Medical Bacteriology- Lecture 16

Gram Negative Rods- Oxidase Positive

Pseudomonadaceae

Pseudomonas

Burkholderia

Vibrionaceae

Vibrio

Gram Negative coccobacilli

Brucella

Francisella

Pasteurella
Pseudomonas

- Gram-negative motile aerobic rods
- Versatile, Catalase +, Oxidase +
- Biofilm (Biofilm mucoid strains of Pseudomonas are also less susceptible to antibiotics).
- Found in water, soil, sewage, vegetatable, human and animal intestine.
- Medical importance species: P. aeruginosa

P. aeruginosa

- Part of normal flora in human and animal intestine, water, soil and moist environment in hospitals.
- Nosocomial pathogen- Opportunistic pathogens
- very simple growth requirement. It is often observed "growing in distilled water", evidence of minimal nutritional needs).
- Its optimum temperature for growth is 37 C, and it is able to grow at temperatures as high as 42C.
- Invasive and toxigenic infections in patients with abnormal host defenses
- Produce soluble fluorescent pyocyanin pigment, refers to "blue pus", which is a characteristic of suppurative infections caused by P. aeruginosa.

- Causes UTI (introduced by catheter)- Wound infection of burn sites, Septicaemia, Otitis externa (Malignant external ear infection in poorly treated diabetic patients) – Pneumonia- Eye infection (injury or surgery)- Endocarditis- bacteremia- meningitis- brain abscesses.
- identification is based on colony morphology, oxidase-positive, growth at 42C- Non lactose fermentor - Bluish-green pigmented large colonies with “fruity” odor on culture media.
- It is resistant to high concentrations of salts and dyes, weak antiseptics, and many antibiotics.
**P. Aeruginosa Virulence Factors**

1. **Pilli** (Adhere to epithelial cells)
2. **Exopolysaccharide capsule** (protected from opsonization by antibodies, complement & phagocytosis)
3. **Motility**: spread through tissues
4. **Fluorescent pigment pyocyanin** (impairs normal function of human nasal cilia, disrupts respiratory epithelium).

**Enzymes**:
5. Two extracellular proteases associated with invasive stage: elastase & alkaline protease
   * **Elastases**: Digests protein (elastin, collagen, IgG, lyse fibronectin for bacterial attachment on the mucosa of the lung, disrupts the respiratory epithelium and interferes with cilia function)
   * **Alkaline protease** lyses fibrin.

**Toxins**:
6. **Haemolysins** (beta haemolysis)
7. **Phospholipase & lecithinase** (destroy lipid and lecithin)
8. **Exdotoxin A** (Cytotoxic by blocking protein synthesis- pore forming protein) same mechanism of action as the **diphtheria toxin**
9. **Toxin S**: interfere with membrane permeability
10. **Lipd A** (Endotoxin): tissue necrosis
11. **Enterotoxins**: food poising
Summary of the Virulence Determinants of Pathogenic *P. aeruginosa*

- **Adhesins**  
  pili  
  polysaccharide capsule  
  biofilm

- **Invasins**  
  elastase  
  alkaline protease  
  hemolysins (phospholipase and lecithinase)  
  cytotoxin (leukocidin)  
  iron uptake systems  
  pyocyanin pigment

**Motility** flagella

**Toxins**  
Exoenzyme S  
Exotoxin A  
LPC

**Antiphagocytic surface properties** capsules  
LPS  
Biofilm

**Defense against serum bactericidal reaction** capsules, biofilm, LPC, protease enzymes

**Ecological criteria**

adapt to minimal nutritional requirements  
metabolic diversity  
widespread occurrence in a variety of habitats
**Burkholderia**

- *Burkholderia mallei* (*pseudomonas mallei*) Human & animal pathogen causing **Glanders**

- **Burkholderia pseudomallei** Human & animal pathogen causing **Melioidosis**

- *Burkholderia cepacia* (Onion bulb rot, **Foot rot of man**)
  - Septicemia, urinary tract infection, wounds, endocarditis pneumonia in immunocompromised individuals

- *Pseudomonas alkaligenes* Very rarely human pathogen- Nosocomial pathogen, wounds, urinary tract infection

- *Pseudomonas fluorescens* unusual cause of disease in humans (usually affects patients with compromised immune system) **Produce yellow pyoveriden pigment, which is soluble in water but not chloroform**

*Burkholderia mallei* grown on sheep blood agar for 72 hours.
Curved Bacilli (Vibrio)

- Actively motile, gram-negative **curved rods**
- Vibrios are distinguished from **enterics** by being **oxidase-positive** and motile by means of polar flagella.
- Vibrios are distinguished from **pseudomonads** by being **fermentative and oxidative** in their metabolism.
- Vibrios are one of the most common organisms in surface waters of the world.
- **Medical importance species:** *V. cholerae* and *V. parahaemolyticus* are pathogens of humans.
  - Both produce diarrhea, but in different ways.
  - *V. parahaemolyticus* is an **invasive** the colon
  - *V. cholerae* is **noninvasive**, affecting the **small intestine** through secretion of an enterotoxin.

