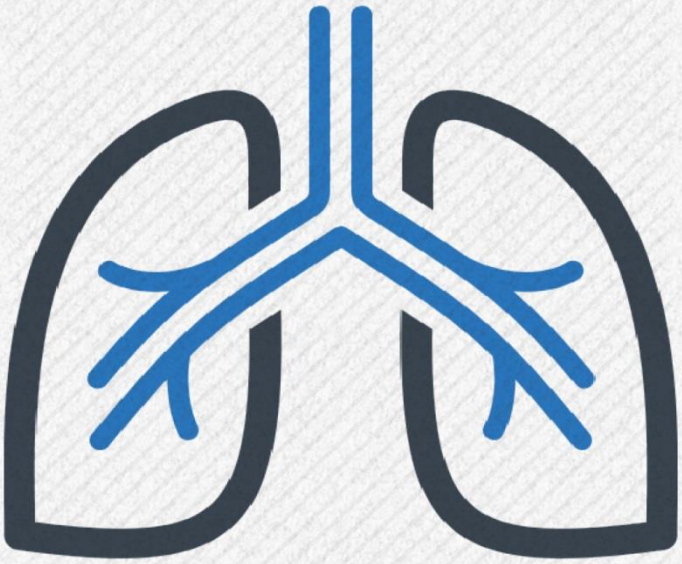

Respiratory system

Microbiology

sheet | handout | slides

Number	3
Doctor	Ashraf Khasawneh
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Pseudomonas Aeruginosa

Pseudomonas Aeruginosa infection is not easy to treat, but it's even more complicated in patients with **cystic fibrosis**, those patients produce thick mucus in the upper respiratory tract and GI tract, this increases their susceptibility to infections, once they are infected with Pseudomonas Aeruginosa, it would be very hard to treat and cause terrific complications for them.

Pseudomonas Aeruginosa:

- **Motile** (by single or multiple polar flagella)
- **Gram-negative rods**
- **Obligate aerobes**
- Oxidase (usually) and catalase positive
- Nonfermentative chemoheterotrophic respiratory metabolism
- Have minimal nutritional requirements; Many organic compounds used as C and N sources, but only a few carbohydrates by oxidative metabolism (Glucose used oxidatively, lactose negative on MacConkey's agar)
- **Some strains produce diffusible pigments:**
  Pyocyanin (blue) . Fluorescein (yellow) . Pyorubin (red)
- **Produces characteristic grape-like odor and blue-green pus & colonies**
  (this smell is felt from the culture but not from patient's breath)
- **It has resistance to broad spectrum of antibiotics**

Structural Components of Pseudomonas Aeruginosa:

- Its adherence to host cells is mediated by pilus and non-pilus adhesins
- It has a lipopolysaccharide that inhibits antibiotic killing and suppresses neutrophil and lymphocyte activity
- **Alginate**: mucoid exopolysaccharide that forms a shiny **biofilm protecting** from antibodies, complement, phagocytosis, and antibiotics. Staphylococcus Aureus, especially MRSA, also forms biofilms.
- Pyocyanin (blue): impairs ciliary function and mediates tissue damage through

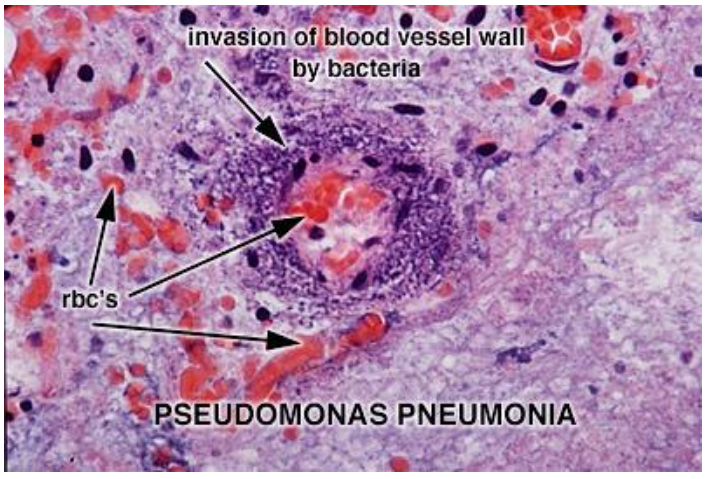
production of **oxygen radicals**

Epidemiology:

- Primary habitat is the environment (water and soil)
- Normal flora 2-10% of healthy individuals
- Infections mostly in **hospitalized** patients with illnesses, such as **cystic fibrosis**, leukemia and burns
- Minimal nutritional requirements and can tolerate broad temperature spectrum (20-42C) and thus contaminate respirator humidifiers, medications and contact lens solutions. **Forming a biofilm also aids** the bacteria to contaminate these areas by becoming resistant to sterilization and autoclave.
- Can transiently colonize the respiratory and GI tract of hospitalized patients
- Respiratory colonization of **cystic fibrosis patients** becomes chronic leading to high morbidity and mortality rates
- No seasonal incidence (**doesn't increase in any season more than others**)

Pathogenesis:

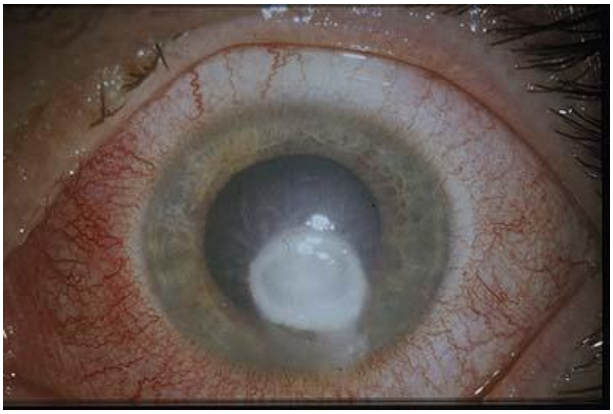
- Require break of first-line defenses (by wound or contaminated solution)
- Attachment by pili, flagella, and extracellular polysaccharide
- Virulence Factors (toxins and enzymes):
  - Exotoxin A
  - Exoenzyme S
  - Endotoxins
  - Phospholipase C
  - Alkaline Protease
  - Elastase



- Can invade blood vessels and spread

Clinical diseases caused by Pseudomonas Aeruginosa:

- **Burn Wound Infections and other skin and soft tissue infections (life threatening)**
- **UTI's (especially catheterized)**
- **External Otitis (malignant otitis externa "swimmer's ear" in diabetic patients, can cause disruption of external ear structure)**
- **Eye Infections and corneal ulceration (in 24-48 hours) via contaminated contact lens cleaning fluids (Pseudomonas Keratitis)**
- **Pseudomonal Endocarditis**



- **Pulmonary infection**

  - Can range from asymptomatic colonization to severe necrotizing bronchopneumonia
  - Colonization is seen in patients with cystic fibrosis, chronic lung disease, and neutropenia
  - Mucoid strains are commonly isolated from chronic pulmonary patients and are more difficult to eradicate
  - Predisposing conditions include previous therapy with broad spectrum antibiotics
  - Mortality rate can be as high as 70% for invasive bronchopneumonia

- **Ecthyma Gangrenosum**

  A well-recognized **cutaneous** manifestation of severe, invasive infection by Pseudomonas aeruginosa that is usually seen in **immunocompromised, burn patients, and other critically ill** patients and cause **black necrotic ulcer**.

Diagnosis:

Oxidase positive colonies, pyocyanin production, and ability to grow at 42C are diagnostic. In addition, **cultures** from bronchoalveolar lavage or sputum and sensitivity tests are also diagnostic.

Treatment:
- Pseudomonas Aeruginosa produces **B-lactamases** so it is resistant to penicillins.
- Inherently resistant to many antibiotics (penicillin, ampicillin, tetracycline, earlier aminoglycosides and sulfonamides)
- They respond to 3rd generation cephalosporins, carbapenems and monobactams (drugs of choice)
- **Combination** of active antibiotics generally required for successful therapy (Anti-β-lactam and aminoglycoside)
- **No vaccine**

Moraxella Catarrhalis

- Gram negative, aerobic coccobacilli, non-motile
- Grows on blood or chocolate agar
- **Oxidase positive, catalase positive,**
- **Most strains produce β-lactamase so they are penicillin resistant**
- formerly classified as Neisseria and more recently as Branhamella
- In elderly patients with chronic pulmonary disease it might cause bronchitis or bronchopneumonia
- **In previously healthy people (their normal flora) it might cause sinusitis or otitis (secondarily), this happens by two mechanisms:**
    - **A viral infection might cause drop in immunity so the normal flora is now able to cause infection and spread from its original site (oropharynx) to the sinuses and middle ear**
    - **Viral infection (or any other condition) might increase secretions causing blockage of airways from the nasopharynx or oropharynx to the middle ear and sinuses, this blockage produces negative pressure in middle ear and sinuses that sucks the secretions (containing normal flora) towards them.**

Note the previous point applies to Haemophilus Influenza and Streptococcus Pneumonia too

