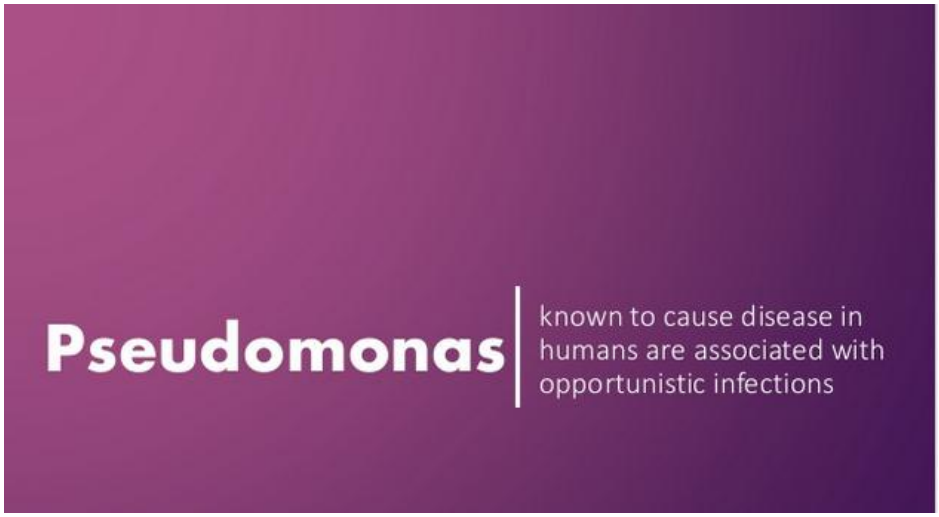


LAB 9:

FAMILY: *PSEUDOMONADACEAE*

Genus: 1- *Pseudomonas*

Spp. A) Pseudomonas aeruginosa

B) Pseudomonas fluorescence

C) Pseudomonas putida

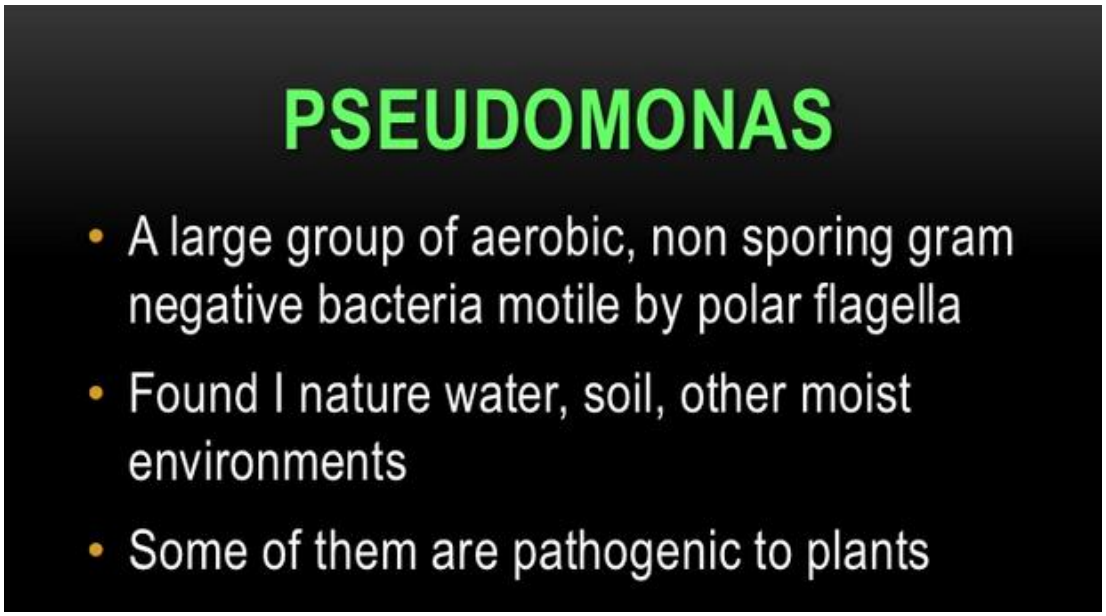
(Fluorescent group)

D) Pseudomonas pseudoalcaligene(opportunistic)

E) Pseudomonas mallei

F) Pseudomonas pseudomallei

G) Pseudomonas cepatia



GENERAL CHARACTERISTICS

- Widely distributed in soil and water
- Gram negative rods
- Aerobic
- Motile
- Produce water-soluble pigments
- Opportunistic pathogens

P. aeruginosa

Forms round colonies with a fluorescent greenish color, sweet odor, and β-hemolysis.

