



# Pseudomonas



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# What are Pseudomonas

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- Family Pseudomonadaceae
  - Aerobic, non-spore forming Gram negative straight or slightly curved rod (1 to 3  $\mu\text{m}$  in length)
  - Motile with polar flagella
  - **Non-fermenters**
  - **Catalase and oxidase positive**
  - Pigment producing bacteria.
  - Mostly causes Hospital acquired infection.
  - Opportunistic pathogens, majorly found in soil, water and sewage.
  - They are highly resistant to chemical disinfectants, antibiotics.

- The most important pseudomonad species responsible for human infections are **Pseudomonas aeruginosa**, **Burkholderia pseudo mallei** and members of the **Burkholderia cepacia complex**.

# Pseudomonas aeruginosa

## • Morphology

- They are slender gram negative bacillus, 1.5 – 3 microbes x 0.5 microns
- Actively motile by polar flagella
- some are pilated
- Non capsulated but many strains have mucoid slime layer.

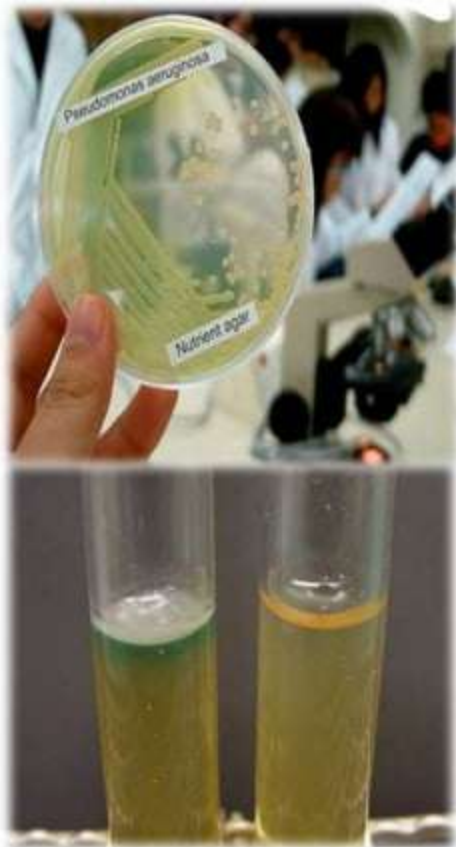


# Cultural Characters

- **Obligate aerobe**
- Growth occurs at wide range of temperatures 6-42°C the optimum being 37°C
- Growth on ordinary media producing large opaque irregular colonies with earthy smell.
- In broth forms dense turbidity with surface pellicle.

## • Nutrient agar-

- Colonies are smooth, large, translucent, low convex, 2-4mm in diameter.
- Produce sweetish aromatic odor
- Greenish blue pigment diffuses



# Growth on

- **Blood agar**

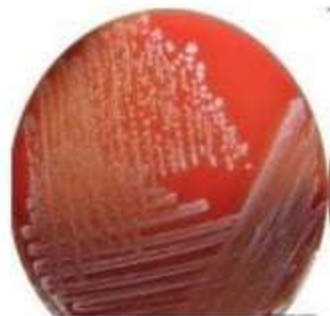
- Similar to nutrient agar
- Many are haemolytic

- **Mac conkey agar**

- Colourless, non lactose fermenters

- **Cetrimide agar**

- selective media



Blood agar



Non lactose fermenting colonies  
on MacConkey agar



Cetrimide agar



*P. aeruginosa*



Muroid *P. aeruginosa*

MacConkey Agar



# Pigment production

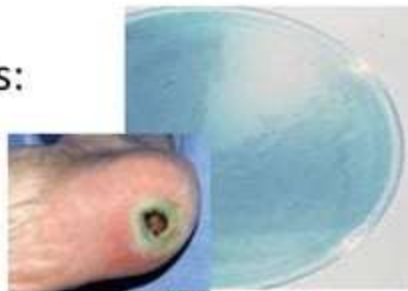
Some strains produce diffusible pigments:

- **Pyocyanin**

- Bluish green phenazine pigment
- Soluble in chloroform and water
- Not produced by other species
- It is diagnostic of *Pseudomonas aeruginosa*

- **Pyoverdin(fluorescein)**

- It is a greenish yellow pigment
- Insoluble in chloroform but soluble in water
- Produced by many other species



Pyocyanin



Pyoverdin

- **Pyorubin**

- Reddish brown pigment
- Insoluble in chloroform but soluble in water

- **Pyomelanin**

- Brown to black pigment
- Production is uncommon

# Biochemical reactions

Catalase test



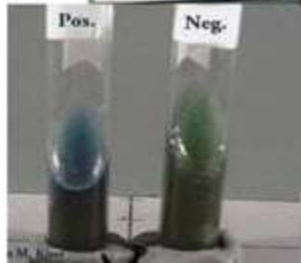
- O/F test-oxidative
- **Catalase-positive**
- Indole, MR and VP and H<sub>2</sub>S tests are negative
- **Oxidase-positive**
- **Nitrate reduction-positive**
- **Citrate test-positive**



Oxidase test



Citrate test



# Resistance

- Killed at 55°C in 1 hour
- High resistance to chemical agents
- Resistance to quaternary ammonium compounds.  
Chlorxylenol
- Grows also in antiseptic bottles
- Dettol and Cetrimide as selective medium(**Cetrimide agar**)
- Sensitive to acids silver salts, (Uses as tropical cream in burns.)
- Intrinsically resistant to commonly used antibiotics.

# Pathogenesis and Immunity

- Common cause of hospital acquired infection.
- *P. aeruginosa* can infect almost any external site or organ.
- *P. aeruginosa* is invasive and toxigenic. It attaches to and colonizes the mucous membrane or skin, invade locally, and produces systemic diseases and septicemia.
- *P. aeruginosa* is resistant to many antibiotics. It becomes dominant when more susceptible bacteria of the normal flora are suppressed.

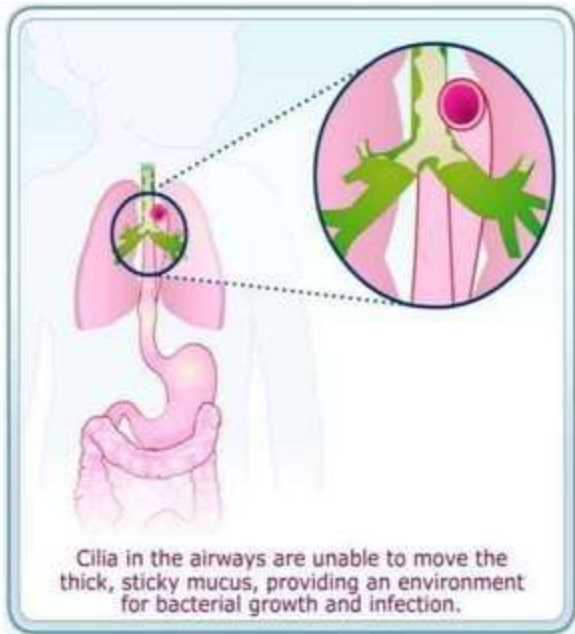
## *P. aeruginosa* is an opportunistic pathogen

- Extremely broad host spectrum
- Hardly any infections in the normal human host
- Severe immunodeficiencies and medical devices predispose the patients to *P. aeruginosa* infections
- Broad spectrum of clinical symptoms
  - Urinary tract infections
  - Pulmonary infections
  - Soft tissue infections
  - Sepsis
  - Bone and joint infections
  - Endocarditis



# Who are more susceptible to infection

- This bacterium is of particular concern to individuals with cystic fibrosis who are highly susceptible to pseudomonas lung infections.
- Pseudomonas aeruginosa is also of grave concern to cancer and burn patients as well as those people who are immunocompromised.
- The case fatality rate for individuals infected with Pseudomonas aeruginosa approaches 50 percent.