- **Treatment**: they are susceptible to Amoxicillin-Clavulanate, second and third

generation cephalosporin

Note: All the species mentioned above don't cause atypical pneumonia

Atypical pneumonia

Atypical pneumonia differs from typical pneumonia in terms of the causative pathogen; previously, atypical pneumonia was defined as non-bacterial pneumonia (and typical pneumonia is the bacterial one), but now we know that atypical pneumonia can be caused by:

- Viruses
- Fungi
- Bacteria (different species from those which cause typical pneumonia)

Typical pneumonia is commonly caused by *Streptococcus Pneumonia*, while atypical pneumonia can be caused by bacteria that **lack cell wall** or are **obligate intracellular**. Because of these two characteristics, they used to classify those bacteria as viruses, and that's why they thought that the causative pathogen of atypical pneumonia is not bacteria.

Bacteria that cause atypical pneumonia:

- Mycoplasma Pneumonia

- Legionella Pneumophila

- Chlamydia Pneumonia

- Chlamydia Psittaci (in dropping of birds)

- Francisella Tularensis

- Coxiella Burnetii

In this lecture we're going to discuss the first two of them only.

Viruses that cause atypical pneumonia:

- Influenza viruses (A & B)
- Parainfluenza virus
- Adenoviruses

- Respiratory syncytial virus
- Herpes viruses

Other differences between typical and atypical pneumonia are in age group of infection and in the clinical presentation; atypical pneumonia can affect any age group but it's manly present in 5-15 years old individuals. Regarding clinical presentation:

	Typical	Atypical
Illness	Patient is severely ill and need to be hospitalized and given IV antibiotics	Patient is still able to walk and move, that's why it's called "walking pneumonia"
Cough	Frequent and productive	Less and dry
Sputum and secretions	More	Less
Chest X-ray	Infection is localized (lobar)	Interstitial infection, widespread, diffuse, ground-glass appearance
WBC count	Elevated	Less elevated

Note: symptoms of atypical pneumonia are more systemic, the patient looks sicker than the actual condition.

Mycoplasma

Mycoplasmataceae family contains two medically important genera:

Mycoplasma and Ureoplasma

Common clinical isolates from those two genera:

- **M. pneumoniae** - M. hominis -M. genitalium -U. urealyticum

These bacteria **do not possess the distinctive cell wall** of bacteria, Plasma membrane is the outermost part of the organism, and what is unique among bacteria in that it has a high content of sterols (acquired from medium or tissue living in) that act to prevent osmotic lysis.

- **Very small in size** (too small to be seen with an ordinary light microscope) and highly pleomorphic
- Don't stain with a Gram stain
- Non-motile
- May possess a capsule
- Although some are free living, most are closely adapted parasites
- Grow on media enriched with serum (need cholesterol)
- Grow best at 35-37^0 C either aerobically or anaerobically
- **Requires long time to grow, one to two weeks**
- M. pneumoniae colonies resemble **fried eggs** and can be stained with Dienes stain (they stain blue)

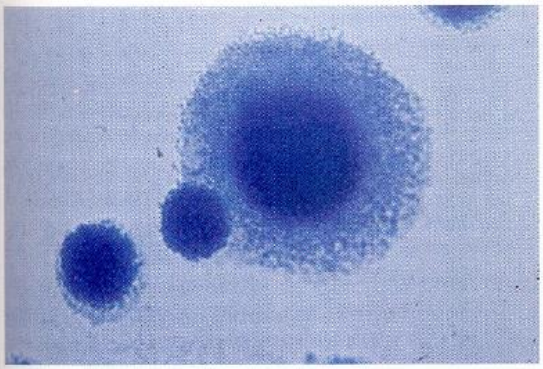


Figure 21-16
Diene's stain of *Mycoplasma* spp. colonies demonstrating typical "fried egg" appearance.

Virulence factors

- Not invasive and simply colonize cell surfaces through specific binding
- It binds to cilia in the bronchus via surface mycoplasmal cytadhesin (P1 protein) to **sialic acid** leading to interference with the ciliary action resulting in its **desquamation**.
- Damage to host tissues may be due to immune response rather than invasion by the organism.
- Organism shed in UR secretions for 2-8 days before onset of symptoms and shedding continue up to 14 weeks

