Pseudomonas sp. &
Burkholderia sp.

General features

- Pseudomonas sp. are medium sized (0.5-1×1.5-5µm)
- Gram negative rods
- Strict aerobes, catalase & oxidase positive
- Mostly motile by one or several polar flagella except P. mallei (non motile)

Natural Habitat

- Exclusively saprophytes including *P. aeruginosa* which is animal importance
- Present in soil, water, skin mucous membrane and in faeces of animals
- *P flourescens* present in soil and water cause food spoilage and lesions in reptiles and fish
- *P folliculitis* is mostly found in swimming pool water and cause skin disease in humans

Pathogenesis

- *P. aeruginosa* produce number of protein enterotoxin that are responsible for diarrhea during initial infections
- Endotoxin and numerous extracellular products like protease and haemolysin also play role in pathogenesis
- *P aeruginosa* possess pili which facilitate adherence to epithelial cells
- Some strains have capsule that is antiphagocytic

- Bacteriocins (pyocins) and pigments exhibit antimicrobial activities
- Blue green pigment can colour pus and stain wool a greenish hue
- *P aeruginosa* is opportunistic and so rarely involved in primary disease
- Predisposing factors include debilitation due to malignancy or immunodeficiency prolonged antibiotic treatment

Diseases associated with pathogenic *P. aeruginosa*

Species	Host(s)	Disease
P. aeruginosa	Cattle	Mastitis, uterine infections, skin infections, abscesses, enteritis and arthritis
	Sheep and goats	Mastitis, pneumonia, lung abscesses and 'green wool' (a skin infection in sheep)
	Pigs	Enteritis, respiratory infections and otitis
	Horses	Metritis, lung abscesses and eye infections
	Dogs and cats	Otitis externa, cystitis, endocarditis, dermatitis, wound infections and conjunctivitis
	Mink	Septicaemia and pneumonia
	Chinchilla	Generalised infection with conjunctivitis, otitis, pneumonia, enteritis and infection of the genital organs
	Reptiles	Necrotic stomatitis and other necrotic lesions, especially in captive snakes
	Many animal species	Infection of burns and other wounds, diarrhoea, genital and nosocomial infections

Lab diagnosis

- **Specimen**
- Variable and depend on clinical signs and lesion site

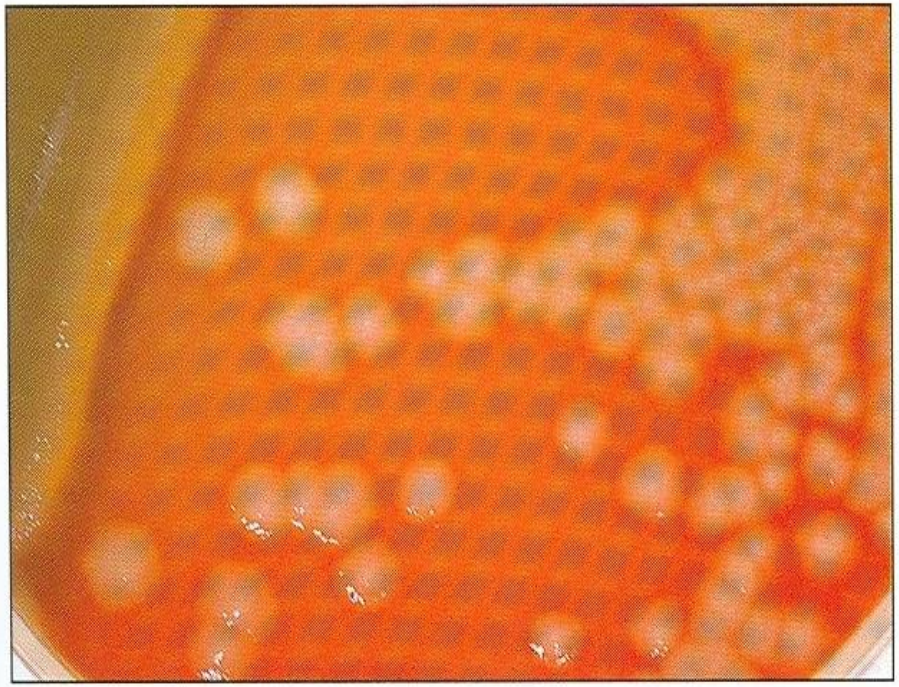
- **Direct microscopy**
- Little importance as pseudomonads are medium sized, Gram negative rods with no distinctive feature

