CPR: Cardiovascular, Pulmonary, and Renal
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Bacterial Respiratory Tract Infections I

Learning Objectives

1. Define Otitis media, acute sinusitis, pharyngitis, epiglottitis, community-acquired (typical vs atypical) pneumonia, hospital-acquired pneumonia, ventilator-associated pneumonia and aspiration pneumonia.
2. Know the most common bacterial pathogens associated with the infections listed above.
3. For the following pathogens: *Streptococcus pyogenes*, *Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae, Moraxella catarrhalis, Pseudomonas aeruginosa Enterobacteriaceae (Klebsiella pneumoniae), Bordetella pertussis, and Mycobacterium tuberculosis,* ***you should:***
    a. Know the basic characteristics of each pathogen.
    b. Know the method of transmission.
    c. Understand the risk factors for acquiring these infections (Ex: age, co-morbidities)
    d. Understand the pathogenesis of infection at the cellular level. – Is the pathogen extracellular or intracellular? If intracellular, what host cells are infected? What virulence factors aid the pathogen in establishing infection? What is the immune response to the pathogen? Does the immune response play a role in the symptoms of the infection?
    e. Describe the clinical presentations of each pathogen.
    f. Know the diagnostic laboratory tests that are used to identify the pathogen (Ex: special media requirements, biochemical assays, serology tests, etc.)
    g. Know the most common antibiotic treatment and/or vaccines that are available for these infections (also see Dr. Karpa's session on treatments for respiratory infections).

Upper Respiratory Tract (UTI) Infections

- **Otitis media**: infection of the middle ear. Common symptoms include: ear pain, fever, hearing difficulties, tugging or pulling at one's ear and irritability may be observed in young children. Bacterial otitis media usually occurs following a viral URT infection. Viral infections alter mucus production, mucocillary action of epithelial cells, pressure and even receptor expression in the Eustachian tube. These changes can allow nasopharyngeal bacteria (*S. pnuemoniae, Moraxella*, *H. influenzae*) access to the Eustachian tube and middle ear where they cause infection.
- **Sinusitis**: The sinuses are lined with respiratory epithelium that includes ciliated cells and mucus-producing goblet cells. Inflammation causes a marked decrease in the beat frequency of the cilia, as well as narrowing or obstruction of the sinuses due to mucosal edema. The resulting disruption of mucociliary transport results in sinusitis. Symptoms of acute bacterial rhinosinusitis include purulent drainage, nasal congestion, and sinus pain or pressure. Studies have shown that in adults over 50% of cases are due to *S. pneumoniae* or nontypeable *H. influenzae*. Other pathogens include other streptococci, anaerobes, *Moraxella catarrhalis*, and rarely *S. aureus*.
- **Pharyngitis**: inflammation of the pharynx or a sore throat. There are an estimated 12 million physician visits per year in the United States due to pharyngitis. The most common cause of bacterial pharyngitis is *Streptococcus pyogenesis.*

Lower Respiratory Tract (LRT) Infections

Bacterial infections of the LRT cause bronchitis, pneumonia and lung abscesses. Pneumonia (bacterial, viral, fungal and parasitic) is a leading cause of morbidity and mortality worldwide and in

the United States. Pneumonia presentations can range from a mild illness to severe, life-threatening infections. In fact, community-acquired pneumonia is the 8th leading cause of death worldwide and the leading infectious cause of death. There are many bacteria that can infect the lower respiratory tract depending upon the patient characteristics (Ex: age, zoonotic exposure, or co-morbidities such as cystic fibrosis), opportunity (Ex: bacterial pneumonia following viral infection), and setting (community or healthcare).

Bacterial pneumonia occurs when the alveoli of the lungs become inflamed due to bacterial growth. The lungs were once considered a sterile environment. We now recognize that bacteria routinely penetrate the lower respiratory tissues **through inhalation or microaspiration of our normal microflora**. The body's mechanical defense (ciliated epithelial cells) and immune system, normally remove these bacteria before they cause damage to the tissues. However, in the case of virulent pathogens or a heavy bacterial inoculum these defense systems can be overwhelmed. **Recent viral respiratory infections** are also a major contributing factor to bacterial pneumonia. Viral damage to ciliated epithelial cells, increased mucus and the inflammatory response to viral pathogens allow bacteria to more easily penetrate the LRT and provide a nutrient rich environment for bacteria to grow. Once bacteria begin to grow in the LRT, activation of the innate immune response will stimulate cytokine (IL6, IL8, TNFα) production and neutrophil recruitment. The actions of macrophages and neutrophils can create capillary leakage, leading to impaired oxygenation, hypoxemia and radiographic infiltrates. Bacterial pathogens may also release species specific toxins that impair tissue function.

