

9. Haemophilus, Bordetella and Pseudomonas

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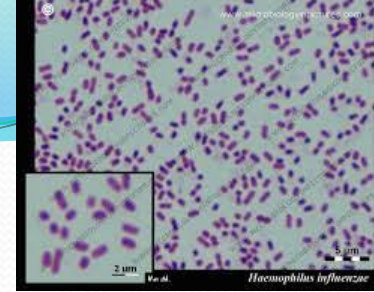
Objectives

- Understand the morphology, epidemiology, pathogenesis, clinical presentations and laboratory diagnosis of *Haemophilus*
- Understand the morphology, epidemiology, pathogenesis, clinical presentations and laboratory diagnosis of *Bordetella*
- Understand the general characteristics, epidemiology, clinical presentation, laboratory diagnosis and treatment of *Pseudomonas*



Haemophilus

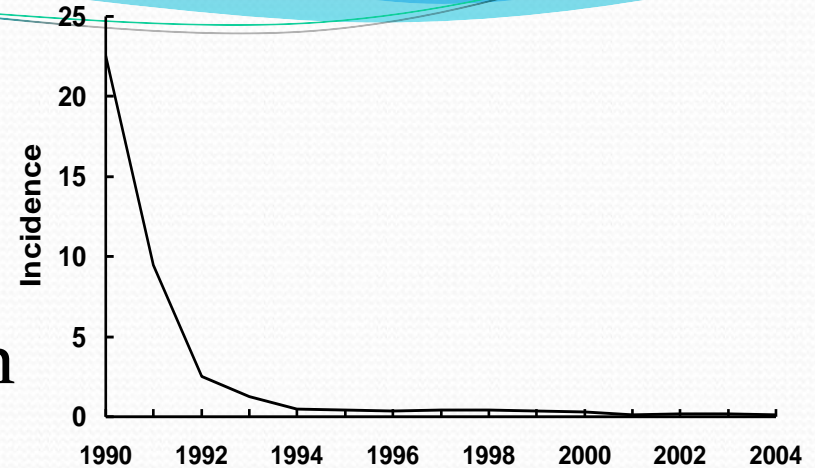
General Characters



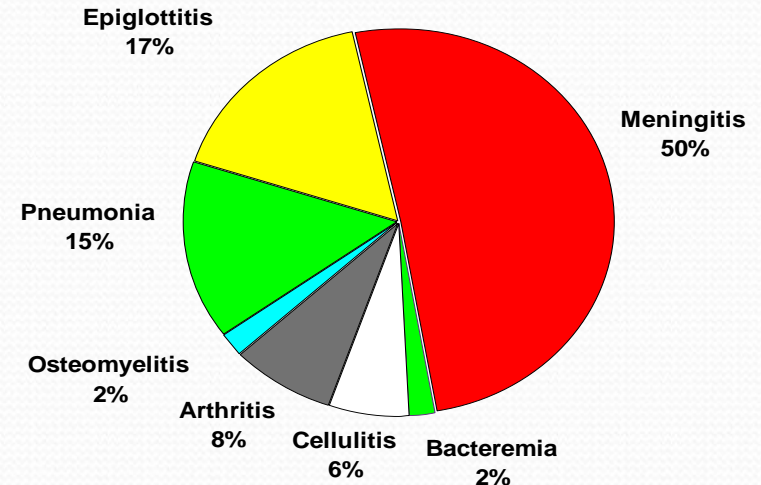
- Aerobic gram-negative bacilli/coccobacilli liking blood
- During late 19th century believed to cause influenza
- Major pathogens for which humans are natural hosts
 1. *Haemophilus influenzae*
 2. *Haemophilus ducreyi*-induce sexually transmitted diseases (chancroid)
- Non motile
- *H. influenzae* has a polysaccharide capsule with six different serotypes (a-f)
- 95% of invasive disease are caused by type b (Hib) which contain polyribose-ribitol phosphate (PRP) capsule

Epidemiology

- Transmitted via respiratory droplets, or direct contact with contaminated secretions
- Normal flora of the human respiratory tract and oral cavity
- Severe bacterial infection, particularly among infants
- Incidence of invasive disease in children <5 years of age has dropped to near zero due to vaccination