Clinical significance

- M. pneumonia – the major cause of primary, atypical pneumonia (walking pneumonia). 10% of all pneumonia
  • Transmitted by droplet infection
  • Age range: 5-15 years old, more severe in older children
  • After a 2-3 week incubation, the disease begins as a mild, upper respiratory tract infection and progresses to fever, headache, malaise, and a dry cough which is usually mild and self-limited. **Most of patients with atypical pneumonia don't even seek medical advice or visit physicians**
  • 3-10% develop clinically apparent pneumonia with occasional complications of arthritis, rashes, cardiovascular problems, or neurological problems.
- Pharyngitis with fever and sore throat
- Myringitis or otitis media (Myringitis is a form of acute otitis media in which vesicles develop on the tympanic membrane)

  Even if a patient presented with these signs, they are commonly misdiagnosed as viral infection and left untreated.
- Genital tract infections - caused by M. hominis and U. ureolyticum which may also be found as part of the NF in the genital tract
- May cause nongonococcal urethritis, PID, post-partum fever, infertility, stillbirth, spontaneous abortion, and acute urethral syndrome

Mycoplasma diagnosis:

- M. pneumoniae
  • Isolation in culture: incubation for more than a week
  • Ability of colonies to hemolyze guinea pig RBCs
  • **4 fold rise in specific antibody titer**; when the patient comes for the physician for the first time due the infection, his antibody titer is measured, then after 10 days for example titer is measured again, if there's a 4-fold (or more) increase in the antibody titer it is considered **primary infection.**
  • **Cold agglutinin test** – a nonspecific test in which the patient produces cold reacting antibodies that agglutinate type O human RBCs at **40 C, but not at 37 C**
  Cold agglutinin is **diagnostic for mycoplasma infection but it's not specific**, some viral infections (like adeno and EBV) can produce cold agglutinin as well
- A single titer of 1:128 is significant and occurs in 7 days and disappears in 6 weeks.

- M. hominis
  - Isolation in culture
  - No hemolysis of guinea pig RBCs
- U. urealyticum
  - Urease production

Treatment:

- M. pneumonia – Clarithromycin and azithromycin and quinolones
- Genital infections – **Tetracycline**, azithromycin and quinolones

Although macrolides and quinolones are drugs of choice for mycoplasma, some strains developed resistant to them (for example, clarithromycin-resistant mycoplasma)

Legionella pneumophilia

There are 21 species of Legionella but we're discussing this one only, the name "legionella" is from "**legionnaire's disease**"

Morphology/cultural characteristics:

- Small, G- pleomorphic rods that stain very poorly alternative Dieterle stain
- Motile
- Requires cysteine, ferric ions and PH 6.9 for growth and, therefore, won't grow on ordinary lab media

- The best media for primary isolation is **buffered charcoal yeast extract** with alpha keto glutarate (BCYE α) and it gives the **ground-glass** appearance colonies.
  • This can be made selective by the addition of cefamandole, anisomycin, and polymyxin B
- Growth might take 2-10 days it is enhanced by incubation in a candle jar or in 2.5% CO_2
- Colonies are pinpoint with a ground-glass appearance

Diagnosis:

- Inoculate BCYE α and CBA and look for growth versus no growth
- Are relatively inert and non-fermenter
- Catalase +
- Direct fluorescent antibody testing (positive in 25-50%)
- PCR

Virulence factors of Legionella:

- **Inhibit phagosome-lysosome fusion which allows for intracellular growth**
- Endotoxin (Lipopolysaccharide) less toxic than other G-ve
- Inhibit generation of bactericidal substances (like peroxide) in phagocytic cells

Clinical significance

Acute pneumonia (Legionnaire's disease):

- Airborne transmission with an incubation of 2-10 days
- The reservoir of infection is often in the **cooling towers of air conditioning systems and in hot water lines as well as in soil and water**
- **No** person to person transmission
- The disease occurs more in **males over 60** years of age and in the **immunocompromised**
- Symptoms are nonspecific including fever, chills, malaise, myalgia, headache, dry cough, vomiting, diarrhea, and abdominal and chest pain.
- **Hospitalization is usually required in 3-5 days.**
- Without antibiotics, the fatality rate is as high as 15%
- Disease rate after exposure is low (very low chance of reinfection)

Pontiac fever:

- An acute, self-limited febrile illness with an incubation of 24-36 hours.
- Symptoms include a high fever, chills, malaise, myalgia, and headache which lasts 2-5

days

- **Reaction to endotoxin or hypersensitivity to Legionella components**

Note that the first disease is caused by the infection while the second is caused by hypersensitivity

Treatment (antimicrobial susceptibility)**:**
Erythromycin, rifampin, clarithromycin or azithromycin

Prevention:
Legionella is resistant to chlorine and heat so this mean of cleaning air conditioners is not effective in prevention.
Prevention can be done by minimizing production of aerosols in public places.

:)