Pyocyanin- nonfluorescent bluish pigment;

pyoverdin- fluorescent greenish pigment;

pyorubin, and pyomelanin

Some strains have a prominent capsule (alginate).

Identification of *P. aeruginosa* is usually based on oxidase test and its colonial morphology: b-hemolysis, the presence of characteristic pigments and sweet odor, and growth at 42 °C.

P. aeruginosa produce blue green pigment → pyocyanin

* *P. fluorescence* produces yellow to green pigment → fluorescen (pyoveridin)

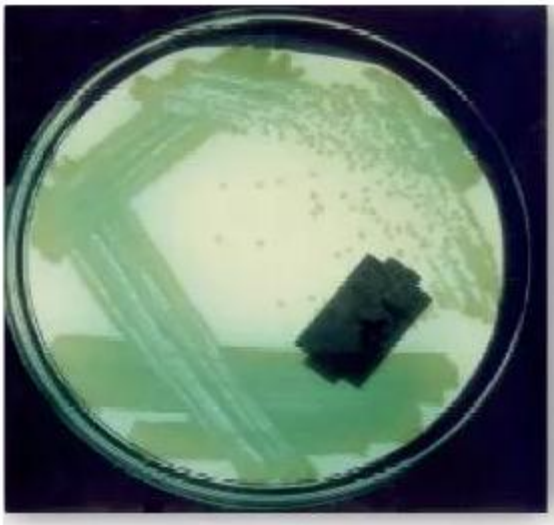
Pyoveridin (composed from 2 pigments fluorescen A and fluorescen B).

Other produces red pigment → pyorubin

Some produce black pigment→ pyomelanin.

PIGMENT PRODUCTION

- Some strains produce diffusible pigments:
  - Pyocyanin (blue); fluorescein (yellow); pyorubin (red)
- *P. aeruginosa* produces characteristic grape-like odor and blue-green pus & colonies
- Broad antibiotic resistance



Pyoverdin

Pyocyanin

Pathogenicity:

P. aeruginosa is the most important species, it is invasive and toxigenic produce infection in patients with abnormal host defense and is an important nosocomial pathogen, they cause UTI, otitis media and septic shock, and the main infection of ***Pseudomonas*** is burn infection and wound infection.

They may found in antiseptic solution, eye drops, grows well in dettol, heating 55 C◦ kill ***Pseudomonas*** ,so it could survive in detergents, it shows also resistant to different and multiple antibiotics.

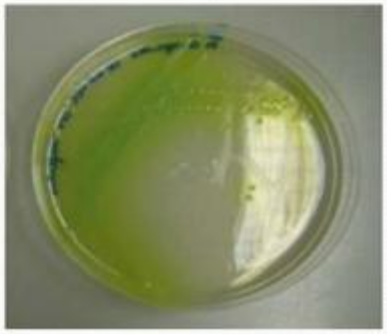
5. *Pseudomonas aeruginosa*

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



Enzymes and toxins:

They are extracellular include hemolysin, lipase,c ollagenase, protease,the most important toxin is exotoxin A which cause blockage of protein synthesis which leads to tissue necrosis.

Classification:

1- Biochemical test.

2- Serological (H-Ag, O-Ag, 110 serotype).

3- Pyocin typing ***Pseudomonas*** produce pyocin which is an antimicrobial agent.

4- Phage typing.

5- Sensitivity pattern antibiotics.

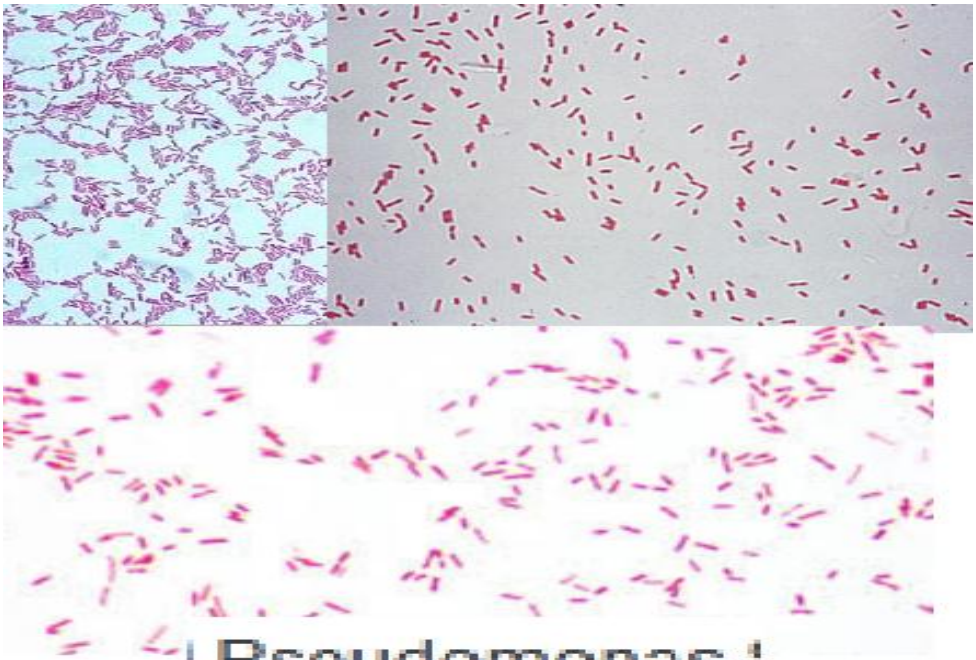
Drug of choice: Carbenicillin (Pyopen)