Incidence of Invasive Hib Disease



Clinical presentations of Hib pre-vaccination

Virulent Factors

- Antiphagocytic polysaccharide capsule is the major pathogenesis factor
- lipid A component from the cell wall (major role in non capsule strains)
- All virulent strains produce **neuraminidase** and an **IgA protease**
- Pathogenesis: Organism colonizes nasopharynx followed by
 1. Local invasion: otitis media and sinusitis
 2. Systemic invasion: ...bacteremia...meningitis

Pre-vaccination: Meningitis

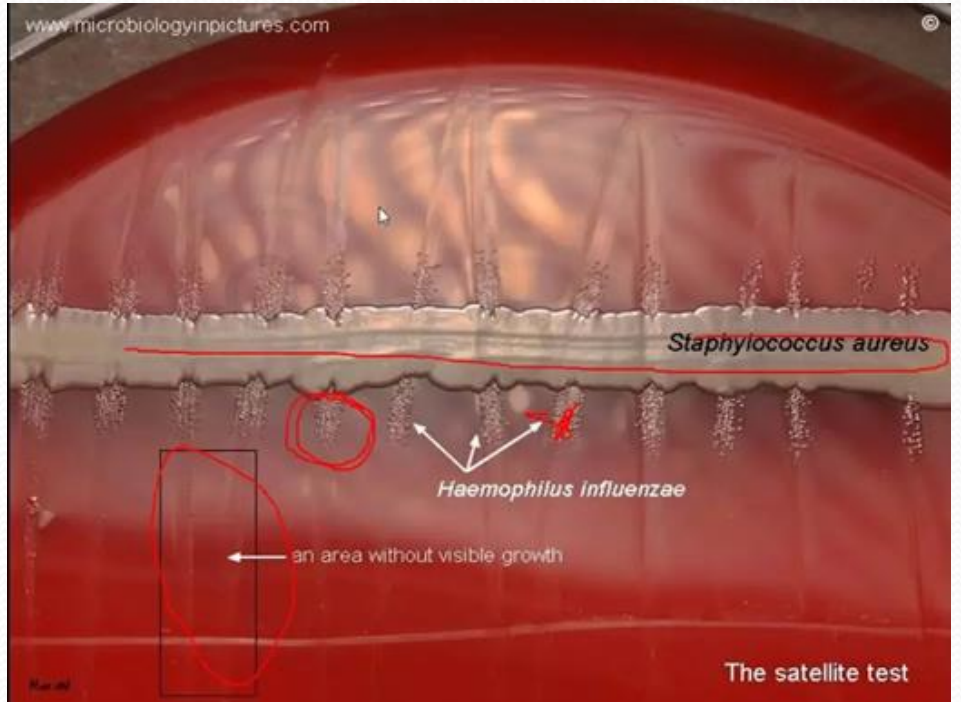
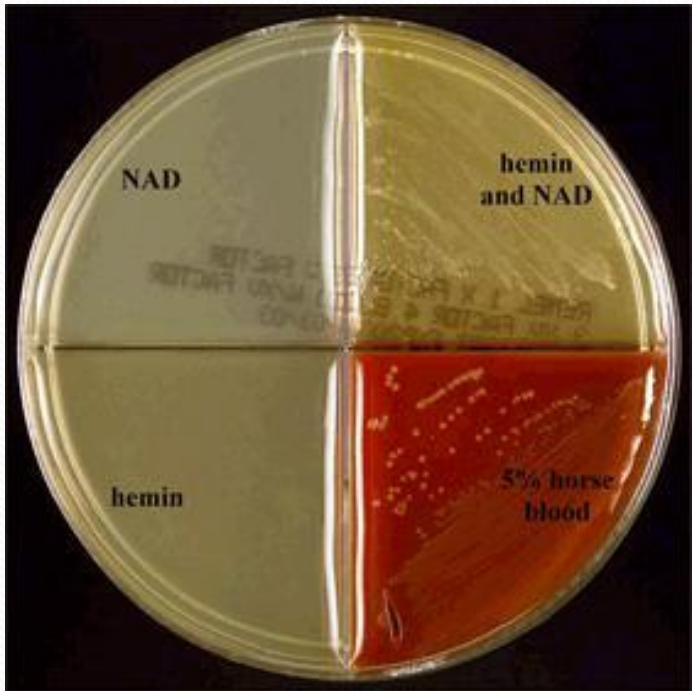