Pneumonia is often classified as community acquired, hospital (healthcare) acquired, or ventilator associated. As described below, some bacterial pathogens are more commonly associated with a specific type of pneumonia. Pneumonia is often empirically treated; thus, knowing the most common pathogens responsible for a particular type of pneumonia helps guide antibiotic treatment choices.

- **Community-acquired pneumonia (CAP):** pneumonia acquired outside of a healthcare/hospital setting. Traditionally, CAP has been classified as **typical** and **atypical** based upon particular laboratory, radiographic and clinical features.
    - **Typical pneumonia** is caused by organisms such as *Streptococcus pneumoniae* (most common). Typical pneumonia is characterized by a sudden onset of fever, shaking chills, chest pain, sputum production, and lobar consolidation. The bacteria can usually be identified in the lab with Gram-staining and/or culture. Sputum can be purulent and contain blood. The description of sputum can sometimes be helpful in determining the pathogen. (Ex: Sputum from *Klebsiella* infections is often described as 'currant jelly.'
    - **Atypical pneumonia** usually refers to pneumonia caused by *Legionella pneumophila, Chlamydia pneumoniae* and *Mycoplasma pneumonia.* Some zoonotic organisms and respiratory viruses are also classified as atypical pneumonia agents. Infections with atypical pneumonia bacteria often present with a dry cough, minimal sputum production, extra-pulmonary symptoms, and patchy lung infiltrates. However, some infections with these organisms (Especially *Legionella*) can resemble infection with typical pneumonia bacteria. Atypical pneumonia agents also do not stain well with the Gram-stain, if at all and are difficult to culture using routine laboratory media.
- **Hospital-acquired pneumonia (HAP):** Also referred to as nosocomial or healthcare-associated pneumonia. Pneumonia that develops at least 48hr after admission to the hospital.

Ventilator-associated pnuemoniae (VAP) is an extension of HAP that often involves multi-drug resistant pathogens, such as MRSA and *Acinetobacter.*

Bacterial Respiratory Tract Pathogens

There are numerous bacteria that can infect the respiratory tract. In the next two sessions we will cover the most common bacterial URT and LRT pathogens. However, these sessions are not all encompassing. You will encounter additional pathogens in your other courses that can cause respiratory tract infections (Ex: *Yersinia pestis* which causes pneumonic plague was covered in Immno/Heme.).

Streptococci

Note Below is a summary of the important features of respiratory *Streptococci*. For a more detailed description of this genus, refer to Dr. Hayman's handout from the Endocarditis session.

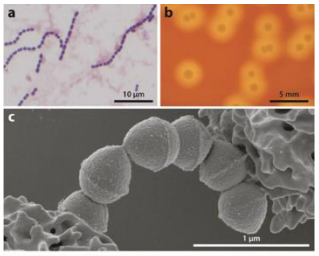
General Characteristics

- Gram-positive cocci growing in pairs (*S. pneumoniae*) or chains (*S. pyogenes*)
- Facultative anaerobes
- Catalase negative (differentiates *Streptococcus* from *Staphylococcus* and other pathogens)

Streptococcus pyogenes (Group A, GAS)

Gram-positive cocci arranged in chains; Lancefield group-specific carbohydrate A antigen and type-specific proteins (M protein) in cell wall, β-hemolytic

Virulence factors and Pathogenesis



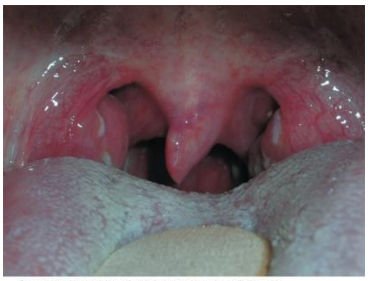
Olsen RJ, Musser JM. 2010. Annu. Rev. Pathol. Mech. Dis. 5:1–31

GAS is the most common cause of bacterial pharyngitis in school-age children (5-15yo). Human-to-human transmission occurs via respiratory droplets. Transmission is most efficient at short distances (<5ft). A small percent (<1%) of the population are asymptomatic carriers of *S. pyogenes*, providing a reservoir of infection. Upon entry into the URT, *S. pyogenes* colonizes the mucosal epithelium using pili, lipoteichoic acids and other surface adhesins to bind to epithelial cells. Surface expressed M-protein also contributes to *S. pyogenes* adhesion. GAS express a wide range of virulence effectors that contribute to nutrient acquisition, dissemination within infected tissues, and immune activation and/or evasion. Listed below are the GAS virulence factors that most contribute to its ability to infect the URT. (The following is not a complete list of GAS virulence factors. GAS express several additional virulence factors (toxins) that contribute to soft tissue and other infections, which are not listed here.)

