



Al-Mustaqbal University College
Dept. Medical Lab. Techniques
Diagnostic Microbiology 20/2021
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Lecture-12, *Pseudomonas aeruginosa* and *Vibrio cholerae*

A-*P. aeruginosa*: It is gram **negative**, **motile** and **rod** shaped.

Specimens: Specimens depend on the site of infection including **skin lesions**, **pus**, **urine**, **blood**, **spinal fluid**, **sputum**, and other material.

Culture: 1- It is an obligate **aerobe**. 2- It does not ferment **lactose**. 3- It produces **grapelike odor**. 4- It forms **smooth round colonies** with a **fluorescent greenish color**. 5- It produces the following pigments:

- 1) non-fluorescent **bluish pigment pyocyanin**, which diffuses into the agar.
- 2) fluorescent pigment **pyoverdin**, which gives a **greenish color** to the agar.
- 3) dark red pigment **pyorubin**.
- 4) black pigment **pyomelanin**.

P. aeruginosa grows well at **37-42° C**; that differentiate it from other *Pseudomonas* species. **Optimum pH- 7.4**. It is **oxidase positive**. **Identification is based on colonial morphology, the presence of pigments, and growth at 42°C**. On **nutrient agar** after aerobic incubation at 37°C for 24 hours, the colonies are **large, smooth, translucent**, irregularly round and characteristic **fruity odor**. The selective medium for *P. aeruginosa* is cetrimide agar.

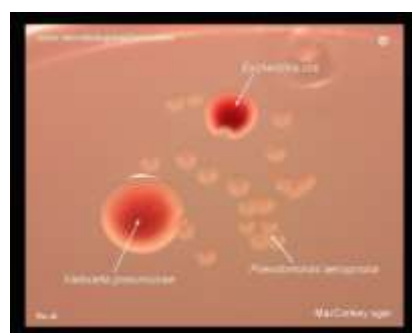
Cultural characteristics of *P. aeruginosa* on different media



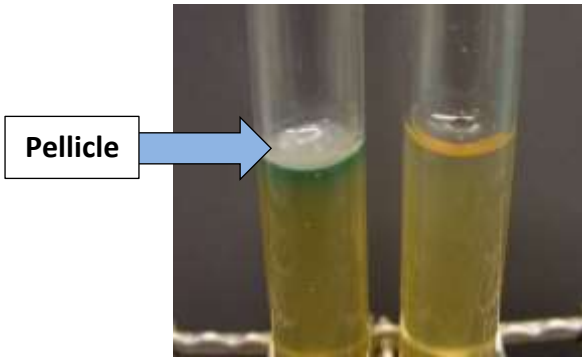
On nutrient agar plate.



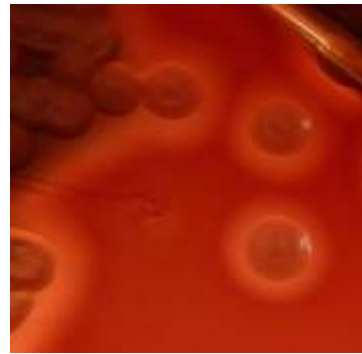
On cetrimide agar plate



On MacConkey agar plates with *E. coli* or *Klebsiella*



In nutrient broth, pellicle



On blood agar, B-hemolysis

P. aeruginosa growing in broth culture. It produces pyocyanin, water-soluble (diffusible) green pigment and (whitish biofilm, pellicle on the surface of the broth, because *Pseudomonas* is aerobic).

....Remember....


5. *Pseudomonas aeruginosa*

On Blood agar: β -hemolysis


On MacConkey agar: Non-lactose fermenting

On Nutrient agar: Large, opaque, irregular colonies with butyrous consistency & fruity odor or earthy smell


- ❖ Produces water soluble pigments which diffuse into the medium
 - i. Pyocyanin- bluish green
 - ii. Fluorescein- yellowish green
 - iii. Pyorubin- red
 - iv. Pyomelanin- black



Blood agar plate



Citrimide plate agar



Nutrient agar plate

B- Vibrio cholera-O1

Morphology: It is **gram-negative**, short, **slightly curved**, cylindrical rods, with rounded or slightly pointed ends. The cell is typically **comma shaped** (hence the old name *V. comma*). **S-shaped** or spiral forms may be seen due to two or more cells lying end to end. The vibrios are seen arranged in parallel rows.



