Pseudomonas aeruginosa and *Burkholderia* species

Characteristics:-

- *Pseudomonas aeruginosa, Burkholderia mallei* and *B. pseudomallei* are Gram-negative rods (0.5 to 1.0 x 1 to *5* µm) (Fig. 1), which are obligate aerobes and oxidase carbohydrates.
- Most isolates are oxidase-positive and catalase positive.
- They are motile by one or more polar flagella, with the exception of *B. mallei* which is non-motile.
- The majority of these organisms have no special growth requirements and grow well on MacConkey agar.
- *Burkholderia mallei* requires 1% glycerol in media for optimal growth.
- *Pseudomonas aeruginosa,* characterized by the production of diffusible pigments, causes a variety of opportunistic infections in a wide range of animals.
- A number of other *Pseudomonas* species may be isolated from clinical specimens.
- *Pseudomonas fluorescence* and *P. putida* occasionally infect fresh water fish.
- *Burkholderia* species, previously classified in the genus *Pseudomonas,* include *B. mallei,* the causes of glanders and *B. pseudomallei,* the causes of melioidosis. Both diseases are zoonosis.

Fig. 1: Pseudomonas aeruginosa in Gram stained smear and Scanning Electron Micrograph

Usual habitat

Pseudomonas species *are* environmental organisms which occur worldwide in water and soil, and on plants. Pseudomonas *aeruginosa* is also found on the skin, on mucous membranes and in feces. *Burkholderia pseudomallei,* which is found in soils, occasionally infects animals and man. Wild rodents can act as reservoirs of this organism. It is widely distributed in some tropical and subtropical regions of Southeast Asia and Australia.

Although *B. mallei* can survive in the environment for up to 6 weeks, its reservoir is infected *Equidae.*

Differentiation of *Pseudomonas* and *Burkholderia* species:-

1-The comparative colonial and biochemical features of these organisms are presented in Table 1.

2- Many *Pseudomonas* species produce pigments. *Pseudomonas aeruginosa* strains can form up to four diffusible pigments (Table 2). Pyocyanin, unique to this organism, is produced by most strains and specifically identifies *P. aeruginosa.* Pyocyanin-enhancing media are available for isolates which are weak pyocyanin producers. Pigment production is observed most dearly on media without dyes such as nutrient agar. Pyorubin and pyomelanin develop slowly and may be detectable only after incubation for 1 to 2 weeks (fig. 2).

3- Colonies of *B. pseudomallei* and *B. mallei* become brownish with *age* but do not produce pigments. The majority of *Pseudomonas* and *Burkholderia* species are motile. Absence of motility distinguishes B*. ma*llei from other members of the group.

Table1*:* Comparative **features of *Pseudomonas aeruginosa, Burkholderia* mallei and *Burkholderia pseudomallei.***

Feature	***P. aeruginosa***	***B. mallei***	***B. pseudomallei***
Colonial morphology	Large and flat with serrated edges	White and smooth becoming granular and brown with age	Ranges from smooth and mucoid to rough and dull becoming yellowish brown with age.
Haemolysis on blood agar	+	-	-
Diffusible pigment production	+	-	-
Colony odour	grape like	none	musty
Growth on MacConkey agar	+	+	+
Growth at 42°C	+	-	+
Motility	+	-	+
Oxidase production	+	-	+
Oxidation of:			
Glucose	+	+	+
lactose	-	-	+
sucrose	-	-	+

Table 2: Pigments produced by *Pseudomonas aeruginosa.*

1-Pyocyanin (blue green)

2-Pyoverdin (greenish-yellow)

3-Pyorubin (red)

4- Pyomelanin (brownish-black)

Fig. 2: *Pseudomonas aeruginosa* pigment on nutrient agar

Clinical infections

Burkholderia mallei pathogen of *Equidae***,** causes both acute and chronic disease. It manifests mainly as lesions in the skin and the respiratory tract, Infection with ***B***. ***pseudomallei*** can cause chronic suppurative lesions in the lungs and other organs of a wide range of species. In contrast, ***P. aeruginosa*** is an opportunistic pathogen which may occasionally cause acute systemic disease.

Pseudomonas aeruginosa **infections:-** (fig. 3)

a- Cattle: Mastitis, metritis, pneumonia, dermatitis, enteritis (calves)
b- Sheep: Mastitis, fleece-rot, pneumonia, otitis
c- Horses: Genital tract infections, pneumonia, ulcerative keratitis
d- Dogs, cats: Otitis externa, cystitis, pneumonia, ulcerative keratitis.

Pseudomonas aeruginosa otitis *Pseudomonas aeruginosa* ulcerative keratitis

Pseudomonas aeruginosa mastitis

Fig. 3: Systemic diseases: (caused by *P. aeruginosa)*

Pathogenesis and pathogenicity:-

Pathogenic strains of *P. aeruginosa* produce a variety of toxins and enzymes which promote tissue invasion and damage.

1- Attachment to host cells is mediated by fimbriae. Colonization and replication are aided by anti-phagocytosis.