- Streptococcal pyrogenic exotoxins (Spe): Superantigens that activate the release of proinflammatory cytokines. This toxin is responsible for many of the severe diseases caused by *S. pyogenes* (Ex: the rash associated with scarlet fever).
- Streptolysin S: Lyses erythrocytes, leukocytes, and platelets. This toxin is responsible for β-hemolysis seen on blood agar plates.
- Streptolysin O: Also lyses erythrocytes, leukocytes, and platelets. It is oxygen-labile. Antibodies are readily formed against this toxin – they are called Anti-Streptolysin O [ASO]

antibodies. They are useful for documenting recent group-A infections (anti-ASO test).

- Streptokinase A and B: Enzymes that mediate the cleavage of plasminogen, which activates the protease plasmin that in turn cleaves fibrin and fibrinogen. This results in the lysis of clots and fibrin deposits, which facilitates the rapid spread of *S. pyogenes* in infected tissues.



Clinical Presentations

1. Pharyngitis (Strep Throat): **Reddened pharynx with exudates generally present**; cervical lymphadenopathy can be prominent. Develops 2-4 days after exposure to pathogen. Symptoms include sore throat, fever, malaise, and headache. Patients with GAS pharyngitis typically do not have cough, rhinorrhea or other symptoms of viral URT infections. However, it is difficult to separate from viral disease based on presentation. Diagnosis made by bacteriologic or serologic tests [Rapid Strep Test, which recognized the Group A antigen].
2. Scarlet Fever: A complication of pharyngitis where the infecting *S. pyogenes* is lysogenized by a temperate bacteriophage that produces a pyrogenic exotoxin [Superantigen]. Within 1-2 days after initial symptoms, a **diffuse erythematous rash appears on upper chest and spreads to extremities**. No rash around mouth or on palms and soles. A raw – strawberry tongue develops after a yellowish-white coating sheds. The rash disappears after 5-7 days and it is followed by desquamation.
3. Rheumatic Fever: A complication of *S. pyogenes* pharyngitis. It is characterized by inflammatory changes involving the heart, joints (arthralgias to arthritis), blood vessels, and subcutaneous tissues. Heart involvement includes pancarditis (endocarditis, pericarditis, myocarditis). Chronic progressive damage to the heart valves may occur. It is believed that antibodies developed to the M-protein cross react with cardiac myofiber protein myosin (an example of molecular mimicry). Symptoms include fever, arthralgia, elevated CRP or sed rate, leukocytosis, rising ASO or DNase titer.
4. Acute Glomerulonephritis: Acute inflammation of the renal glomeruli with edema, hypertension, hematuria, and proteinuria (general symptoms include fever, headache, malaise, nausea, vomiting). Can arise following pharyngitis or pyodermal infections (10-14 days later). Diagnosis is based on clinical presentation and evidence of recent *S. pyogenes* infection. The exact pathology remains unclear, but it is believed to be a type III hypersensitivity reaction. Immune complexes (antigen-antibody formed during infection) become lodged in the glomerular basement membrane. Complement activation leads to destruction of the basement membrane.

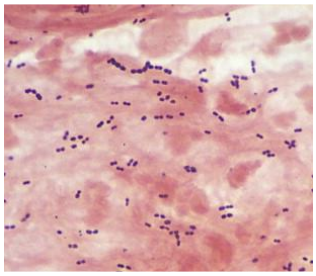
Diagnostic Tests

- Microscopy is not useful in pharyngitis or nonsuppurative complications because streptococcus species exist in the nasopharynx as normal flora.
- Direct tests for the group A antigen (Rapid Strep Test) are useful for the diagnosis of streptococcal pharyngitis (Antibodies in test recognize antigens of GAS), but negative results must be confirmed by culture or molecular assays.
- Culture: β-hemolytic colonies on blood agar. Isolates are identified by catalase (negative), susceptibility to bacitracin, and presence of group-specific antigen (group A antigen) (See the Staph/Strep laboratory flow chart provided in Dr. Hayman's Endocarditis Session)
- Antistreptolysin O test is useful for confirming rheumatic fever or glomerulonephritis associated with streptococcal pharyngitis; anti-DNase B test should be performed for glomerulonephritis associated with pharyngitis or soft-tissue infections because development of ASO Ab is less

prevalent from soft-tissue infections due to it being oxygen-labile and streptolysin O is irreversibly inhibited by cholesterol in skin lipids. Patients with cutaneous infections do NOT produce streptolysin O, and don't produce ASO Ab.