- **Isolation**
- Non fastidious
- Grow on trypticase soy agar, blood agar or less complex media
- Commercial selective media available that contain 0.03% cetrimide
- *P. aeruginosa* grow well on MacConkey, Brilliant Green agar and XLD
- Cultures of pseudomonas are incubated at 37 C aerobically fro 24 – 48 hrs

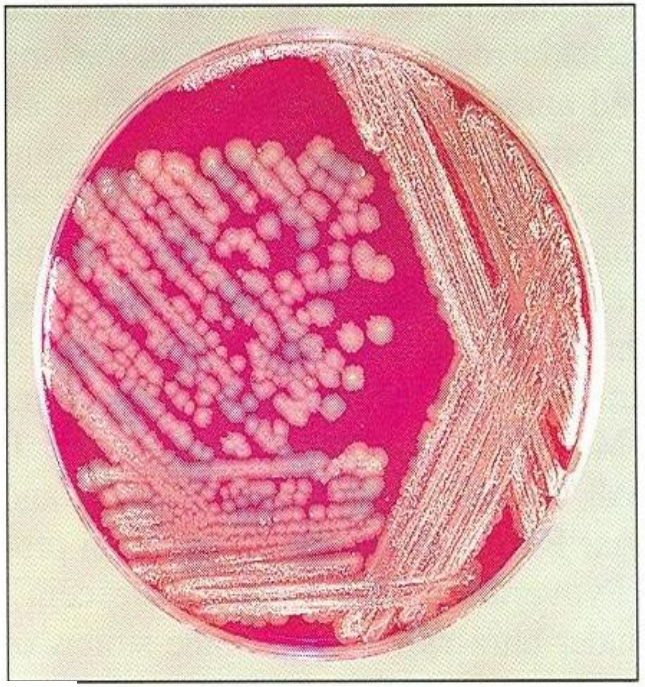
- **Identification**
- Colonies are large (3-4 mm)
- Flat, greyish-blue with characteristic fruity, grape like odour of aminoacetophenone
- Most strains give clear zone of haemolysis on Blood agar
- Pyocyanin, a bluish pigment unique of *P. aeruginosa*

- Colonial variation includes S-form (soft and shiny)
- R-form (dry and granular)
- M-form (mucoid)
- Some colonies have metallic sheen

- P aeruginosa produces large, pale colonies on MacConkey agar (unable to utilise lactose) with greenish-blue pigment
- Red colonies and medium indicative of an alkaline reaction
- No Hydrogen sulphidenproduced on XLD medium



Pseudomonas aeruginosa on sheep blood agar snowing large, flat, irregular-edged colonies resembling those of some *Bacillus* species. The green-blue pyocyanin pigment is most obvious in areas of heaviest growth.

