

جامعة الانبار

كلية: الصيدلة

قسم: العلوم المخبرية السريرية

اسم المادة باللغة العربية: احياء مجهرية طبية I

اسم المادة باللغة الإنكليزية: **Medical Microbiology I**

المرحلة: الثانية

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عنوان المحاضرة باللغة العربية: المتقلبات والزوائف الزنجارية

عنوان المحاضرة باللغة الإنكليزية: **Proteus spp & Pseudomonas**

Proteus spp.

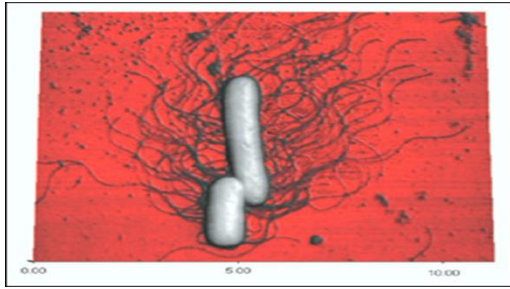
Bacteria, which belong to enterobacteriaceae, are commensal in human intestine .However this group also becomes pathogenic under opportunistic conditions causing infections such as (UTI following catheter & surgery, wound infection, otitis media and septicemia.

The species of proteus are differentiated in to:

1. *Proteus mirabilis.*
2. *Proteus vulgaris.*
3. *Proteus penneri*
4. *Proteus hauseri*
5. *Proteus myxofaciens*

Morphology:

Gram-negative, facultatively anaerobic rod-shaped, pleomorphic , noncapsulated, non spore forming and motile show Swarming motility.



Swarming motility of *proteus* spp.

Cultural Characteristics:-

They grow readily on all ordinary media, producing fishy smell. Swarming type of growth is observed on solid media such as nutrient agar and blood agar. Swarming is inhibited on macConkey's agar and produce pale yellow colonies which moderate in size, moist and low convex, Non-fastidious bacteria. Urease producing organisms which decompose urea.



How to prevent swarming????

- ❖ Increase the agar concentration (from 1.2-1.5% to 6%).
- ❖ CLED agar (electrolytes deficiency).
- ❖ To add chemicals: chloral hydrate, sodium azide, sulphonamide & paranitroglycerol.
- ❖ MacConkey agar (Bile salt).

LAB- Diagnosis:-

- Specimen: according to the site of infection (Pus, swabs, blood, urine, sputum.....etc).
- Colonial morphology. □ Culture & incubation.
- Gram stain
- Biochemical reaction.
 - It forms acids and gas from glucose fermentation (except *P. rettgeri*).
 - It characteristically deaminates phenylalanine to phenyl pyruvic acid (PPA). Phenylalanine deaminase test is used to differentiate members of the genera *Proteus*, (+ve) from other members of **Enterobacteriaceae** which give negative results.

It is used to test the ability of microorganism to produce enzyme deaminase this enzyme remove amine group from phenylalanine and produce phenyl pyruvic acid. Phenylalanine agar, also known as phenylalanine deaminase medium is used as test medium. Positive result gives green color while negative result no change in media yellow color.

- Hydrolysis of urea is another characteristic property of proteus spp.
- It is MR positive and VP negative.
- It is non lactose fermenter.
- *Proteus mirabilis* and *Proteus vulgaris* can produce H₂S
- Indole is not produced by *Proteus mirabilis*
- Citrate utilization test is positive in *Proteus mirabilis* and variable (+ or -) in *Proteus vulgaris*

Pseudomonas

They are saprophytic and innately resistant bacteria causing opportunist infections in man, found in nature water, soil and other moist environments, causing suppurative and inflammatory lesions.

Pseudomonas spp:-

1. *Pseudomonas aeruginosa*
2. *Pseudomonas fluorescens*.
3. *Pseudomonas putida*
4. *Pseudomonas stutzeri*
5. *Pseudomonas alcaligenes*
6. *Pseudomonas pseudomallei*

The species most common associated with diseases is *Pseudomonas aeruginosa* .It is commonly encountered in secondary infections of wounds, burns and chronic ulcer of skin as well as urinary tract infections.

Pseudomonas aeruginosa

Morphology

It is slender gram negative bacilli, 1.5 – 3 x 0.5 microns in size, actively motile by a by single or multiple polar flagella, non capsulated and its non-spore forming.



Cultural Characters

It is aerobic, growing on simple media with optimum temperature of 37 °C.

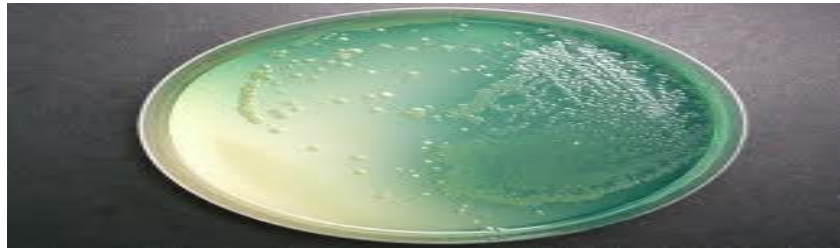
In broth forms dense turbidity with surface pellicle. Bluish green pigment due to water soluble Pyocyanin is seen.

Nutrient agar: producing large opaque irregular colonies with distinctive musty or earthy smell.

Pseudomonas spp. is Produce water soluble pigments which diffuses in medium. There are several types of pigments:

- a) Pyocyanin (bluish green).
- b) Pyoverdin (yellowish green).
- c) Pyorubin (Reddish- Brown)
- d) Pyomelanin (Brown – Black).

Some strain may be non –pigmented.



Blood agar: It shows beta type of hemolysis.

MacConkey agar: It produce non lactose fermenting colonies.

Laboratory diagnosis:

- 1- **Specimens:** According to the site of infection (swabs, blood, urine, sputum).
- 2- **Smears:** *pseudomonas aeruginosa* is gram negative bacillus often seen.
- 3- **Culture :** on nutrient agar media characteristic bluish green colonies appear.
- 4- **Biochemical tests**
 - Glucose is utilized oxidatively forming acid only.
 - Indole, MR and VP and H₂ S tests are negative.
 - Utilizes Citrate as a sole source of Carbon.
 - Catalase and Oxidase are positive.

OXIDASE TEST

The oxidase test is a key test to differentiate between the families of Pseudomonadaceae and Enterobacteriaceae. Cytochrome oxidase is an enzyme found in some bacteria that transfers electrons to oxygen, the final electron acceptor in some electron transport chains. Presence of cytochrome oxidase can be detected through the use of oxidase test reagent, **1% tetramethyl-para-phenylenediamine dihydrochloride**. This reagent acts as an electron donor to cytochrome oxidase. If the bacteria oxidize the Oxidase test reagent (remove electrons) the reagent will turn purple indicating a positive test. No color change in one minute indicates a negative test.

THE PROCEDURE:

1. Pick a good-sized amount of inoculum (already incubated and grown) from a plate culture or slant culture and place it on a piece of filter paper.
- 2- Add one drop of the reagent. A positive reaction will usually occur within 10-15 seconds be a bluish-purple color that progressively becomes more purple.