Treatment and prevention

- Penicillin V (oral) or amoxicillin. Starting antibiotic therapy within 10 days in patients with pharyngitis prevents rheumatic fever. For patients with a history of rheumatic fever, antibiotic prophylaxis is required before procedures (e.g., dental) that can induce bacteremia leading to endocarditis. No specific antibiotic treatment or prophylaxis is indicated for preventing glomerulonephritis.



Streptococcus pnuemoniae

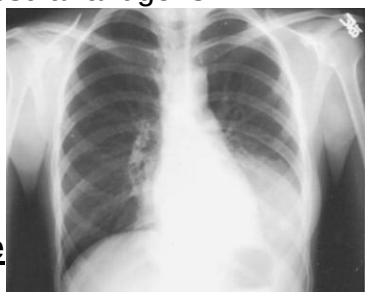
Gram-positive cocci arranged in pairs (lancet-shaped diplococci) and short chains; α-hemolytic

Virulence and Pathogenesis

S. pnuemoniae virulence determined by its ability to colonize the oropharynx (surface protein adhesions) as a member of the microbiota from a very early age. When given the opportunity (Ex: following viral infection) the bacteria spread into normally sterile tissues using virulence factors such as pneumolysin and IgA protease. Person-to-person spread through infectious droplets is rare. A characteristic of pneumoncoccal infections is the mobilization of inflammatory cells to the focus of infection. Teichoic acid, peptidoglycan fragments, and pneumolysin activate the immune response while the bacterium's polysaccharide capsule allows it to avoid phagocytosis and killing. Listed below are some important *S. pneumoniae* virulence factors.

Virulence factors

- Amidase augments the inflammatory process by enhancing the release of cell wall components.
- Pneumonlysin: Activates the classic complement pathway. It binds cholesterol in the host membrane and creates pores leading to destruction of ciliated epithelial cells. Is responsible for the α-hemolysis on blood agar plates (BAP).
- Polysaccharide Capsule: Prevents phagocytosis. Encapsulated strains are more virulent because of this protection. Non-capsulated strains are avirulent. Antibody production is directed to capsule antigens. Vaccines are also based on specific capsular antigens.



Clinical presentation

1. **Sinusitis and otitis media:** *S. pneumoniae* is a common cause and is usually preceded by a viral infection of the upper respiratory tract, after PMN infiltrate and obstruct the sinuses and ear canal. Otitis media is usually in young children, but sinusitis can occur in all ages.
2. **Pneumonia:** Pneumococcal pneumonia is the **most common cause of community-acquired pneumonia.** Individuals with antecedent viral respiratory tract disease or other conditions that interfere with bacterial clearance from respiratory tract are at increased risk for pulmonary disease. Pneumonia develops when the bacteria multiply in the alveolar spaces where there is nutrient-rich edema fluid. Erythrocytes, leaking from congested capillaries, accumulate, followed by neutrophils, then alveolar

macrophages. Resolution occurs when specific anti-capsular antibodies form, facilitating phagocytosis and killing. Pneumonia symptoms: abrupt onset, consisting of severe shaking chill and sustained fever. Often the bacterial pneumonia is secondary to viral pneumonia. Cough is productive (blood-tinged sputum). Often have chest pain (pleurisy). It is generally localized in the lower lobes (lobar pneumonia), but bronchopneumonia is common in children and elderly, too. Patients generally respond rapidly to antibiotic therapy. Mortality rate is ~5%.

3. **Bacteremia:** Occurs in 25-30% of patients with pneumococcal pneumonia and in more than 80% of patients with meningitis (does not occur with sinusitis or otitis media). Endocarditis can occur in those with previous valve tissue damage. People with hematologic disorder (e.g., malignancy, sickle cell disease) or functional asplenia are at risk for fulminant sepsis
4. **Meningitis:** Caused by spread to CNS after bacteremia, infections of ear or sinuses, or head trauma that causes a communication between the subarachnoid space and the nasopharynx. Pneumococcal meningitis is uncommon in neonates. Children and the elderly are at greatest risk for meningitis Neurologic deficits from *S. pneumoniae* meningitis is 4-20X more likely than from meningitis resulting from other organisms.