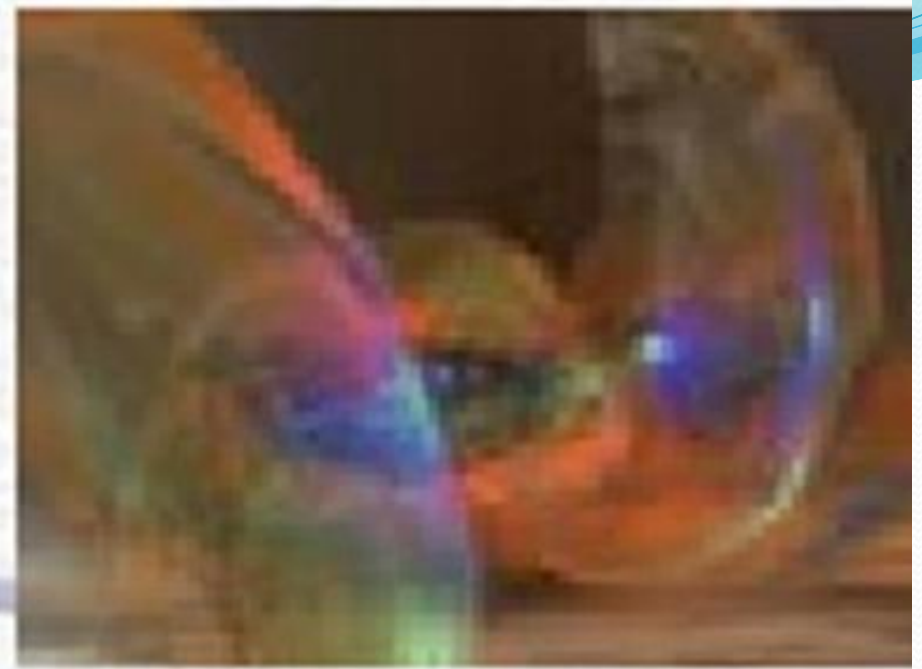
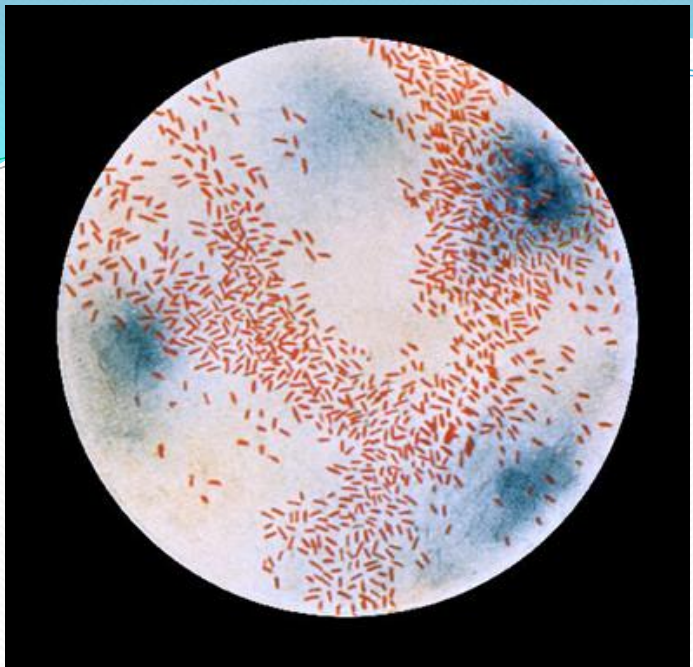
- Accounted for approximately 50%-65% of cases in the prevaccine era
- Hearing impairment or neurologic sequelae in 15%-30%
- Case-fatality rate 2-5% despite of effective antimicrobial therapy

Post-vaccination

- Most cases in unvaccinated or incompletely vaccinated children.
- Non-encapsulated, non-typeable strains and serotype f are the most common
- Children - Pneumonia and meningitis are less common
- Hib are among the commonest causes of bacterial otitis media and sinusitis