# Laboratory diagnosis

## ***Specimens:***

- Wound discharge
- sputum
- Blood
- Urine
- CSF
- Pus





# Culture

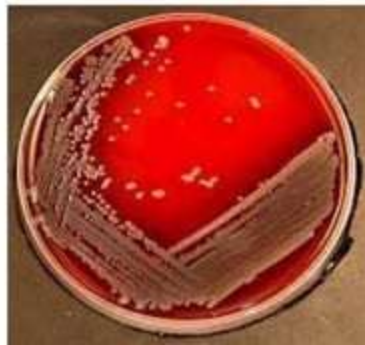
## On nutrient agar

- Colonies are smooth, large, translucent
- Greenish blue diffusible pigment



## On blood agar

- Grayish colonies
- Many are haemolytic (beta hemolysis).



## On MacConkey agar

- Non-lactose fermenting (colourless colonies)



# Pigment production on Cetrimide agar

- is a type of agar used for the selective isolation of the gram-negative bacterium, *Pseudomonas aeruginosa*.
- As the name suggests, it contains cetrimide, which is the selective agent against alternate microbial



*Pseudomonas aeruginosa*  
fluorescence under UV illumination



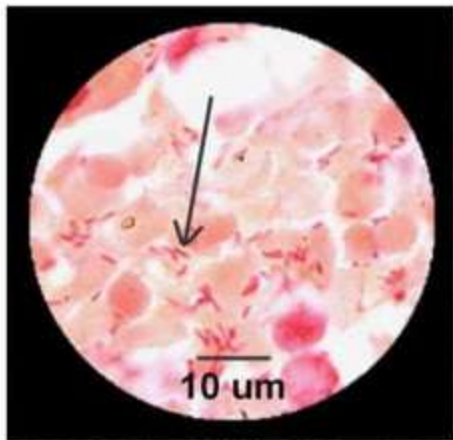
# Microscopy

## -Gram staining

Gram negative bacili seen.

## -Hanging drop preparation:

Actively Motile



# Biochemical Reactions

## Oxidase test

- Oxidase positive

## Catalase test

- Catalase positive

## Automated methods

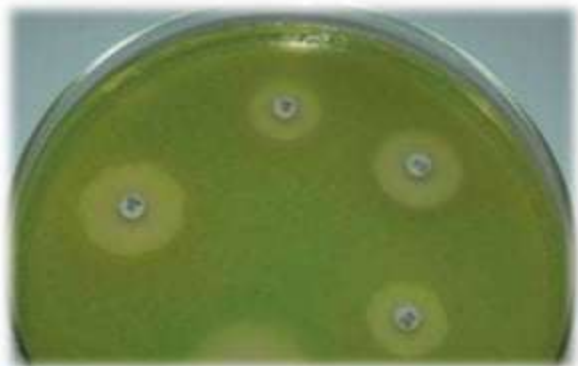
VITEK test: for identification and antibiotic sensitivity of bacteria

## Molecular methods

The bacterial genome can be identified by PCR.

# Antibiotic sensitivity testing

Helpful to select out appropriate antibiotic for treatment.



# Treatment of *P. aeruginosa* infections

- *P. aeruginosa* is frequently resistant to many commonly used antibiotics.
- Although many strains are susceptible to **gentamicin, tobramycin, colistin, and amikacin**, resistant forms have developed.
- The combination of **gentamicin and carbenicillin** is frequently used to treat severe Pseudomonas infections.
- No vaccines so far

## Prevention and Control

*Pseudomonas* spp. normally inhabit soil, water, and vegetation and can be isolated from the skin, throat, and stool of healthy persons.

Spread is mainly via contaminated sterile equipment's and cross-contamination of patients by medical personnel.



## Prevention and Control

- High risk population: patients receiving broad-spectrum antibiotics, with leukaemia, burns, cystic fibrosis, and immunosuppression.
- Methods for control of infection are similar to those for other nosocomial pathogens. Special attention should be paid to sinks, water baths, showers, hot tubs, and other wet areas.

## Other *Pseudomonas*

- Clinically relevant *pseudomonas* in increasing percentage are:
  - *Pseudomonas putida*
  - *Pseudomonas stutzeri*
  - *Pseudomonas fluorescens*