Rods : Gram – Negative Rods : Nonenteric Rods

The organisms not part of a closely related family, they share two significant features:

1) They all have a G– cell envelope and, therefore contain lipopolysaccharide (LPs) which is a virulence factor.

2) They are aerobic, grow in the presence of oxygen cause infections at sites where O₂ tension is high (e.g. lung …).

3) It’s helpful to consider these organisms as
   a) Those are primarily pathogen of human respiratory tract (Haemophilus, Bordetella, Legionella).
   b) One genus that includes successful opportunistic pathogen (Pseudomonas).
   c) Those are primarily pathogen of animals (as zoonotic organisms such as Brucella, Francisella, Pasteurella, which human are accidental hosts. Yersinia pestis is included in this group because it’s a nongastrointestinal G– rod. Bartonella, unusual G– rod responsible for trench fever and cats cratch disease).

A- Pseudomonas

*Pseudomonas aeruginosa*, the primary human pathogen in the genus Pseudomonas, is distributed in soil, water, plant, animals. Although it may colonize healthy human without causing disease, it’s an opportunistic pathogen and major cause of nosocomial infections (hospital – acquired), such as nosocomial pneumonia, nosocomial urinary tract infections, surgical site infections, infection of severe burns, and infections of patients undergoing either chemotherapy for neoplastic disease or antibiotic therapy.

*P. aeruginosa* is G– rods, motile has polar flagella, encapsulated , obligately aerobic (it oxidizes but does not ferment carbohydrates). Nutritional requirements are minimal so it’s can grow on a wide variety of organic substrate, also can even grow in laboratory water baths, hot tubs, wet IV tubing and other water-containing vessels. This explains why the organism is responsible for so many nosocomial infections.

**Pathogenesis**

*P. aeruginosa* disease begins with attachment to and colonization of host tissue. **Pili** on the *P.* mediate adherence, and a **capsule** reduces the effectiveness of normal clearance mechanisms. *P. aeruginosa* produces numerous toxins and extracellular products that support local invasion and dissemination of it.
Virulence factors
Most strains produce:
(A) 2 exotoxins (exotoxin A + exoenzyme S)
(B) variety of cytotoxic substances, including proteases, phospholipases, rhamnolipids and the blue pigment (pyocyanin).
(C) exopolysaccharide, composed of D-mannuronic acid and L-glucuronic acid is responsible for the mucoid phenotype.
These virulence factors depends upon the site and nature infection.
- Proteases play role in corneal ulceration.
- Exotoxin and proteases in burn infections and septicemia.
- Alginate and quorum sensing molecules in chronic pulmonary colonization.
- Pyochelin and fluorescein (pyoverdin) act as important bacterial siderophores.
- Fluorescein in vivo, allows P. aeruginosa to compete with transferrin (mammalian iron-binding proteins).
- Pyocyanin in infected wounds.
- Exotoxin A is similar to dipheria toxin subunit A.

Clinical Significance

_P. aeruginosa_ causes both localized and systemic illness. Individuals must at risk include those with impaired immune defenses.

1- **Localized infections:** (Localized infections have the potential to lead disseminated infection, it can invade blood vessel walls). These may occur in the _eye_ (Keratitis, endophthalmitis, following trauma) _ear_ (external otitis or swimmer’s ear, particularly in elderly diabetic patients or trauma patients) _skin_ (wound sepsis, pustular rashes occurring in epidemics associated with use of contaminated whirlpools, hot tubs, swimming pools). _Urinary tract_ (in hospitalized patients who have been subjected to catheterization, instrumentation, surgery or renal transplantation). _Respiratory tract_ (pneumonia in individuals with chronic lung disease, congestive heart failure or cystic fibrosis, and in patients who have been incubated or are on ventilators). _Gastrointestinal tract_ (infection range from mild diarrheal illness in children to severe, necrotizing enterocolitis in infants and neutropenic cancer patients). _Central nervous system_ (CNS, meningitis, brain abscesses association with trauma, surgery, or tumors of the head or neck).

2- **Systemic infections:** This include _bacteremia_ (in compromised patients), _secondary pneumonia, bone and joint infections_ (in IV drug users and patients with urinary tract or pelvic infections), _endocarditis_ (in IV drug users and patients with prosthetic heart valves), _CNS_ (in meninges are breached) and _skin/soft tissue_
infections. So, *P.aeruginosa* is feared because (1) it can cause severe hospital–acquired infections, especially in immunocompromised hosts, (2) it’s often antibiotic resistant, complicating the choice of therapy.

**Laboratory identification**

Gram stain: *G*– rods, encapsulated, motile. Culture: *P.aeruginosa* can be isolated by plating on a variety of media, both nonselective (Nutrient agar, Blood agar) and moderately selective (MacConkey agar, Fig 1). On nutrient agar → produces diffusible = blue–green pigments (pyocyanin) or yellow–green fluorescent pigment (fluorescein or pyoverdin) or pyoruben (red)/melanin (brown), with fruity odor (sweet grape like) from the culture and at the beside. On MacConkey agar → Lac⁺ as oxidizes but does not ferment. For enrichment culture from soil or water, selective medium containing acetamide as carbon and nitrogen source may be used.

Biochemical tests: **Catalase +, oxidase +, nitrate –, glucose + (oxidation).**

Serological test: serum Abs against *P* have no place in diagnosis except in patients with cystic fibrosis or chronic obstructive pulmonary disease, where Ab levels increasing correlate directly with immune-mediated lung damage.