Laboratory Diagnosis

- **Gram stain: Gram-negative bacilli/coccobacilli**
- **Requires 2 erythrocyte factors for growth: X (hemin) and V (NAD).** X & V factors are released following lysis of RBCs
- **Culture:**
 - IsoVitaleX-enriched chocolate agar
 - Blood agar with *S. aureus* (satellite)
- **Biochemical tests:**
 - Catalase, oxidase, nitrate reduction, and glucose fermentation are all positive
- **Iridescence:** different colors on transparent media due to the optical properties of the capsule
- **Serological tests** for serotyping (anti-a, b..)



Treatment and Prevention

- Hospitalization required
- Treatment with an effective 3rd generation cephalosporin
- Ampicillin-resistant strains now common
- Prevention by vaccination



Bordetella

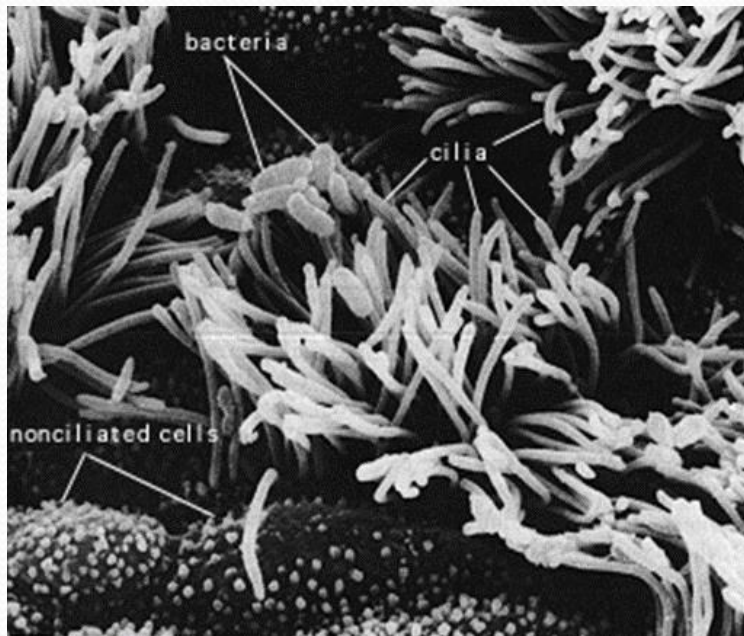
General Characteristics



- Gram-negative coccobacilli (rod-shaped) single or paired
- Obligate aerobe, Optimum growth 35-37 °C , grow better on media with a slightly acidic reaction
- Colonizes the respiratory tract
- Incidence dropped significantly after vaccination
- Specific to human hosts
- The main species
 1. *B. pertussis*: whooping Cough (Pertussis)
 2. *B. parapertussis*
 3. *B. bronchiseptica*

Route of Transmission

- Spread through direct contact of respiratory secretions
- Most contagious during first few days of infection
- Resides in upper airway pathways, mostly the trachea and bronchi.
- Very contagious