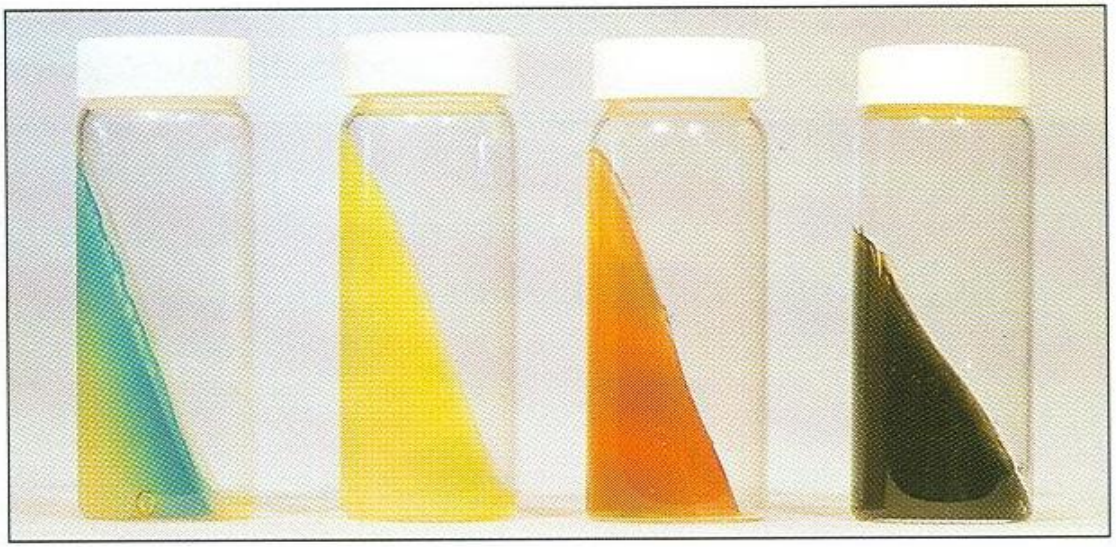


P. aeruginosa on brilliant green agar where the alkaline reaction is similar to that given by *Salmonella* species. The metallic sheen displayed by this strain is a feature of some isolates.

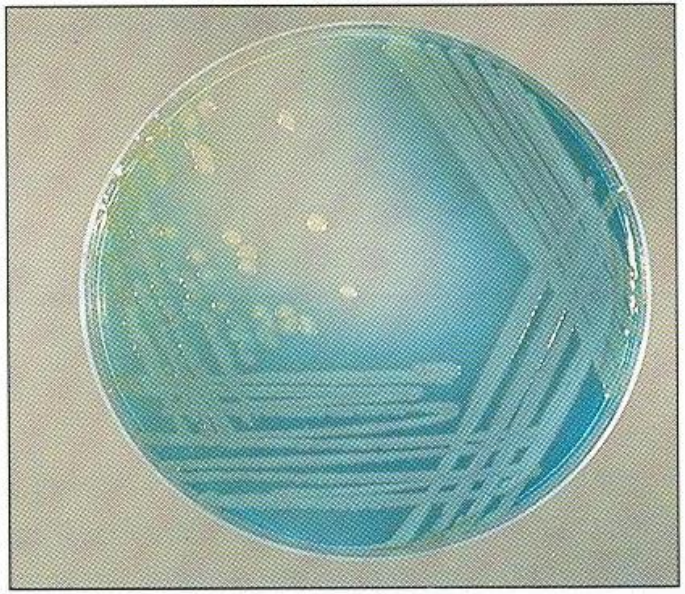
P. aeruginosa on MacConkey agar. It has the pale colonies of a non-lactose-fermenter with green-blue, pyocyanin pigment superimposed.

Pigments of *Pseudomonas*

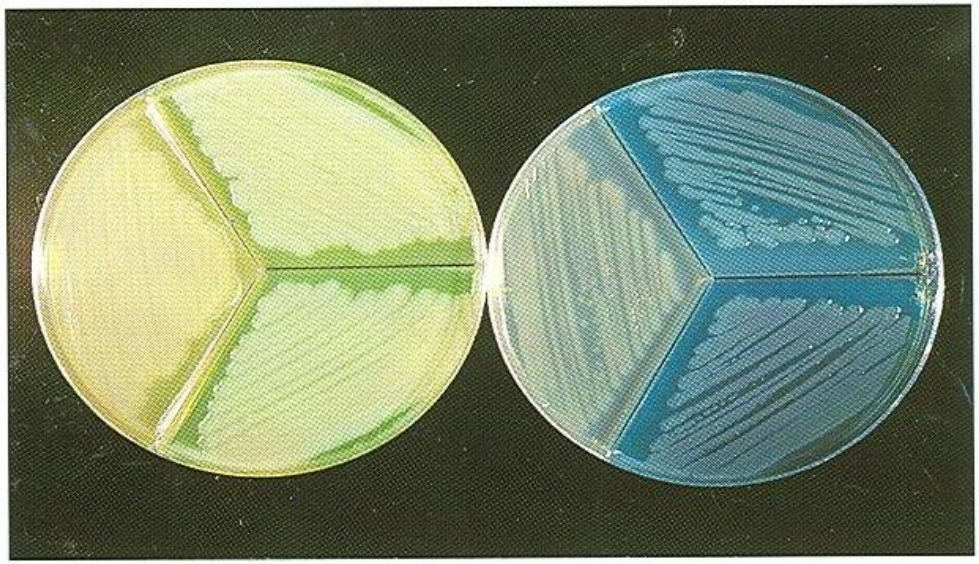
- *P. aeruginosa* produce diffusible four pigments
  - Pyocyanin (greenish Blue)
  - Pyoverdin (Yellow)
  - Pyorubin (red)
  - Pyomelanin (dark brown)
- Pyorubin and pyomelanin less commonly produced, develop slowly.
- Pyocyanin is unique in *P. aeruginosa* (diagnostic importance)
- Pseudomonas agar F enhances pyoverdin production
- Pyoverdin fluoresce under UV light.



