Medical Bacteriology- Lecture 16

Gram Negative Rods- Oxidase Positive

Pseudomonadaceae

- Pseudomonas
- *Burkholderia*

Vibrionaceae

- Vibrio

Gram Negative coccobacilli

- Brucella
- *Fracncisella*
- *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 fermintor** - **Bluish-green pigmented large colonies with "fruity" odor on culture media.**

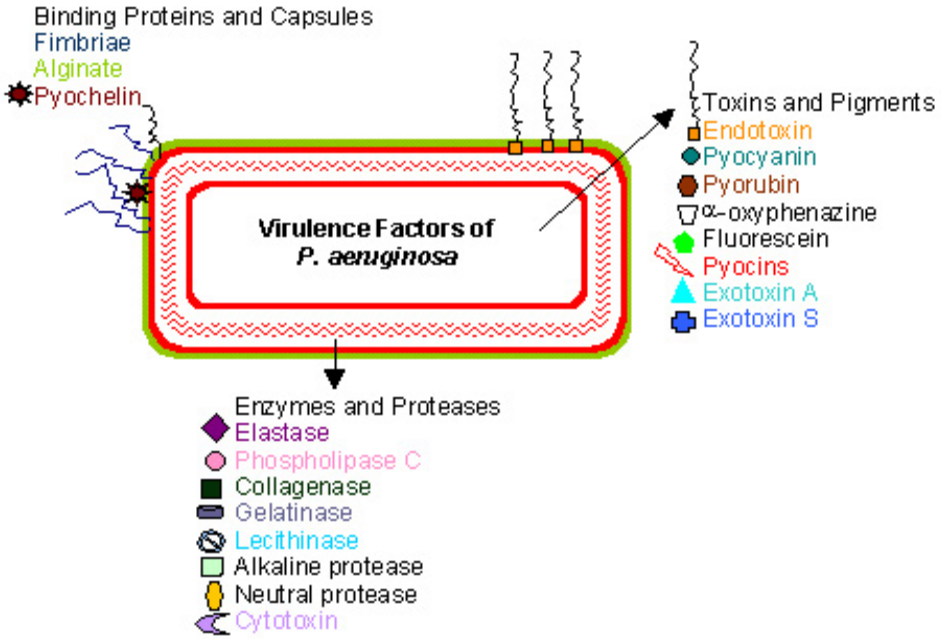
- It is resistant to high concentrations of salts and dyes, weak antiseptics, and many antibiotics.

P. Aeruginosa Virulence Factors

- 1- **Pill**i (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

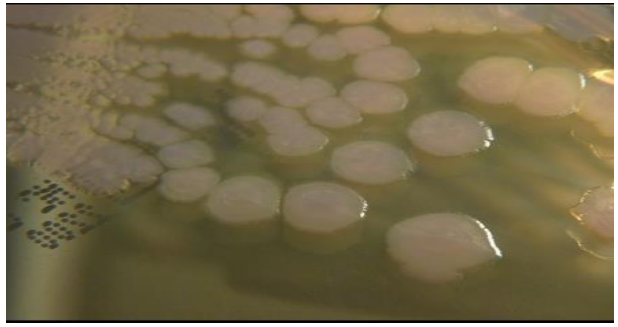
Toxins Exoenzyme S
Exotoxin A
LPC

Antiphagocytic surface properties capsules
LPS
Biofilm

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

***P. aeruginosa* gram stain**

Florescent pigment of *P. aeruginosa on* Cetrimide agar



***P. aeruginosa* on MacConkey agar (non lactose fermintor)**

Ecological criteria

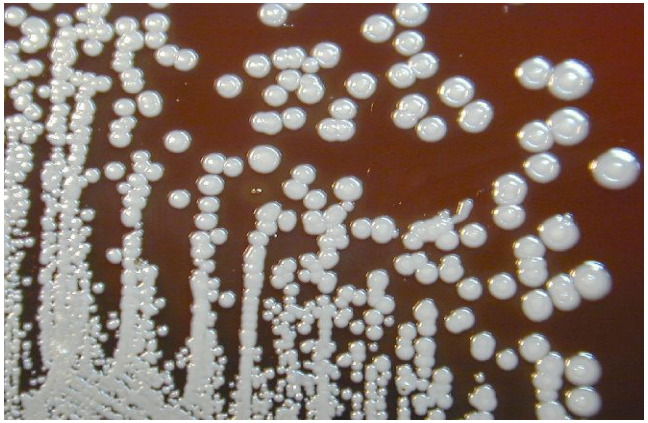
adapt to minimal nutritional requirements
metabolic diversity
widespread occurrence in a variety of habitats