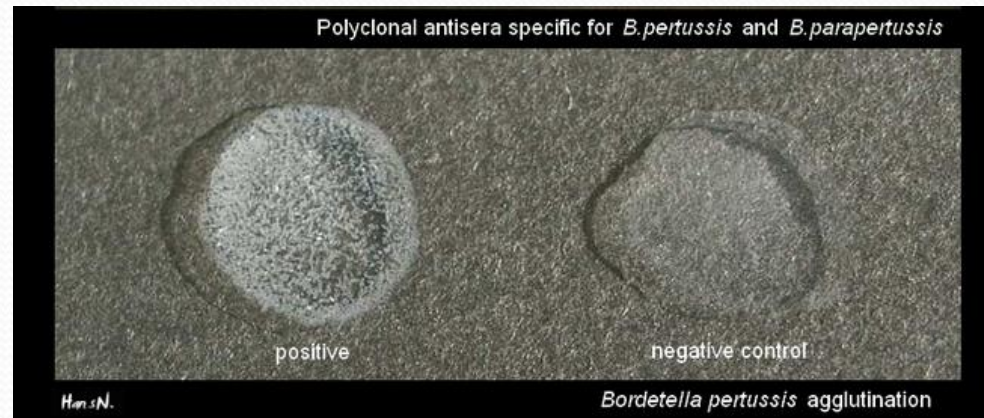
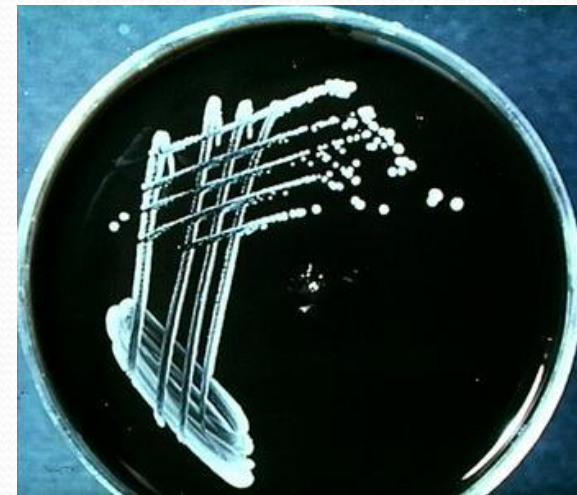
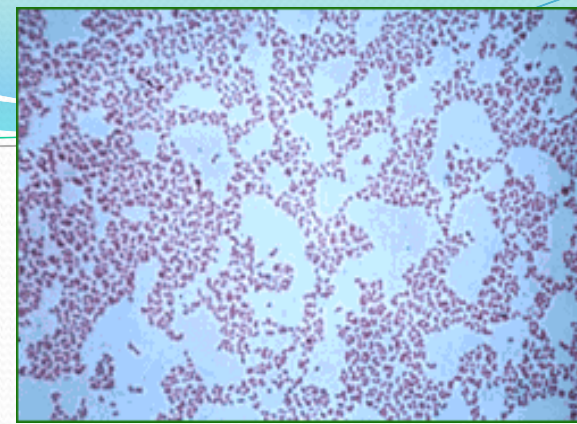
Clinical Presentation: Whooping Cough

- Incubation period 4-21 days
- 3 Stages:
 1. Catarrhal Stage 1-2 weeks: runny nose, sneezing, low fever, and a mild cough (common mistaken for cold)
 2. Paroxysmal Stage 1-6 weeks: whooping cough, which consists of bursts or paroxysms of numerous, rapid coughs, that end with a long inspiratory effort with high pitched whoop, severity of the infection is at its greatest
 3. Convalescent Stage: weeks-months, gradual recovery starts



Laboratory Diagnosis

1. **Specimen:** posterior nasopharynx
2. **Gram stain:** Gram-negative coccobacilli
3. **Culture:**
Media: Bordet-Gengou, or charcoal agar
4. **Polymerase Chain Reaction:** rapid, sensitive, specific
5. **Bordetella antibodies detection by ELISA**
6. **Slide agglutination**



Treatment and Prevention

- Antibiotic Therapy
 - Erythromycin
 - Azithromycin
 - Clarithromycin
- Pertussis vaccine
 - 1st Pertussis vaccine- whole cell
 - Acellular vaccine now used
 - Combination vaccines
- CDC recommends children be given the Diphtheria, Tetanus, and Pertussis (DTaP) vaccine as early as 6 weeks but no later than 6 y/o