Pigments produced by *P. aeruginosa*. The nutrient agar slopes show from left: pyocyanin (blue-green), pyoverdin (greenish-yellow), pyorubin (red) and pyomelanin (dark brown).



304 *P. aeruginosa* on 'Pseudomonas agar P'. This medium enhances the production of pyocyanin.



305 Three strains of *P. aeruginosa* on 'Pseudomonas agar F' (left) and on 'Pseudomonas agar P' (right). These media are used to enhance pigment production.

- **Microscopic appearance**
- All pseudomonads are medium sized Gram negative rods

- **Biochemical characteristics**
- Pyocyanin production , odour, a strong oxidase reaction, sugar fermentation tests and nitrate reduction test

Main characteristics of Pathogenic *Pseudomonas aeruginosa*

Characteristic	*P. aeruginosa*
Pigment produced	++
Odour	'fruity', grape-like
Growth on MacConkey agar	+
Growth at 5°C	–
Growth at 42°C	+
Oxidation of: glucose	+
lactose	–
Arginine dihydrolase	+
Reduction of nitrate to nitrite	+
Reduction of nitrate to N_2 gas	v
Motility	+

Differentiation of *Pseudomonas aerugionosa* and other saprophytic pseudomonads

	Growth on MacConkey agar	Pyoverdin produced	Oxidase	Growth at 5°C	Growth at 42°C	Gelatin	Urease	Oxidation of Glu.	Oxidation of Lact.	Oxidation of Malt.	Arginine dihydro-lase
P. aeruginosa	+	+	+	–	+	+	+	+	–	–	+
P. fluorescens	+	+	+	+	–	+	(+)	+	–	–	+
P. putida	+	+	+	(+)	–	–	(+)	+	–	(–)	+
P. cepacia	+	–*	+	–	(+)	+	+	+	+	+	–
P. maltophilia	+	–	(–)	–	–	+	–	(–)	–	+	–
P. stutzeri	+	–	+	(+)	(+)	–	(+)	+	–	(+)	(+)

+ = positive reaction, (+) = most strains positive, (–) = most strains negative, – = negative reaction, * = some strains produce a yellowish non-fluorescent pigment, Glu. = glucose, Lact. = lactose, Malt. = maltose.

Burkholderia sp

- Previously refered as Pseudomonas
- Two species of veterinary importance
  - *B. malei*
  - *B. pseudomalei*

- **Pathogenesis**
- B. pseudomalei wide host range including humans and cause melioidosisTor pseudoglanders.

- Infection usually systemic and manifestation depend on extent and distribution of lesion

- Lesion are nodules

- Suppuration can occur in any organ
- Most infections are chronic but acute disease may occur

- Toxin include a lethal factor with anticoagulant activity and skin necrotising proteolytic agent
- Meliodosis occurs in tropical regions between 20 C northern and southern lattitudes
- P mallei causes glanders or farcy in the equidae

- Humans and members of cat family are susceptible and occasional infections in dog, goat, sheep and camels
- Cattle, pigs, rats and birds are resistant
- Transmission occurs from infected animals via contaminated food and water and less commonly from aerosols and infection of wounds

- Toxins are suspected in pathogenesis
- But mode of action uncertain

- Primary lesion is at portal of entry with dissemination via the lymphatic system and bloodstream

- Disease can be acute or chronic and many infections are fatal

- Infection characterised by formation of tubercle like nodules that ulcerate

Diseases and main hosts of *Burkholderia sp*

Species	Host	Disease
P. pseudomallei	Many animal species	Melioidosis (pseudoglanders):
	Horses	The disease can mimic glanders
	Cattle	Acute and chronic forms with localisation of lesions in lungs, joints and uterus
	Sheep	Arthritis and lymphangitis predominate
	Goats	Loss of condition, respiratory and central nervous disturbances, arthritis and mastitis
	Pigs	As for goats but in addition diarrhoea and abortion
	Dogs	Febrile disease with localising suppurative foci
P. mallei	Horses and other equids	Glanders: acute form with high fever, mucopurulent nasal discharge, respiratory signs, septicaemia and death within 2 weeks
		Chronic forms of glanders • Pulmonary: small nodules in lungs that break down and discharge *P. mallei* into the bronchioles • Cutaneous form: Farcy, which is a lymphangitis with ulcers along lymphatic vessels of the limbs and chest. The ulcers eventually heal leaving 'star-shaped' scars
	Humans, cats and other animals	Acute, septicaemic disease

- Glanders had wide geographical distribution but now is seen in China and Mongolia with pockets of infection in India, Iraq, the Phillippines and Eastern Europe

- **Laboratory Diagnosis**

- P mallei and P pseudomallei are among the most dangerous bacteria to work in a Lab.