Specimens:

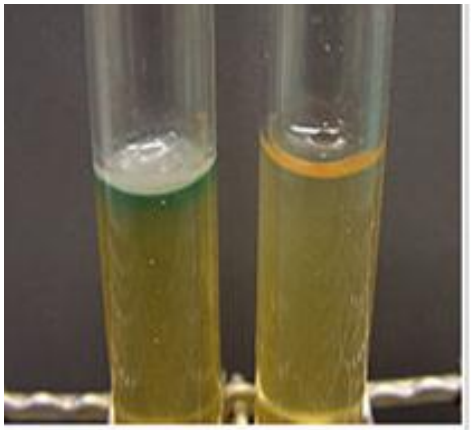
skin lesion, pus, spinal fluid, sputum and urine.

Laboratory Diagnostic tests:

1- Gram stain: G -ve bacilli .
2- Milk agar (for pigmentation).
3- Blood agar (for hemolysis).
4- Kling A,King B (selective and differential)
5- MacConkey agar.
6- TSI
7- IMVIC
8- Motility
9- O/F (oxidation-fermentation) contain 1% glucose, bromothymol blue, K_2HPO_4 buffering, add paraffin on the slant to produce anaerobic condition,inoculation by stabing, the colour change to yellow.
10- Nitrate broth.
11- oxidase and catalase.