Diagnostic Tests

- Microscopy is highly sensitive, as is culture, unless the patient has been treated with antibiotics
- Nucleic acid–based tests are not commonly used for diagnosis
- Culture requires use of enriched-nutrient media (e.g., sheep blood agar); organism highly susceptible to many antibiotics, so culture can be negative in partially treated patients
    - Isolates identified by catalase (negative), susceptibility to optochin, and solubility in bile (a drop of bile is placed onto a colony. Autolysins will be activated and most of the colony will dissolve within minutes; called “bile solubility test”).

Treatment, and Prevention

- If susceptible, a penicillin is the drug of choice, although resistance is increasingly common.
- Ceftriaxone in the case of community acquired pneumonia with complications.
- Vancomycin combined with ceftriaxone is used for empiric therapy; monotherapy with a cephalosporin, fluoroquinolone, or vancomycin can be used in patients with susceptible isolates.
- **Vaccines:**
    - PCV: Pneumococcal Conjugated Vaccine (PCV13, PCV15 and PCV20)
    - PPSV23: Pneumococcal polysaccharide vaccine (PPSV23)
    - Prevnar-20 (a conjugated vaccine): Approved by FDA in June 2021. Covers the original 13 plus 7 more serotypes. The continued approval is contingent upon verification and description of clinical benefit. Antigens are conjugated to diphtheria toxoid; induces Th2 cells and class switching for long-lived memory. PCVs cover 80-90% of strains common in US, but not all serotypes are covered. PCV vaccines are recommended for immunization in most children under 2 years old because these young children fail to have a good immunological response to the PPSV. A PCV vaccine or PPSV23 is recommended for adults. (See https://www.cdc.gov/vaccines/vpd/pneumo/index.html if you would like more information about specific pneumococcal vaccine recommendations for different age groups.)

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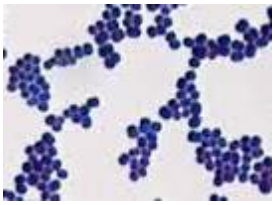
Staphylococcus aureus

Note The following is a summary of *Staphylococcus aureus* characteristics, pathogenesis and diagnostic features as they relate to pneumonia. For a detailed description of the genus *Staphylococcus*, see Dr. Hayman's Endocarditis handout.

***S. aureus* is the most virulent of all staphylococcus species and is one of the most important of all human bacterial pathogens.** It is a major cause of serious nosocomial as well as community-acquired infections, including LRT infections.



General Characteristics

Gram-positive cocci in Grape-like clusters
Facultative anaerobe
Common member of microbiome, particularly the skin and nasopharynx.

Virulence and Pathogenesis

S. aureus is a major cause of both minor and severe disease in humans. It causes disease through the production of toxins or through the direct invasion of and destruction of tissue. The clinical manifestations of some staphylococcal diseases are almost exclusively the result of toxin activity (e.g. SSSS, staphylococcal food poisoning, and TSS), whereas other **diseases result from the proliferation of the organisms, leading to abscess formation and tissue destruction** (e.g. cutaneous infections (impetigo, furuncles, carbuncles), endocarditis**,** pneumonia, empyema, osteomyelitis, septic arthritis).

Clinical Presentation

Pneumonia and Empyema

- *S. aureus* respiratory disease can develop after aspiration of oral secretion or from the hematogenous spread of the organism from a distant site. **Aspiration pneumonia is seen primarily in the very young, the elderly, and patients with cystic fibrosis, influenza, chronic obstructive pulmonary disease**. Empyema, collection of pus in the cavity between the lung and the membrane that surrounds it (pleural space), is caused by an infection that spreads from the lung. The accumulation of pus and fluid in the pleural space causes shortness of breath and pain. Empyema occurs in 10% of patients with pneumonia, and *S. aureus* is responsible for one third of all cases.

Diagnostic Tests

- **Culture:** growth of white-yellow, γ or β-hemolytic colonies on BAP
- **Mannitol Salt Agar:** Medium contains high NaCl and Mannitol. The high salt will inhibit most bacteria from growing but *S. aureus* can grow. *S. aureus* will ferment the carbohydrate mannitol and produce acid, which will change the color of the agar to yellow.
- **Catalase Enzyme:** Catalases are enzymes that catabolize hydrogen peroxide into water and oxygen gas. If a drop of hydrogen peroxide is placed on bacteria with catalase enzyme, bubbles of oxygen gas will be formed. This separates *Staphylococcus* from *Streptococcus*. **Staph are catalase (+); Strep. are catalase (-).**
- **Coagulase Enzyme:** Coagulase is a virulence factor that converts fibrinogen to fibrin. It is used by *S. auerus* to coat itself in fibrin molecules, which prevents immune effector cells from

accessing the bacteria. **This test separates *S. aureus (coagulase positive)* from all other Staphylococci**.