- **Vibrio parahaemolyticus**  Two biotypes (*Parahaemolyticus* and *Alginolyticus*)
  - Parahaemolyticus causes **food poising in Japan**
  - Gastroenteritis (enterotoxin): Explosive or mild diarrhea

- **Aeromonas hydrophila** Septicemia  (Produce phospholipase + haemolysin)

- **Pleisioomonas shigelloides**
  - Septicemia, wound infections, and gastroenteritis
  - Haemolysin (kanagawa test)
**Vibrio cholerae**

- Found in fresh water-sea food. most often in communities with poor sewage and water treatment.
- **Grow in asparagine** (as a sole source of carbon and nitrogen)
- **Optimum pH growth range (8.5-9.5)**- Sensitive to acidic pH
- Causes **Cholera** (**epidemic cholera**) is a severe diarrheal disease
- Transmission to humans is by contaminated water or food- Route of infection is (fecal-oral)- Incubation period 1-4 days
- Large inoculum (108) is required to cause disease because the bacteria are susceptible to acidic stomach environment

Cholera is one of the most rapidly fatal illnesses. Human may become hypotensive within an hour of the onset of symptoms and may die within 2-3 h if no treatment The disease progresses from the first liquid stool to shock in 4-12 hours, with death following in 18 h to several days.

Abrupt **watery diarrhea and vomiting** (Rice-water stool is characteristic) contains enormous numbers of vibrios- result in severe fluids and electrolytes loss- dehydration, can lead to coma and death- The loss of potassium ions may result in cardiac complications and circulatory failure. Untreated cholera results in high (50-60%) mortality.

**Cholera toxin** (**enterotoxin**) is the most important virulence factor of **V. cholerae**. action on the mucosal epithelium- it is responsible for the characteristic diarrhea of cholera disease.

Cholera toxin activates the adenylate cyclase enzyme of the intestinal mucosa leading to increased the secretion of $H_2O$, $Na^+$, $K^+$, $Cl^-$, and $HCO_3^-$ into the lumen of the small intestine.

The bacterium produces an **invasin, neuraminidase**, during the colonization stage which is degrade N-acetyl-neuraminic acid.

- **Cultures:** 1- TCBS (thiosulphate citrate bile salt sucrose agar) **Selective media** for primary isolation of **V. cholerae** (Observe for large yellow sucrose-fermenting colonies after 18-24 hrs of incubation)
  - 2- Alkaline peptone water: **Enrichment media** for **V. cholerae**

- **Treatment**: fluids and electrolytes replacement - Antibiotics are not important because they are lost in watery stool – Tetracycline shorten the duration of diarrhea and reduce fluid loss.
Disease progression

Ingestion - Pass HCl Barrier of the stomach
(100,000,000 acidic, 10,000 neutral)

↓

Multiply in the small intestine (predisposes malnutrition, vitamin B drop)

↓

1 - Motility (for contact)
2 - Envelope (for adhesion)
3 - Mucinase (break the mucosal layer to allow attachment to epithelial cells)

↓

4 - Endotoxin
5 - Enterotoxin
6 - Neuraminidase to break N-acetyl-neuraminic acid

↓

diarrhea (plasma → lumen)

Loss of fluids (decrease of blood volume + pressure)

↓

Shock

• **Loss of electrolytes:** Potassium → Disturb heart rhythm
  Bicarbonate → Acidosis → weak heart

• **Rice water stools:** mucosa, epithelial cells, and lots of bacteria

• **Give solution containing:** Glucose + Bicarbonate + Potassium
Brucella

- Gram-negative, non-motile, non-sporeforming, **zoonotic**, obligate **intracellular** aerobic coccobacilli

**Major human pathogenic species**

<table>
<thead>
<tr>
<th>Species</th>
<th>Primary animal host</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. abortus</em></td>
<td>Cattle</td>
</tr>
<tr>
<td><em>B. melitensis</em></td>
<td>Goat / Sheep</td>
</tr>
<tr>
<td><em>B. suis</em></td>
<td>Swine</td>
</tr>
<tr>
<td><em>B.canis</em></td>
<td>Dogs (pathogenic to humans with immunodeficiency)</td>
</tr>
<tr>
<td><em>Brucella neotomae</em></td>
<td>Wood rat (Neotoma lepida), desert mice and fleas</td>
</tr>
<tr>
<td><em>Brucella ovis</em></td>
<td>Sheep</td>
</tr>
</tbody>
</table>

**Brucellosis (Undulant fever)** is a zoonotic disease transmitted to human by direct contact with infected tissue via skin and mucus membrane, or ingestion of infected milk via intestinal tract.