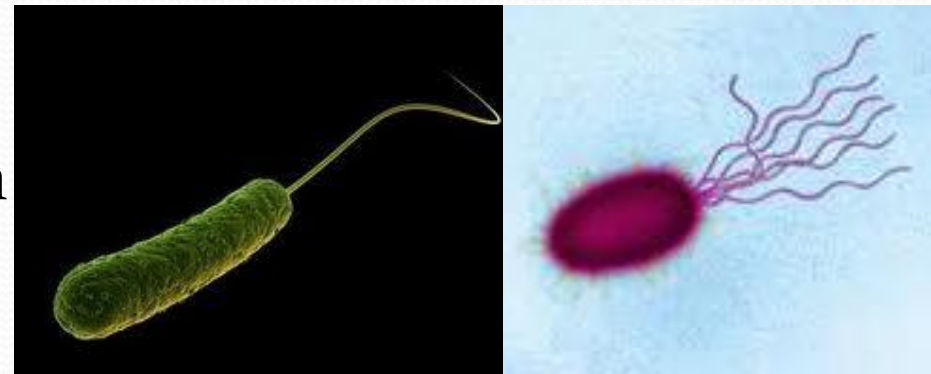




Pseudomonads

General Characters

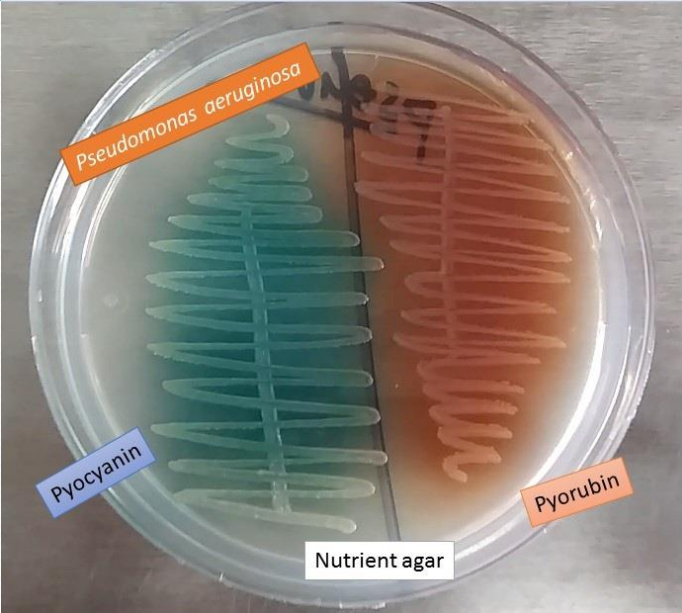
- *P. aeruginosa* is the most common pseudomonad
- Gram-ve rods arranged in pairs
- Motile by single or multiple polar flagella
- Non-fermentative
- Oxidase-positive
- An obligate aerobes
- Some strains are mucoid contain (polysaccharide capsule)
- Pseudomonads produce diffusible pigments, as:
 1. Blue pyocyanin
 2. Yellow fluorescein
 3. Reddish-brown pyorubin
 4. Black pyomelanin



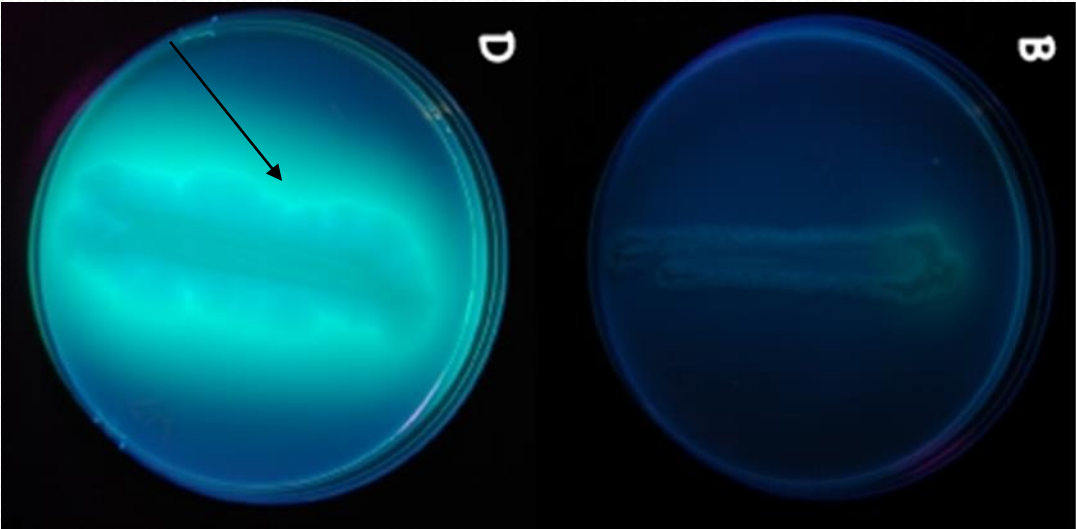
Fluorescein



Pyomelanin



UV light causes fluorescence of colonies.