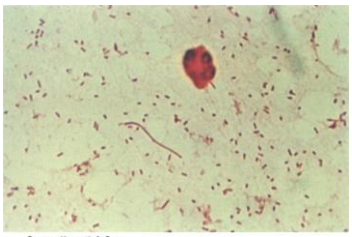
- **Nucleic Acid-Based Tests:** Commercial nucleic acid amplification tests are available for the direct detection of *S. aureus*.
- **Protein A Test:** *S. aureus* is **the only Staph strain** that has Protein A. It is located either in the peptidoglycan layer or on the cytoplasmic membrane. It has a unique affinity for binding the Fc receptor of immunoglobulin. Lab tests for protein A involves latex agglutination.

Treatment and Prevention
Penicillins (nafcillin, oxacillin) or Vancomycin (MRSA)

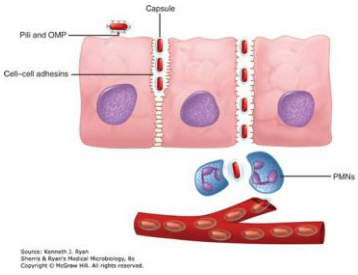
Haemophilus influenzae

Haemophilus influenzae is a common cause of upper and lower respiratory tract infections. Encapsulated *H. influenzae* are classified based on their capsule serotype, types A-F. Encapsulated strains, particularly serotype B (Hib), is often associated with more invasive diseases such as meningitis, cellulitis, epiglottitis and bacteremia in young children and infants. Non-typeable strains (no capsule) are common members of the microbiota and are often associated with otitis media, pneumonia, sinusitis and bronchitis.

General characteristics
Gram-negative pleomorphic bacterium (it can be found as coccobacilli or as long filamentous rods)
Fastidious growth requirements – requires X (hemin) and V (NAD+) factors
Catalase positive



Virulence and Pathogenesis
H. influenzae is transmitted via respiratory droplets. People are colonized by Non-typeable strains at an early age. Studies suggest that there is a temporal pattern to *H. influenzae* infections with a peak in Sept-Dec. and March-May. *H. influenzae* has a number of virulence factors including pili, outer membrane proteins, hemolysin, and sIgA protease which help with attachment to and invasion between mucosal epithelial cells. Typable strains (Types A-F) have a polysaccharide capsule made of polyribitol-ribosyl phosphate (PRP). This capsule is antiphagocytic allowing these strains to produce a more virulent infection than non-typeable strains, which lack a capsule.

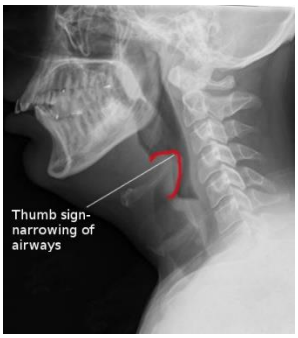


Clinical presentations

1. **Otitis media and sinusitis:** *H. influenzae* is a common cause of sinusitis and otitis media. These infections are usually opportunistic infections of non-typeable strains following viral URT infections or other inflammatory conditions like allergic rhinitis.
2. **Cellulitis** -inflammation of cellular tissue; presents as tender, reddish-blue swelling in the cheek or periorbital areas. Often follows a viral URT infection or otitis media.
3. **Epiglottitis** is a life-threatening condition that occurs when the epiglottis and surrounding tissues become inflamed, swollen and damaged due to infection. The initial presentation includes sudden onset of fever, sore throat, hoarseness and cough. This is followed quickly by

https://www.ncbi.nlm.nih.gov/books/NBK430960/

airway obstruction. A '**cherry-red' epiglottis** may be visualized upon examination and can be viewed on lateral X-rays as a **'thumb' sign**. Epiglottitis can occur in both children and adults but it is more common in children due to their smaller respiratory passageways. Hib historically has been the most common cause of epiglottitis. However, these cases have decreased with the introduction of a Hib vaccine for children. Other bacteria (Ex: Streptococci) and fungi have also been implicated in epiglottitis. Often the infection is secondary to a viral URT infection, but not always.