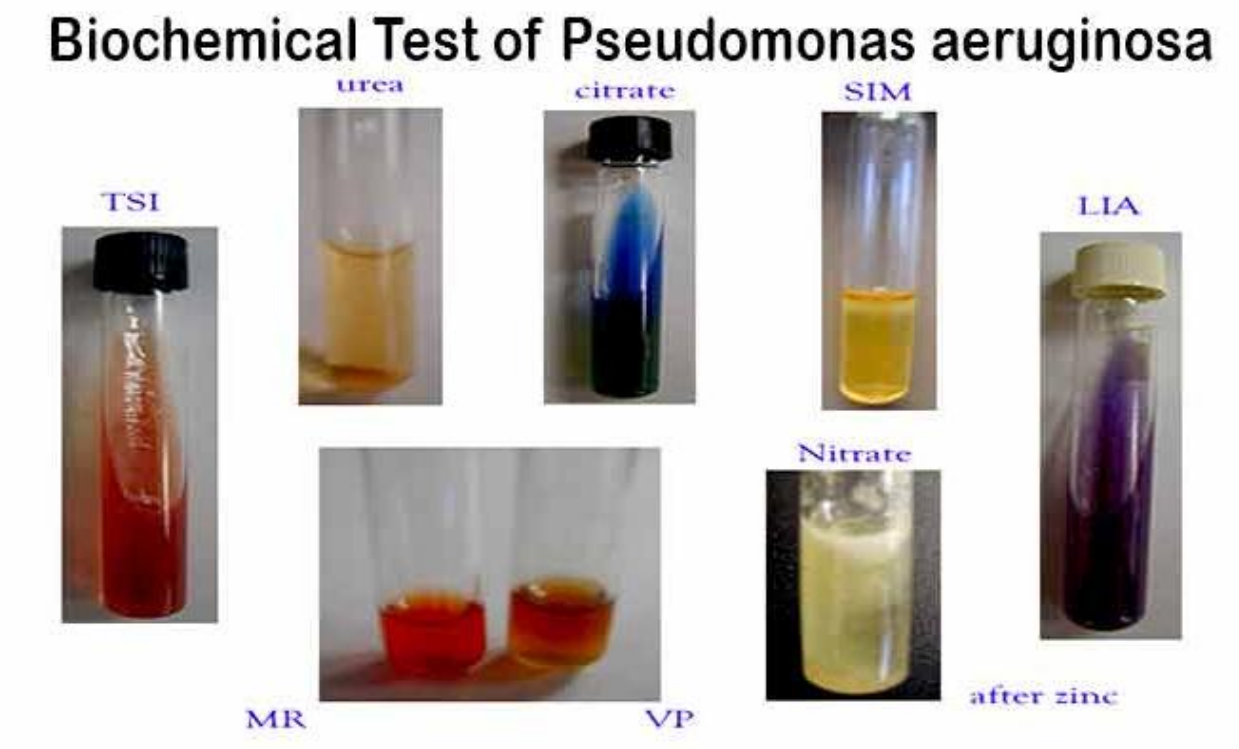


Pseudomonas :

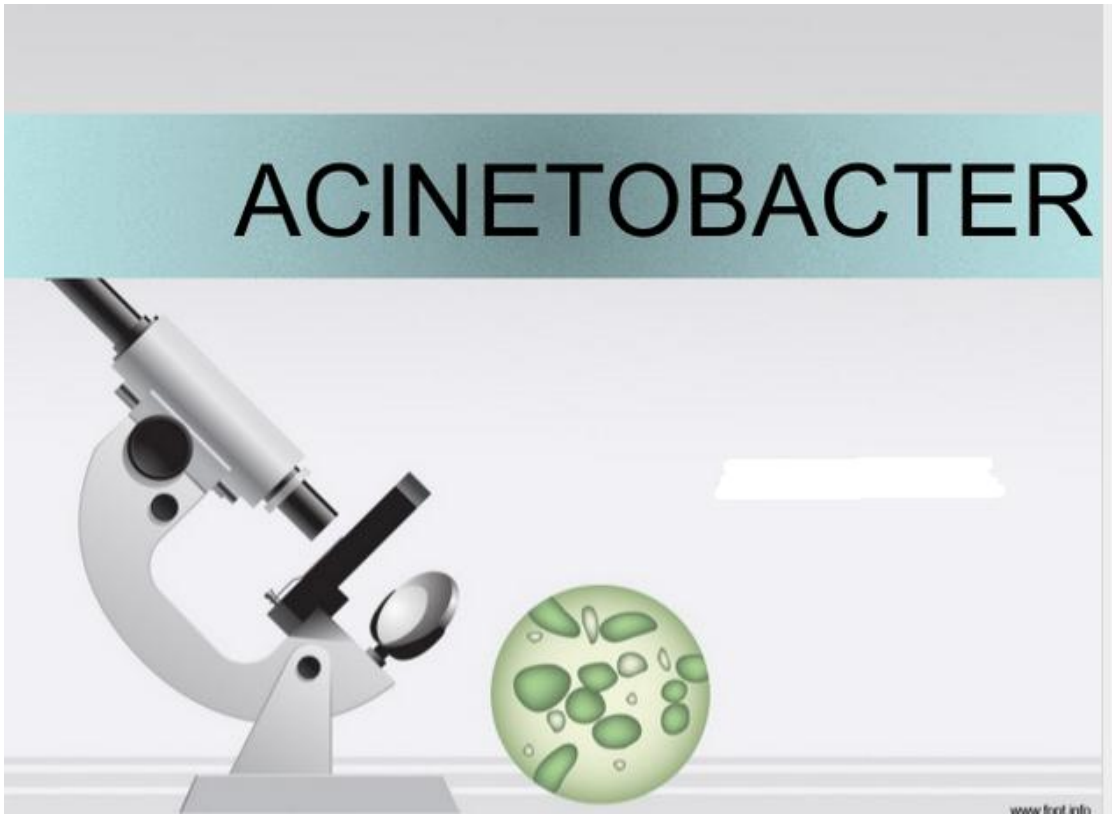


إنتاجبايوسيانين،تذوب في الماء الصبغة الخضراء الزائفة الزنجارية (الأنبوب الايسر)

Test	*P. aeruginosa*	*P. fluorescence*
Indol	-	-
MR	-	-
VP	-	-
SC	+	+
TSI	K/K - -	K/K - -
Nitrate	+	+
Motility	+	+
Growth at 42 C°	+	-
Growth at 4 C°	-	+
King A	+ pyocianin	(- +) - pigment
King B	+ fluorescen	+ fluorescen
MacConkey	N.L.F. transparent,irregular	N.L.F. transparent,irregular
oxidase	+	+
catalase	+	+
OF medium	O (+)/F (-)	O (+)/F (-)



methyl red-, voges proskaeur, tolerate salt 4%, SIM in 30c+, SIM in 37c –



Taxonomy

The genus *Acinetobacter* comprises 38 validly named species.