Epidemiology

- Habitats in soil, water and plants
- Survive well in domestic environments like contact lenses and solutions, and in hospital (food, sinks, toilets, floor mops, respiratory therapy and dialysis equip, and even in some disinfectant solutions)
- Rarely it can be a part of normal flora in healthy individuals
- Transmission by:
 1. Ingestion of contaminated food or water
 2. Exposure to contaminated medical devices and solutions
 3. Penetrating wounds
 4. Rarely might be transmitted by person to person

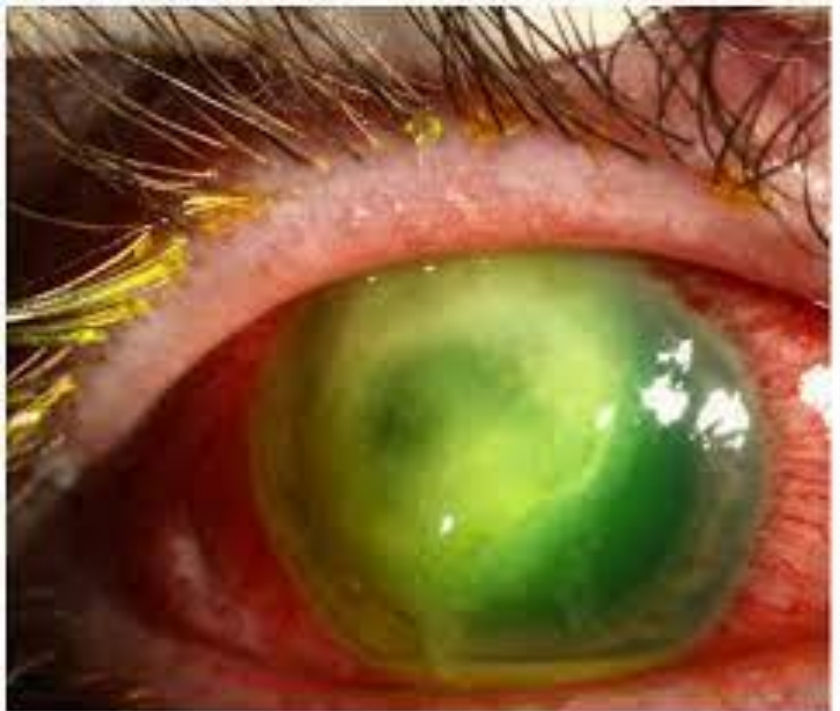
Virulence Factors

- **Polysaccharide capsule:** Mediated bacteria adhesion to epithelial cells, prevent phagocytosis and inhibits antibiotics
- **Endotoxin (lipid A)**
- **Exotoxin A:** Disrupts protein synthesis by blocking peptide chain elongation leading to necrosis, ulceration and tissue damage. The toxin is also immunosuppressive
- **Pyocyanin :** Can mediate tissue damage
- **Exoenzymes S and T:** Cause epithelial cell damage
- **Elastases:** Cause damage to elastin-containing tissues and lung parenchymal
- **Alkaline Protease:** Causes tissue destruction and interferes with host immune response
- **Phospholipase C:** Heat-labile hemolysin
- **Rhamnolipid:** Heat-stable hemolysin.

- **Resistance to antibiotics:** *P. aeruginosa* is one of the most resistant bacteria to many groups of antibiotics
- Mechanism of resistance:
 1. Mainly due to outer membrane porin proteins mutation.
 2. Production of many β -lactamases and carbapenemase