It is **actively motile**, by means of a **single, polar flagellum (monotrichous)**. The motility is of the **darting type (darting movement)**. They are non-sporing, and non-capsulated.

Most important pathogenic members in human are:

1. *Vibrio cholerae*. (**classic, El Tor**... Inaba or Ogawa)
2. Non-agglutinable vibrio (NAV) or non- O1.
3. *Vibrio parahemolyticus*

Bio Chemical Reactions

V.cholerae(Classical)

Hemolysis	-ve
Voges -proskauer test	-ve
Polymyxin sensitivity	+ve
Group IV phage	
Susceptibility	+ve
Chick erythrocyte	
Agglutination	-ve

V.cholrae (El Tor)

+ve
+ve
-ve
-ve
+ve

Specimens: Specimens for culture consist of **mucus flecks from stools**.

Cultural Characteristics: Cholera vibrio is **strongly aerobic**. It grows within a temperature range of 16-40°C (**optimum 37°C**). **Optimum pH 8.2**. Growth is better in an **alkaline medium**.

On nutrient agar, after overnight growth, colonies are **smooth, convex, moist, translucent round disks**. On blood agar, colonies are initially surrounded by a zone of greening. Liquefaction of gelatin begins at the top which spreads downwards in **forming a funnel shaped**. When incubated at 37°C in liquid media, such as **Alkaline peptone water (which is the enrichment medium for vibrios)**, it forms a **fine surface pellicle** because of its **affinity for oxygen**, which on shaking breaks up into **membranous pieces' turbidity** and a **powdery deposit** develop on continued incubation.

Transport Media: The stool samples should be transported in transport media if the cultures cannot examine immediately. The transport medium for **V. cholerae** is **Cary-Blair Medium**.

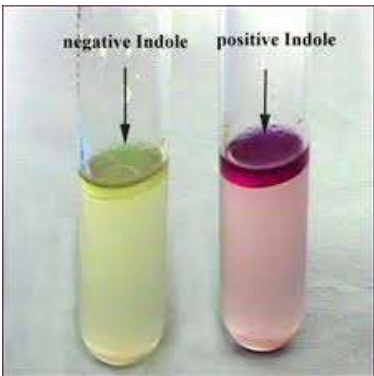


The selective medium for **V. cholera** is **Thiosulphate-Citrate-Bile-Sucrose (TCBS) Agar**

Selective Medium – TCBS

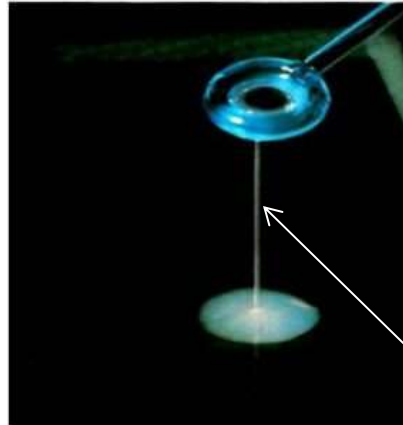
V.cholerae grows well on Thiosulphate citrate bile sucrose (TCBS) agar, on which it produces yellow colonies that are readily visible against the dark green background of the agar.

Cholera red reaction is diagnostic test for V. cholera (Indole). This test is done by adding few drops of H₂SO₄ to 24-hour growth in peptone water. With *Vibrio cholerae*, appearance of red pink color indicates for positive result.



Cholera red reaction

String Test: When isolated colony of *V. cholera* is mixed in Sodium deoxycholate (bile salt), it lyses the cell wall of the bacterium releasing the DNA. The suspension loses turbidity and the mixture becomes viscous. A mucoid “string” is formed when an inoculating loop is drawn slowly away from the suspension



Mucoid string

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