Domain	Bacteria
Phylum	Proteobacteria
Class:	Gammaproteobacteria
Order:	Pseudomonadales
Family:	Moraxellaceae
Genus:	*Acinetobacter*

Species

Acinetobacter albensis
Acinetobacter apis
Acinetobacter bohemicus
Acinetobacter boissieri
Acinetobacter bouvetii
[1]*Acinetobacter equi*
Acinetobacter gandensis

The Acinetobacter has more than 50 species, most of which are nonpathogenic environmental organisms. The most common infection-causing species is A baumannii, followed by Acinetobactercalcoaceticus and Acinetobacterlwoffii

Acinetobacter is often cultured from hospitalized patients' sputum or respiratory secretions, wounds, and urine. In a hospital setting, *Acinetobacter* commonly colonizes irrigating solutions and intravenous solutions.

Introduction

- The name, *Acinetobacter*, comes from the Latin word for **"motionless,"** because they lack cilia or flagella with which to move.
- Have 32 species, *A. baumanii* and *A. lwoffii* have greatest clinical importance.

2

Introduction

- Most species are not significant sources of infection. However, one opportunistic species, ***Acinetobacter baumannii***, is found primarily in **hospitals** and poses a risk to people who have suppressed immunity.
- >2/3 of *Acinetobacter* infections are due to *A. baumannii*

3

Acinetobacter

- Gram-Negative
- Coccobacilli
- Strictly aerobic
- Nonmotile
- Catalase positive
- Oxidase negative

4

Epidemiology

Environmental reservoirs

- Soil
- Fresh water
- Vegetables
- Animals
- Body lice, fleas, ticks

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Epidemiology

In the hospital…

- Environmental surface
- Ventilators, dialysis machines, air ventilation systems, water sources
- Hands
- Contaminated suction equipment
- Respiratory, urinary, GI tracts & wounds of patients

6

Growth Requirment

Aerobic

Grow at 44° C

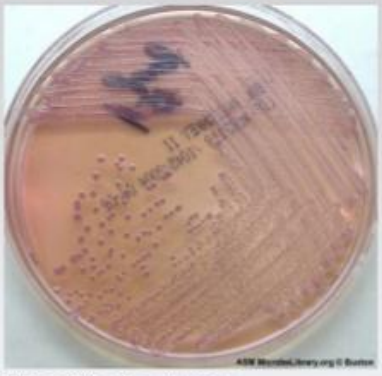
Differential Media

- MacConky Agar

Selective Media

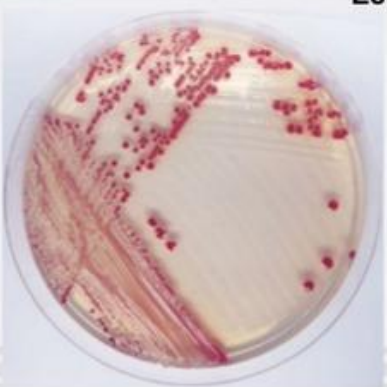
- CHROM Agar
- Leeds Acinetobacter Agar

7



MacConky Agar

Leeds Acinetobacter Agar



CHROM Agar

8

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Biochemical Reactions

- **Oxidase negative** (opposite to *Neisseria spp.* or *Moraxella spp.*)
- Haemolytic
- **Indole negative.**
- **Catalase positive.**

Biochemical Profile

- Both *A.baumennii* and *A.lwofii* are Catalase positive and Oxidase Negative.
- *A.baumennii* ferment glucose, xylose and lactose but *A.lwofii* cannot ferment.

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Molecular Detection

- *A.baumennii* and *A.lwofii* can be detected by PCR.
- *recA* specific primers are used to detect *recA* gene in *A.baumennii*, giving a 382 bp fragment
- *est* specific primers are used to detect *est* gene in *A.lwofii*, giving a 309 bp product.

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Pathogenesis

- Opportunistic pathogen…
- Survive under dry conditions

Virulence Factors

- Polysaccharide capsule, prevent complement activation, delay phagocytosis
- Fimbriae (adhere to human bronchial epithelium)
- Pilli (colonization of environmental surface to form biofilms)

12

Transmission

- *Acinetobacter* can be spread from person to person (infected or colonized patients), contact with contaminated surfaces of exposure to the environment.

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Antibiotic Resistance

- *Acinetobacter* species are capable of accumulating multiple antibiotic resistance genes, leading to the development of multidrug-resistant or even panresistant strains.

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