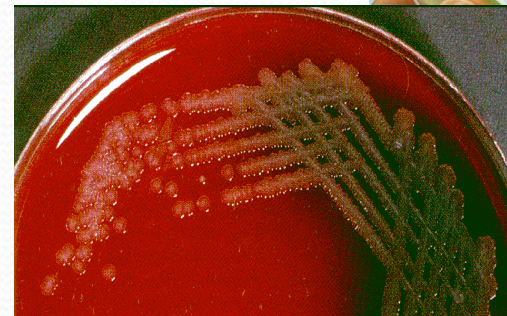
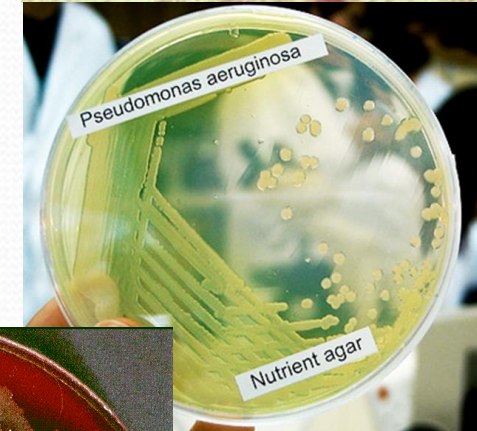
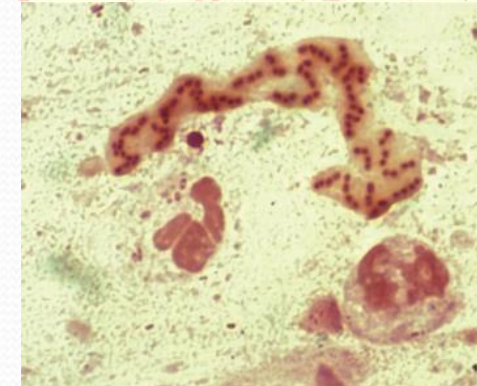
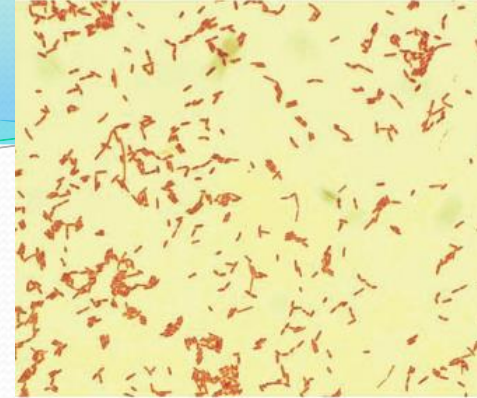
Clinical Presentation

- Pulmonary infections, common in cystic fibrosis patients
- Primary skin infections: Opportunistic infections of existing wounds (e.g., burns) to localized infections of hair follicles
- UTI infections: Opportunistic infections in patients with indwelling urinary catheters
- Ear infections: range from mild irritation of external ear "swimmer's ear" to invasive destruction of cranial bones
- Eye infections: Opportunistic infections of exposed, mildly damaged corneas e.g., contact lens wearer
- Bacteremia: Dissemination of bacteria from primary infection to other organs and tissues



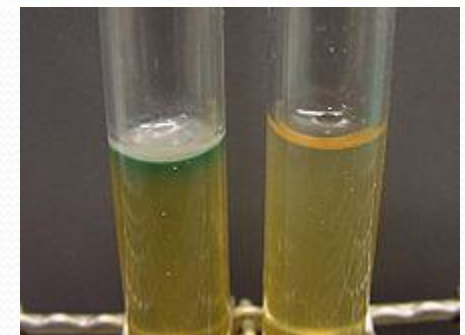
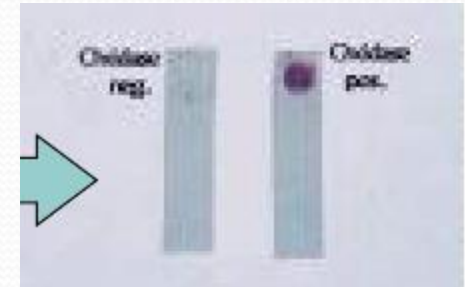
Laboratory Diagnosis

- **Specimens:** according to infection
- **Gram stain:** Gram-negative bacilli
- **Culture:** nutrient or blood
can grow at 42 °C
Green color and fruity smell
- **Biochemical tests:** Oxidase, Catalase,
and Nitrate reduction-positive
None fermenters
- **Antibiotics sensitivity tests:**



- **Biochemical tests:**

- Oxidase-positive
- Catalase-positive
- None fermenters
- Nitrate reduction-positive
- Pyocyanin green pigment production



- **Serological test:** not usually used
- **Antibiotics sensitivity tests:** important for guiding therapy to prevent using resistant antibiotics commonly associated with *P. aeruginosa*