Burkholderia

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

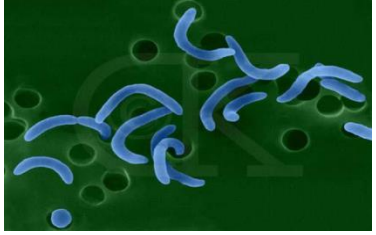
- ***Burkholderia cepacia*** (Onion bulb rot, **Foot rot of man**)
- Septicemia, urinary tract infection, wounds, endocarditis pneumonia in immunocompromised individuals

- ***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)

- ***Pleisiomonas 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_20, Na^+, K^+, Cl^-, and HCO_3^- into the lumen of the small intestine.

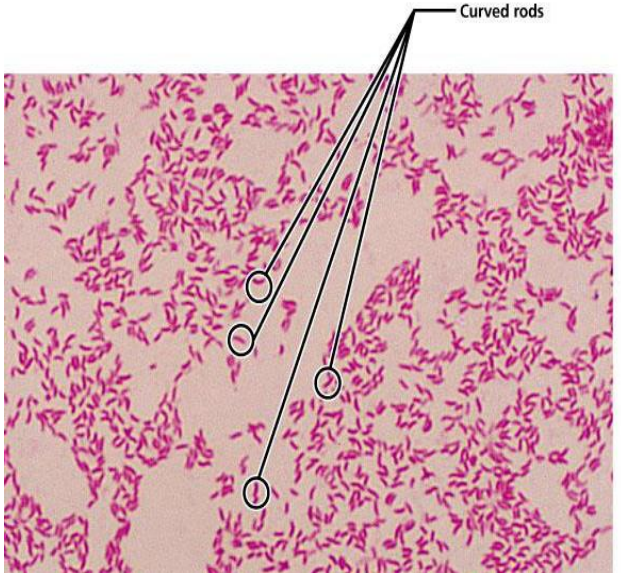
The bacterium produces an invasin, neuraminidase, during the colonization stage which is degrade N-acetyl-neuraminic acid.

- **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

Species	**Primary animal host**
B. abortus	**Cattle**
B. melitensis	Goat / Sheep
B. suis	Swine
B.canis	Dogs (pathogenic to humans with immunodeficiency)
Brucella neotomae	Wood rat (Neotoma lepida), desert mice and fleas
Brucella ovis	Sheep

- **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)

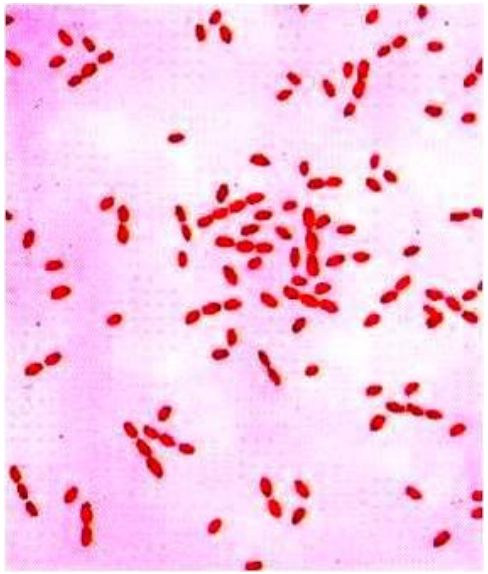
↓

Septicemia

↓

Generalized infections

(Meningitis, L-forms in bone marrow)



Fracncisella

- ***Francisella tularensis***
- Small, facutative **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 intery
↓
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**