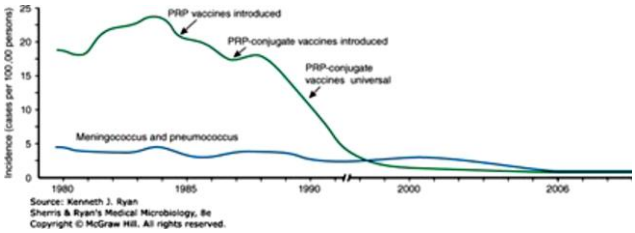


4. **Bronchitis/pneumonia:** *H. influnezae* may cause or exacerbate chronic bronchitis, particularly in patients with underlying illness (Ex: COPD) or who smoke. *H. influenzae* pneumonia presents similarly to pneumococcal pneumonia. It can be caused by both encapsulated and non-typeable strains, with encapsulated organisms being more common.
5. **Meningitis**- Dissemination of *H. influenzae* from local infections can lead to bacteremia and meningitis. The presentation is similar to other causes of bacterial meningitis. Hib is the most common strain responsible for *H. influenzae* meningitis. These infections are most common in children (2-5yo). Prior to vaccine development 1 of 200 children under 5yo developed invasive *H. influenzae* infections.

Diagnostic tests

- **Microscopy:** Gram-negative pleomorphic rods – elongated rods or tiny coccobacilli
- **Culture:** No growth on BAP. Positive growth on chocolate agar. Chocolate agar is boiled sheep's blood agar. Boiling the agar releases hemin (X-factor) and NAD (V factor) that is required for *H. influenzae* growth.

Treatment and Prevention



- Cephalosporins: ceftriaxone or cefotaxime.
- **Vaccine:** There is a vaccine for *H. influenzae* serotype B. It is a conjugate vaccine composed of capsular PRP and tetanus toxoid OR diphtheria toxoid. Conjugation of the capsular polysaccharides to a protein toxoid increases the T cell dependent response. The vaccine is given at 2 months old with boosters at 4 and 6 months, followed by one booster at 12-15 months. Prior to 1990 Hib was one of the most common causes of meningitis in infants aged 2-3 months. This vaccine is very effective having reduced the incidence of *H. influenzae* meningitis to a rarity in the U.S (shown in figure (green line)) More than 95% of infants have protective antibodies by the second or third booster.

Moraxella catarrhalis

Common opportunistic pathogen originating from the normal URT microflora. *M. catarrhalis* Colonizes the UTR within in 28-100% of humans in the first year of life. Infections often follow viral URT infections or occur in individuals with underlying illness or immunocompromised patients.

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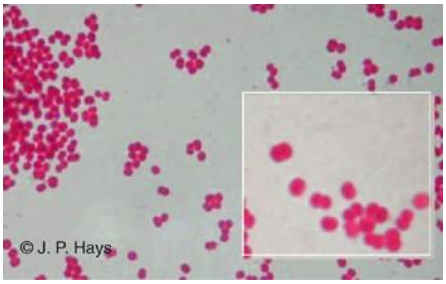
General Characteristics



Gram negative diplococci, can be mistaken for Neisseria sp.
Strictly aerobic, oxidase and catalase positive
In adults the colonization rate is 1-10.4%, predominantly those patients with COPD.

Virulence and Pathogenesis

M. catarrhalis has a large repertoire of adhesins that allow it to bind to and colonize respiratory epithelial. *M. catarrhalis* was previously thought to be an exclusively extracellular pathogen. Recently, it has been demonstrated that the organism can invade multiple cell types, including bronchial epithelial cells and type2 alveolar cells.

Clinical presentations

1. **Otitis media**- can occur in any age group but most often associated with children 1-48 mo. 15-20% of cases involve M catarrhalis.
2. **Sinusitis**- can occur in children and adults
3. **Bronchitis and pneumonia:** Present similarly to pneumococcal pneumonia. Children and adults that are immunocompromised or have underlying lung disease are most susceptible.

Diagnostic Tests

- **Microscopy:** small Gram-negative diplococci
- **Culture**: Appear as small, non-hemolytic, smooth white to grey, opaque, colonies on blood agar. The colonies are relatively dense and solid, a transfer loop can push a colony around without disruption on the agar surface “hockey puck sign”.