2- Properties of exoenzyme S, extracellular slime and outer membrane lipopolysaccharides. Resistance to complement-mediated damage and the ability to obtain iron from host tissues**.**

3- Toxins produced by Pseudomonas aeruginosa.

a- Exotoxin A : prevent protein synthesis lead to die of cells

b- Exoenzyme S: causes necrosis and damage of mucus membrane of respiratory track and blood vessels.

c- Proteases: causes lysis of fibrin, damage of tissue and inhibit production of TNF and Gamma interferon

d- Urease

e- Haemolysin.

Diagnostic procedures:-

1- Specimens for laboratory examination include pus, respiratory aspirates, mid-stream urine, mastitic milk and ear swabs.

2- Blood agar and MacConkey agar plates, inoculated with suspect material, are incubated aerobically at 37°C for 24 to 48 hours.

3- Identification criteria for isolates :

a- ColoniaI morphology and characteristic fruity, grape-like odour.

b- Pyocyanin production.

c- Lactose-negative, pale colonies on MacConkey agar.

d- Oxidase-positive.

e- Triple sugar iron agar unchanged.

f- Biochemical profile (Table1).

Glanders

1- Glanders, caused by *B. mallei*, is a contagious disease of Equidae. Characterized by the formation of nodules and ulcers in the respiratory tract or on the skin.
2- Transmission follows ingestion of food or water contaminated by nasal discharges of infected Equidae. Less commonly, infection may be acquired by inhalation or through skin abrasions.
3- An acute septicemic form of the disease is characterized by fever, mucopurulent nasal discharge and respiratory signs.
4- Death usually follows within a few weeks.
5- Chronic disease is more common and presents as nasal, pulmonary and cutaneous forms, all of which may be observed in an affected animal. It is appear in three form:

1- **Nasal form:** ulcerative nodules develop on the mucosa of the nasal septum. A purulent, blood-stained nasal discharge and regional lymphadenopathy are usually present. The ulcers eventually heal leaving star-shaped scars.
2- **The respiratory form:** characterized by respiratory distress and the development of tubercle-like lesions throughout the lungs.
3- **The cutaneous form** (termed farcy): is a lymphangitis in which nodules occur along the course of the lymphatic vessels and Ulcers develop and discharge yellowish pus (Fig. 4).

Fig. 4: Glander disease in cutaneous form (farcy)

Pathogenesis

Glanders in the horse is usually a chronic, disseminated, debilitating disease but the mechanisms of pathogenicity are not known. The presence of *B. mallei* in the host gives rise to a hypersensitivity reaction, the basis of the mullein test.

Diagnostic procedures

1- In regions where the disease is endemic, clinical signs may be diagnostic.
2- Specimens for laboratory diagnosis should include discharges from lesions and blood for serology. Specimens must be processed in a biohazard cabinet.
3- *Burkholderia mallei* grow on media containing 1% glycerol and most strains will grow on MacConkey agar. Plates are incubated aerobically at 37^0C for 2 to 3days.
4- Identification criteria for isolates:
   a- Colonial characteristics
   b- Majority of strains grow on MacConkey agar without utilizing lactose
   c- Comparatively unreactive biochemically and non-motile.
   d- Suitable serological tests include the complement fixation test and agglutination techniques.
   e- The mallein test is an efficient field test both for confirmation and for screening in-contact animals. Mallein, a glycoprotein extract of *B. mallei*, is injected intradermally (0.1 ml) just below the lower eyelid. A positive reaction is indicated by local swelling and mucopurulent ocular discharge after 24 hours.

Melioidosis

1- caused by *B. Pseudomallei*,
2- Infection may follow ingestion, inhalation or skin contamination from environmental sources.
3- The bacterium is an opportunistic pathogen and stress factors or immunosuppression may predispose to clinical disease.
4- Abscesses develop in many organs including lungs, spleen, liver, joints and central nervous system.

Pathogenesis and pathogenicity:-

The pathogenesis of Melioidosis is poorly understood. Extracellular products of *B. pseudomallei* such as an exotoxin, a dermonecrotic protease and a Lecithinase have been implicated in disease production. Both strain virulence and host immunosuppression may influence the establishment and outcome of infection.

Diagnostic procedures

1- In regions where the disease is encountered, gross pathological findings may aid diagnosis.
2- Specimens for laboratory diagnosis should include pus from abscesses, affected tissues and blood for serology. A biohazard cabinet must be used for processing specimens.
3- A fluorescent antibody technique for demonstrating the organism in tissue smears is available in some reference laboratories.
4- Blood agar and MacConkey agar plates, inoculated with suspect material, are incubated aerobically at 37°C for 24-48 hours.
5- Identification criteria for isolates:
   a- Colonial morphology and characteristic musty odour
   b- Lactose utilized in MacConkey agar
   c- Biochemical characteristics.
   d- Slide agglutination test using specific antiserum
   e- ELISA**,** complement fixation and indirect haemagglutination tests can be used for detecting serum antibodies