**Complication:** Brucella spondylitis (Vertebral brucellosis)

**Brucella human symptoms:** chills, fever (undulant) sweats, weakens, myalgia and headache.

- no vaccine to humans.
Mechanism of pathogenesis

Skin, contaminated milk and cheese, Aerosols to the mucosa of (nose, mouth and conjunctiva)

↓
Local multiplication
(Slight ulceration of mucosa, PMN phagocytize but *Brucella* multiply in them)

↓
Lymphatic system (local lymph nodes)

↓
Reticulo-endothelial system
(Liver, spleen, and bone marrow)
chronic inflammation (granulomas → abscesses)

↓
Septicemia

↓
Generalized infections
(Meningitis, L-forms in bone marrow)
Francisella

- **Francisella tularensis**
  - Small, facultative intracellular, gram negative, nonmotile coccobacillus, grow in blood-cysteine agar

Tularemia (Rabbit Fever) is a major zoonotic disease and transmitted to human by
- 1- biting (rabbit)
- 2- direct contact with infected animal tissue
- 3- inhalation of aerosols
- 4- ingestion of contaminated food and water.
- 5- by fly or Ticks

- **Ulceroglandular tularemia**: Ulceration of arms and hands with lymphadenitis after tick bite or direct contact of broken skin with infected tissue or blood

- **Oculoglandular tularemia**: Accidental contamination of conjunctiva with infected droplets/aerosols

- **Pneumonic tularemia**: Contracted through contaminated aerosols

- **Typhoidal tularemia**: Following ingestion of inadequately cooked food
Mechanism of pathogenesis

Site of injury
↓
Ulcerating papule
↓
Lymphatics
↓
Regional lymph nodes
(Enlarged, tender, may suppurate),
(Survival for long times in the monocytes)
↓
Blood (transitory bacteremia)
↓
Lungs
Liver
Spleen

Francisella tularensis, Colonization on Cysteine Heart Agar after 72hrs
**Pasteurella**

- Gram-negative, small, non-motile, aerobic or facultative anaerobic coccobacilli or rods shaped
- Grow in ordinary media
- Catalase positive- Oxidase positive
- Primarily parasites of domestic and wild animals and birds.

- *Pasteurella multocida* (Shipping fever and cat bite fever)
  Occur in gastrointestinal and respiratory tract of many domestic and wild animals-
  Most common organism in human wounds inflicted by bites from cats and dogs.
Mechanism of pathogenesis

Bite or scratch
↓
18-24 hrs red, swollen, tender
↓
Local abscess
↓
Lymphatics (Severe local pain and swelling)
↓
Survive in the phagocytes
and able to spread through
the lymphatic system
↓
Septicemia (endotoxic symptoms)
↓
Osteomyelitis
Meningitis
Endocarditis
Pneumonia
Abscesses (Cerebral)

Droplets→ Conjunctivitis
↓
Respiratory
↓
Chronic bronchitis
↓
Sinusitis,
mouth ulcers,
pneumonia
↓
(Severe local pain and swelling)
↓
Survive in the phagocytes
and able to spread through
the lymphatic system
↓
Septicemia (endotoxic symptoms)
Review Questions

• What is the causative agent of: Brucellosis (Undulant fever)- cholera- tularemia?
• What is the major characteristic of P. aeroginasea- how can identify it?
• Give six examples of P. aeroginosa virulence factors? Give three examples of its toxins, with its roles? Its exotoxin A is similar with other bacteria toxin. What is it?
• Why P. aeroginosa can grow on distilled water? The bacteria can grow at two different temperature. Explain. What it’s the special pigment?
• Give three examples of P. aeroginosa infections? (nosocomial urinary tract infection introduced by catheter- Wound infection of burn sites- eye infection)
• You studied two species under the genus Burkholderia. Give the species name and disease for each?
• What is the different between endotoxins, enetrotoxins and neurotoxins?
• Write the Latin name of the bacteria that cause 50% of the food poisoning cases in Japan?
• Differentiate between Vibrio cholera and Vibrio parahaemolyticus?
• Grow in asparagine (as a sole source of carbon and nitrogen, optimum growth at alkhlaine pH, sensitive to acidity. What is the bacteria?
• Why V. cholera needs a large numbers of cells to initiate their infections?
• Cholera fatality or death may results from severe fluids and electrolytes loss due to dehydration, explain? How can lead the loss of potassium and bicarbonates- How can you treat a patient with severe diarrhea as a result of V. cholera infection?
• What is the characteristic of cholera diarrhea? Explain the mechanism of the V. cholerae enterotoxin?
• What is the major virulence factor for V. cholera?
• What are the types of tularemia diseases. How can transmitted to human?