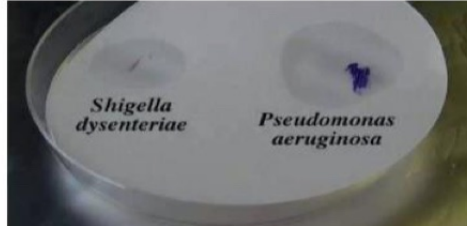
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Test	What it tests for ?	Principle ( How it works)	Indication
7. Phenylalanine deaminase	determining whether the sample contains Salmonella or Shigella or not.	as only these two contain Phenylalanine deaminase enzyme so green color would indicate the presence of salmonella or shigella	<b>green</b> indicates (+ve Phenylalanine).
Ornithine decarboxylase	to distinguish between <i>P. rettgeri</i> & <i>M. morgani</i>	tests for the presence of ornithine decarboxylase in <i>M. morgani</i> bacteria.	<b>yellow</b> , <i>Providencia rettgeri</i> (-ve). <b>purple</b> , <i>Morganella morgani</i> (+ve).
The analytical profile index (API)	A lot of tests combined	We inoculate each tube, place them in an incubator, and after 24 hours, we record the color changes according to the catalog. This allows us to identify the type of bacteria.	
10. oxidase	Used to differentiate Enterobacteriaceae from pseudomonas	All Enterobacteriaceae are oxidase- negative, except Pseudomonas which is oxidase-positive	a <b>purple</b> color → indicates the presence of Pseudomonas bacteria.
11. Catalase test	Used to differentiate between staphylococci and streptococci	Staphylococci are catalase-positive streptococci are catalase-negative	bubbles forming, indicates that the bacteria are catalase-positive.
12. Coagulase test	Used to differentiate staphylococcus aureus from other species under the staphylococci genus	Staphylococcus aureus is the only member of the Staphylococcus family that is coagulase-positive.	forming of fiber clot, indicate Coagulase-positive bacteria.

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## Oxidase Test

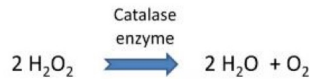
- All Enterobacteriaceae are **oxidase-negative**.
- This test is used to differentiate enterobacteriaceae from *Pseudomonas* which is **oxidase positive**.



### - Catalase test:

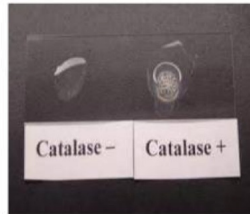
- Is used to differentiate between staphylococci(catalase +ve) and streptococci(catalase -ve).

- Principle:



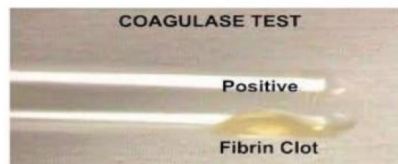
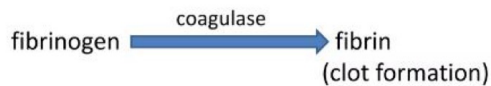
- Procedure

- Smear a colony of the organism to a slide
- Drop  $\text{H}_2\text{O}_2$  onto smear
- Observe



## Coagulase test

is used to differentiate *Staphylococcus aureus* from coagulase-negative staphylococci.





- تم بجد الله تعالى -



ما اذكرك الله سبحانه وتعالى  
ببارك يوقتي ولاكم بالملك  
واحب 4/4 🥺🥺🥺

