

PSEUDOMONAS

Learning objectives

After reading this chapter, the learner will understand the following,

- Diseases produced by the pseudomonas in domestic animals
- Define white more's bacillus, Glanders, Farcy and fleece rot
- Morphology, cultural and biochemical characters of pseudomonas species
- Pigments produced by the strains of *Pseudomonas aeruginosa*
- Antigens, toxins and pathogenesis of *Pseudomonas*
- Strauss reaction and Mallein test
- General approaches used to isolate and identify *Pseudomonas* species

SYSTEMATICS

- The *Pseudomonas* is Gram –ve, aerobic, medium-sized rods, motile by one or several polar flagella (except *P.mallei*).
- Catalase and oxidase positive and some species produce water-soluble pigments and most will grow on MacConkey agar.

Domain	Bacteria
Phylum	<i>Proteobacteria</i>
Class	<i>Gamma proteobacteria</i>
Order	<i>Pseudomonadales</i>
Family	<i>Pseudomonadaceae</i>
Genus	<i>Pseudomonas</i>

HISTORY AND HABITAT

History

- *P. aeruginosa* was first isolated by Gessary 1882
- *P. mallei* was discovered by Loeffler and Schutz –1882
- *P.pseudomallei* was first described by Whitmore and Krishnaswamy 1912

Habitat

- Most of the species in this genus are saprophytes, including *P.aeruginosa* and *P.pseudomallei*.
- The *P.aeruginosa* is ubiquitous in the environment and is found in water, soil and on plants, as well as on skin and mucous membrane of healthy animals.
- The *P.pseudomallei* mainly is present in the tropics and it is considered to be a soil organism.
- Wild rodents act as important reservoir of this organism. *P.mallei* is an obligate parasite.

MORPHOLOGY

- Gram negative, rod shaped bacteria. They have parallel sides and rounded ends.
- They are arranged singly, in small bundles, in short chains or in filaments.
- All except *P.mallei* are motile by means of polar flagella at one or both ends.
- Some strains are lopotrichate and some are monotrichate. *P.pseudomallei* is peritrichous.
- Non spore forming and non acid fast.
- It is non capsulated but when grown in the absence of sucrose an extracellular polysaccharide slime layer may be formed.
- Most of the strains possess fimbriae.

CULTURAL CHARACTERISTICS

- Very strict aerobes, growth occurs at wide range of temperature 5-42°C.
- *Pseudomonas* species are non fastidious and will grown on TSA, blood agar and on less complex media.
- Agar that contains cetrimide is the selective medium for *P.aeruginosa*.
- On nutrient agar, the colonies are grayish blue with a characteristic fruity, grape like odour (amino acetophenone) and gives different types of colonies (Smooth form, Muroid form and rough form). Some strains have colonies with distinct metallic sheen.

- The strains of *P.aeruginosa* produce diffusible pigments
 - Pyocyanin – bluish green in color, which dissolves in Chloroform
 - Pyoverdin – greenish yellow in color which is soluble in water but not in chloroform (Fluorescin)
 - Pyorubin – red
 - Pyomelanin – dark brown to black pigment
- Some strains produce all 4 pigments, pyorubin and pyomelanin are less commonly produced.
- The pigment production is best seen by growing the strains on nutrient agar slants at room temperature for up to two weeks.
- Pyocyanin-, which is unique to *P.aeruginosa*, gives the blue color associated with many cultures.
- Most strains give a clear zone of haemolysis on blood agar.
- On MacConkey agar, *P.aeruginosa* produce (non-lactose fermenter) pale colonies with greenish blue pigment superimposed.
- They also grow on Brilliant green agar and XLD medium.
- *P.pseudomallei* colonial growth varies from smooth and mucoid, to rough form with a dull, wrinkled, corrugated, honeycomb surface.
- In smooth form the colonies are shiny and grayish yellow. After several days the colonies become yellowish brown and umbonate.
- The growth has a characteristic earthy or musty odour due to the production of ammonia.
- Partial and later complete haemolysis is observed on sheep blood agar.
- It is a lactose fermenter on MacConkey agar but there is no growth on deoxycholate or salmonella shigella (SS) agar.
- *P.mallei* growth is very slow; the colonies are smooth, and white to cream. *P.mallei* cannot be grown on MacConkey agar.

BIOCHEMICAL PROPERTIES, ANTIGENS AND TOXINS

Biochemical properties

- In addition to pigment production (*P.aeruginosa* only) and characteristic odour the pseudomonads are strongly oxidase positive, reduce nitrate to nitrite, liquefy gelatin, IMViC -, -, -, +.
- All ferment glucose and produce less acid. *P.pseudomallei* ferments lactose. OF test is positive for *P.aeruginosa*.

Antigens and toxins

- The *Pseudomonas* is highly heterogenous. Serotyping, immunotyping, pyocin typing and phage typing are all used for characterization of isolates.
- Based on somatic antigens 17 serotypes have been described in *P.aeruginosa*.
- *P.aeruginosa* produces numerous extracellular toxins and enzymes. Those that may play role in pathogenesis are fibrinolysin, elastase, lecithinase, lipase, protease, haemolysin (heat stable and heat labile), leucocidin, alginate, phospholipase C, esterase and exotoxin A.
- In addition to this, the pigment produced by *P.aeruginosa* exhibits antimicrobial activities against a wide range of bacteria and some fungi. *P.pseudomallei* produces very potent exotoxin (similar to exotoxin A), protease, lipase and a lecithinase.
- No exotoxin has been described in *Pseudomonas mallei*.