![Pseudomonas species](image)

**Treatment and Prevention**

It’s difficult to find antibiotics effective against *P.aeruginosa* because of (1) it’s rapid development of resistance mutations (2) its own innate mechanisms of antibiotic resistance. *P.* infections typically occur in patients with impaired defenses, so, aggressive antimicrobial therapy is generally required (Figure 1) (combination of 2
bactericidal antibiotics such as aminoglycoside+ an antipseudomonal β-lactam, or a quinolone).

**B- Brucella**

Members of the genus *Brucella* are primarily pathogens of animals, is a zoonosis. It’s includes which associated with particular animal spps:-

*B. abortus*→ cattle

*B. melitensis*→ goats and sheep

*B. suis*→ swine

*B. canis*→ dogs

*B. ovis*→ sheep

All but *B. ovis* are known to cause disease in humans. The *Brucella* are unencapsulated, G- small coccobacilli, arranged singular or in pairs (Figure 2), nonmotile. LPS and cell wall Ag are the major virulence factor. Its aerobic, facultative intracellular parasites that can survive and multiply within host phagocytes.

![Image of Brucella species](image.png)

**Epidemiology**

Brucellosis is a chronic infection in animals. Organism localize in **reproductive organs (male and female)** and release in large numbers in milk, urine, and the placenta and other tissues discharged during delivery or spontaneous abortion. Causes in animals sterility or abortion. Transmission to human occurs as a result of either **direct contact** with infected animal tissue or ingestion of unpasteurized milk or milk products. Person to person transmission is rare.
Pathogenesis

Brucella typically enter the body through cuts in the skin or the gastrointestinal (GI) tract or Inhalation of infected aerosols among abattoir workers, veterinarians and farmers. Once the organism gain entry → they transported via the lymphatic system → to the regional lymph nodes → they multiply → carried by the blood to organs that are involved in the reticuloendothelial system, including liver, spleen, kidneys, bone marrow and other lymph nodes.

Antigenic Structure

Somatic Ag (O Ag) has to components A and M. B abortus contain 20 times as much (A and M). B. melitensis contain 20 times as much (M and A). B. suis has intermediate Ag pattern.

Ag cross reaction occurs between Brucella and V. cholerae. In addition, L-Ag has been demonstrated that resemble the Vi-Ag of Salmonella. Phage typing = one at strain phage is Tb, is specific as lysis strains having character of B. abortus., which is of great value in identification Brucella.

Clinical Significance

The incubation period for B. infections ranges from 5 days – several months, but typically lasts several weeks. Symptoms are nonspecific and flulike (malaise, fever, sweats, anorexia, GI symptoms, headache, and back pains) and depression. Untreated patients may develop an undulating pattern of fever (temperatures repeatedly rise then fall, hence the name undulant fever is the traditional name for brucellosis). Subclinical infections occur. Brucellosis may involve any of a variety of organ system, including the GI tract, the skeletal, neurologic cardiovascular and pulmonary systems.

Laboratory identification

Although the nonspecific symptoms may not point to a diagnosis of brucellosis, a detailed history is important, including the patients’ occupation+ exposure to animals+ travel to countries where Brucella infections is prevalent + ingestion of contaminated foods.

Specimen : blood and other body fluids or from tissue. Multiple blood specimens should be cultured.

Culture : In liquid media ⇒ uniform turbidity, in old culture there may be powdery deposits (a tryptose broth or tryppticase – soya broth).

On Blood culture ⇒ most defined method for brucellosis. Colonies may appear in 4-5 days longer times and these cultures are routinely examined for up to
one month before declared –ve, its strict aerobic, addition of 10% CO$_2$ improves the growth of $B.\ abortus$, $B.\ melitensis$.

On nutrient agar $\Rightarrow$ colonies are small, moist, translucent and glistening with butyrous consistency. On liver infusion agar $\Rightarrow$ after 48-72 hs. Colonies similar to above in N.A. On MacConkey agar $\Rightarrow$ after 7 days, colonies Lac$^{-}$ yellowish appear.

Biochemical reactions: catalase $+$ve, oxidase $+$ve, urease $+$ve, nitrate $+$ve, no carbohydrate is fermented. Basic Fuchsine and thionin are the dyes which can be used to differentiate spp. ($B\ abortus$ and $B\ melitensis$ growth in basic Fuchsine 1:50.000, while $B.\ suis$ growth in thionin 1:250.000. Casterdas method $\Rightarrow$ gives $+$ ve results in 50% of cases. This done from CSF, lymph node, bone marrow, urine, abscess.

Hematological investigation: Total leukocyte count shows leukocytosis in early acute phase of disease. Differential leukocyte count shows lymphocytosis.

Serological diagnosis: such as

- Agglutination test: it’s $+ve$ about week after onset of the infection, titer 1/100 or more indicates active infection. Slide agglutination method as Rose Bengal test for 4 mins only.
- Radio immunoassay and ELISA $\Rightarrow$ to distinguish acute brucellosis.
- 2-mercaptoethanol test $\Rightarrow$ gives good result with chronic disease.
- Complement fixation test $\Rightarrow$ used in chronic disease.
- Indirect immunoflorescent test $\Rightarrow$ specific and sensitive.
- Indirect hemaglutination test $\Rightarrow$ very sensitive.
- Skin test (Brucillin test) $\Rightarrow$non specific used in chronic cases, give an indicator for post or present infection.
- Rapid plate agglutination test.

**Treatment**

Combination therapy involving doxycycline and gentamicin (or streptomycin) is used (Figure 2) for six weeks is generally necessary to prevent relapse and reduce the incidence of complications.