- A biohazard cabinet must be used and all necessary safety procedures taken

- **Direct Microscopy**
- Flourescent antibody technique can be useful for P. mallei and P. pseudomallei

- **Isolation**
- Trypticase soy agar, blood agar

- Growth enhanced by 1 % glycerol

- Selective media for P. malei contain 1000 units of Polymyxine, 1250 units bacitracin and 0.25 mg actidione to 100 ml of trypticase soy agar

- **Identification**
- ***P. pseudomallei*** –
- Colonial growth varies from smooth and mucoid to rough with a dull, wrinkled, corrugated surface.
- Smooth form-colonies are round, low convex, entire, shiny and greyish-yellow
- After several days, colonies become opaque, yellowish-brown and umbonate
- Growth has characteristic earthy or musty odour
- Partial and later complete haemolysis occurs on sheep blood agar

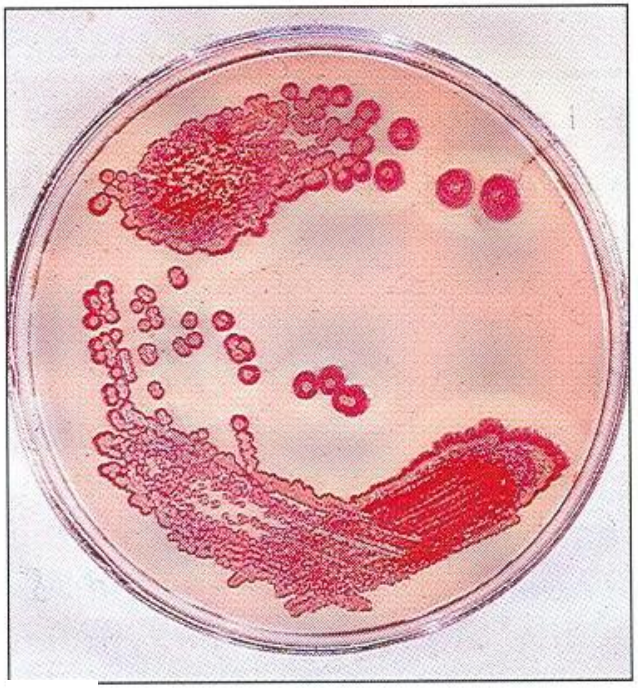
- *P. pseudomallei* grows on MacConkey agar utilising lactose but there is no growth on Deoxycholate or Salmonella-Shigella agar

- ***P. mallei***
- Growth is slower than that of P. aeruginosa and P. pseudomallei

- After 24-48 hrs the colonies are 1-2 mm in diameter, smooth and white to yellowish or brown in colour

- Unable to grow on MacConkey

Pseudomonas pseudomallei: smooth colonial form on sheep blood agar after several days' incubation. The colonies are smooth, glistening, opaque, yellowish-brown and umbonate with a zone of clear haemolysis.



P. pseudomallei on MacConkey agar (*P. mallei* does not grow on this medium).

Main characteristics of pathogenic *Burkholderia sp.*

Characteristic	*P. pseudomallei*	*P. mallei*
Pigment produced	– but colonies become orange to cream	– but colonies are yellow to brown
Odour	putrid becoming earthy	–
Growth on MacConkey agar	+	–
Growth at 5°C	–	–
Growth at 42°C	+	–
Oxidation of: glucose	+	+
lactose	+	–
Arginine dihydrolase	+	(+)
Reduction of nitrate to nitrite	+	+
Reduction of nitrate to N_2 gas	+	–
Motility	+	–

- **Immunological tests**

- **Meliodosis**
- Complement fixing and indirect haemagglutination

- **Glanders**
- Complement fixation, agglutination, indirect haemagglutination and counter immunoelectrophoresis tests

The end