RESISTANCE

- They are more resistant to high dilutions of quaternary ammonium compounds and phenolic compounds.
- *P.aeruginosa* can survive for long periods on water faucets, utensils, floors, instruments, baths, humidifiers and respiratory equipments.
- They grow very well in antiseptic lotions kept for use in hospitals. *Pseudomonas* is susceptible to ethylene oxide and heat (55°C for one hour).
- Pseudomonads are resistant to wide range of antimicrobial drugs.
- Much of this intrinsic resistance is attributed to the outer membrane porins, which restrict passage of many antimicrobial agents into the periplasm.
- Resistance to penicillin, Ampicillin, tetracycline, first and second-generation cephalosporin, sulfonamides, neomycin, streptomycin, kanamycin, chloramphenicol, nitrofurantoin and trimethoprim-sulfonamide.
- Susceptible to gentamicin, amikacin, colistin, Polymyxin B, carbenicillin, cefoxime, third generation cephalosporins and Ciprofloxacin

PATHOGENESIS

- In *P.aeruginosa* the source of infection may be either endogenous or exogenous.
- Some defect in local defense or generalized defense is necessary for this opportunistic pathogen to cause disease.
 - *P.aeruginosa* + Breach in host defense
 - (Ubiquitous in environment)
 - Adhere
 - (Pili, exoenzymes)
 - Multiply
 - Antiphagocytic LPS and slime
 - Iron scavenging system (Pyocyanin and Pyoverdine)
 - Resistance to killing by serum
 - Produce toxins
 - (Exotoxin A, Elastase and Protease)
 - Tissue damage due to toxins
 - Tissue damage due to immune complex mechanism
 - (Acute disease) (Chronic disease)
 - Elimination of bacteria Persistence of bacteria
- In *P.pseudomallei*, the animal gets infections mainly occurring as a result of ingestion, inhalation or skin contamination. The pathogenesis of melioidosis is not known.
- In *P.mallei* (Cattle, swine, birds and rats etc are resistant to infection) equidae gets infections most commonly by ingestion and also by inhalation and skin contamination.
- After ingestion, there is invasion of the gut wall and a subsequent septicaemia.
- The endotoxin plays a major role. Inhalation usually leads to a bronchopneumonia and lesions in the nasal mucosa. Infections of abraded skin leads to Farcy.
- Glanders is a contagious, usually chronic disease of horse, characterized by the formation of tubercle like nodules (granulomas) that frequently break down to form ulcers.

Diseases produced by the Pseudomonads

Species	Host	Disease
<i>P. aeruginosa</i>	<ul style="list-style-type: none"> • All species • Dogs and cats • Cattle • Horses • Pigs • Sheep 	<ul style="list-style-type: none"> • Wound infections • Otitis externa, keratitis, dermatitis and cystitis • Mastitis, enteritis and arthritis • Ulcerative keratitis, metritis and abortion • Enteritis and respiratory infection

		<ul style="list-style-type: none"> • Mastitis, pneumonia, green wool (Fleece rot/Skin infection)
<i>P.pseudomallei</i> (Whitemore's bacillus)	Horses, cattle, sheep, goats, pigs and dogs	Melioidosis (Pseudoglanders)
<i>P. mallei</i> (<i>Malleomyces mallei</i> or <i>Loefflerella mallei</i> or <i>Pfeiferella mallei</i>)	Horses, mules and donkeys	Glanders Farcy (Cutaneous glanders)

PATHOGENECITY

Symptoms and lesions

- Depends upon the degree and site, the *P.aeruginosa* produces wide variety of suppurative infections.
- In localized lesions there will be yellowish green pus.
- The acute form of *P.pseudomallei* occurs more often in young animals.
- The chronic form is characterized by nodules in the lungs, liver, spleen, lymphnodes and subcutis, the lesions resembling those of caseous lymphadenitis and numerous visceral abscesses.
- Three forms of glanders are seen

in horse. They are classified as

Pulmonary form: rise in temperature, coughing with blood stained mucosa.

Nasal form

- Nasal form initially there is reddening of the nasal mucosa and mucoid discharge from nostrils.
- It will become purulent, blood stained and adherent as brown crust. Ulcers may form on the nasal mucosa.

Cutaneous form: (Farcy):

- Development of small cutaneous or subcutaneous nodules in the limbs and flanks.
- These nodules develop into hollow ulcers exuding yellow or oily pus.
- The local lymphatic vessels become corded and tubercle like nodules are seen in lungs and other visceral organs.

DIAGNOSIS

Specimens

- Urine, pus, affected tissues and swab from infected tissue surfaces are suitable for *P.aeruginosa*.
- In case of *P.mallei*, collect tissue containing early nodules or pus from ulcers.

Diagnosis

- Based on symptoms and lesions
- Based on pigment production
- Based on cultural and Biochemical characters
- Based on animal inoculation
 - **Straus reaction**
 - The Straus reaction is seen in male guinea pigs inoculated intra peritoneally with infective material containing either *P.pseudomallei* or *P.mallei*.
 - The reactions of swelling of the testes, inflammation of the tunica vaginalis and ulceration of the scrotal skin develops in 2-3 days. (Not confirmatory one.
 - Because Straus reaction is also produced by *Brucella*, *Preisz-nocard bacillus*, and *Actinobacillus ligniersii*)
- Mallein test
 - This is used to demonstrate the hypersensitivity developed after infection with *P.mallei*.
 - Mallein is a glycoprotein extracted from the bacterium.

- **Subcutaneous test:** swelling at the injection site and fever.
 - **Ophthalmic test:** Instilled on conjunctival sac. Inflammatory and purulent reaction occurs within 6-12 hrs.
 - **Intrapalpebral:** Inoculate at skin of the lower eyelid. Localized, oedematous swelling and purulent conjunctivitis
- Serological tests
 - CFT, Agglutination, IHA, and CIE are used in the diagnosis of glanders.
 - CFT and IHA are useful in melioidosis.