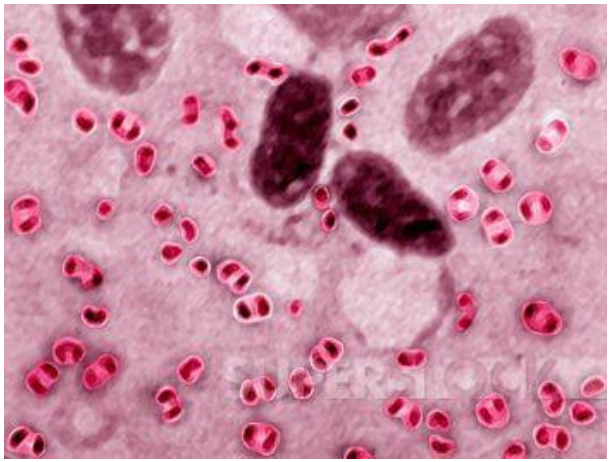
Pasteurella

- 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.

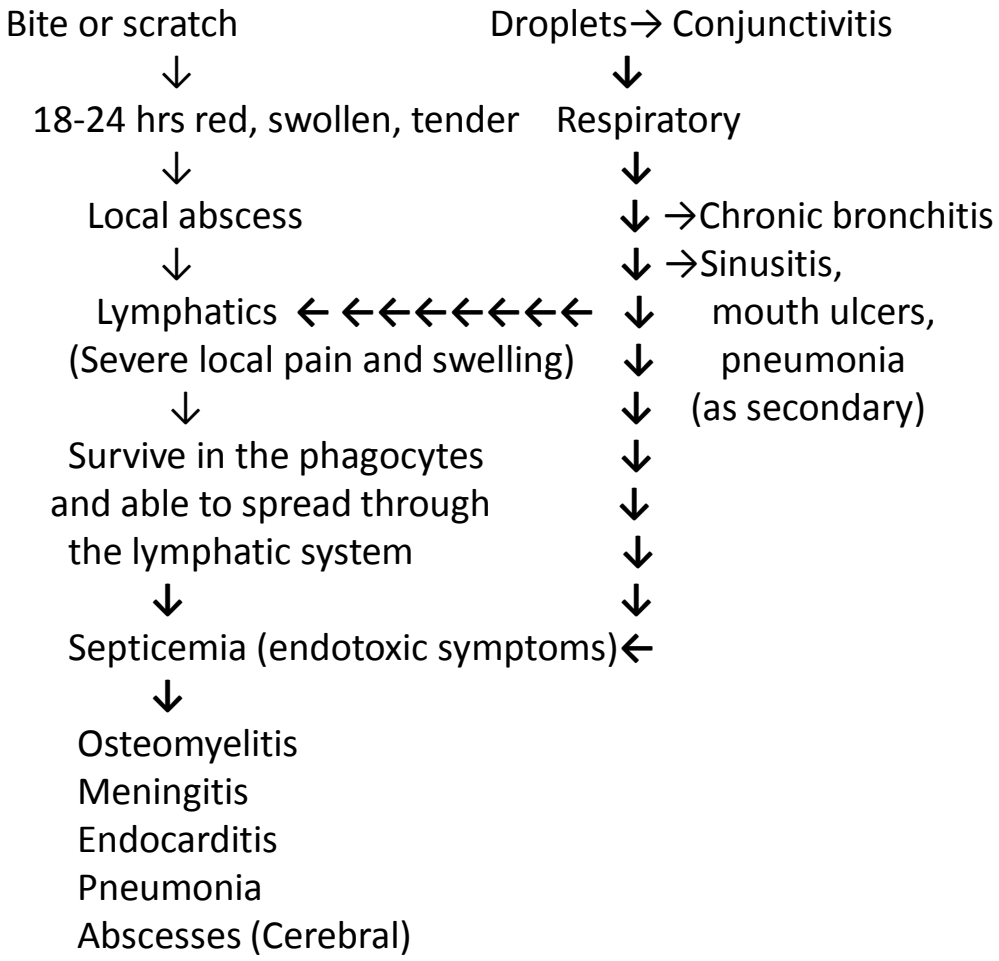
- ***Pasteurella multocida*** (Shipping fever and cat bite fever)

Occur in gasrointestinal 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



Review Questions

- What is the causative agent of: Brucellosis (Undulant fever)- cholera- tularemia?
- What is the major characterstic of *P. aeroginasea*- how can identify it?
- Give six examples of *P. aerginosa* 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. aerginosa* 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. aerginosa 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?