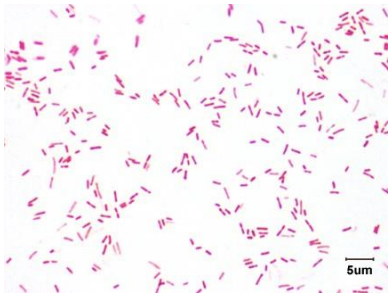
Treatment

Several antibiotics can be used, including Augmentin (amoxicillin/clavulanate) and cephalosporins. Infections are often treated empirically. Agents that are active against *S. pneumoniae* and *H. influenzae* in addition to *M. catarrhalis* are administered.

Pseudomonas aeruginosa

Ubiquitous organism found in soil and water. It is often found in non-sterile fluids such as humidifiers, contact lens solutions, and tap water. In healthcare settings *Pseudomonas* can be found in moist reservoirs such as sinks, respiratory or dialysis equipment, food, cut flowers, floor mops and even disinfectant solutions.

General Characteristics



- Motile, thin, aerobic Gram-negative rod
- Oxidase Positive –
- Catalase positive
- Associated with water.
- Minimal nutrition requirements and wide temperature growth range (4-42oC) – This allows *P. aeruginosa* to grow in many environments.
- Resistant to many antibiotics and disinfectants

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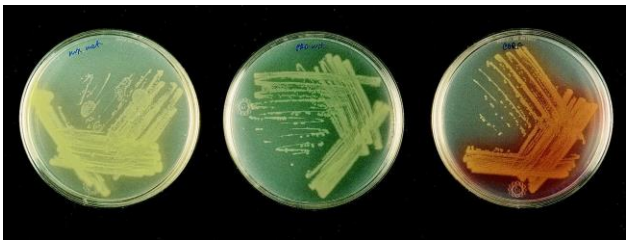
Virulence and Pathogenesis



P. aeruginosa express a wide variety of virulence factors (major ones listed below) and are notoriously resistant to antimicrobial treatments. Expression of some of its major virulence factors (Ex: Alginate and Exotoxin A) are coordinated by quorum sensing. Autoinducers are released into the environment. When the cell population reaches a threshold, the autoinducer concentration will be high enough it will activate a response regulator that will alter gene expression. Using this system *P. aeruginosa* can maximize the impact of its virulence factors by only releasing them when there are a lot of bacteria present to increase the concentration of that virulence factor.

Virulence factors

- **Adhesins**: Pili bind to epithelial cells promoting colonization
- **Capsule - Alginate**: Mucoid polysaccharide adhesin that forms the polysaccharide matrix for Pseudomonas biofilms. Anchors bacteria to epithelial cells and tracheobronchial mucin. Protects against phagocytosis and some antibiotics.
- **Pyocyanin**: Blue pigment that mediates tissue damage by impairing ciliary function and increases release of IL-8, stimulating the inflammatory response and production of toxic oxygen radicals
- **Exotoxin A:** An A/B subunit toxin that ADP-ribosylates host EF2. This disrupts protein synthesis by blocking peptide chain elongation in eukaryotic cells, leading to cell death. (Function is similar to diphtheria toxin but less potent.)
- **Elastases: LasA (serine protease) and LasB (zinc metalloprotease)** degrade elastin, which damages elastin-containing tissues (blood vessels, lung tissue, skin)
- **Phospholipase C**: Heat-labile hemolysin that breaks down lipids and lecithin

Clinical presentations

1. **Otitis externa (Swimmer's ear):** Infection of the outer ear canal. Symptoms include itching and redness of the ear canal, ear pain, and drainage of fluid from the ear.
2. **Pulmonary Infections:** Range from asymptomatic colonization to tracheobronchitis to severe necrotizing bronchopneumonia. Invasive disease usually presents as bilateral bronchopneumonia with microabscesses and tissue necrosis. Bacteremia and sepsis are possible if the infections are not treated successfully. Individuals with cystic fibrosis (CF), other chronic lung diseases, immunocompromised and those who use respiratory therapy equipment (nosocomial infections) are at increased risk for *P. aeruginosa* pulmonary infections.
    a. Upwards of 60% of cystic fibrosis patients are colonized with *P. aeruginosa.* Pseudomonas infections in CF patients are the leading cause of morbidity and mortality. Due to altered NaCl and water movement, cystic fibrosis patients develop thick mucus secretions that block the airways and provide an ideal breeding ground for Pseudomonas. In these patients, *P. aeruginosa* infections alternate between colonization and overt bronchitis or pneumonia. *Pseudomonas* often form biofilms in the lungs of CF patients which can't be cleared. This exacerbates the underlying disease by expressing a thick extracellular matrix (alginate).

Diagnostic Tests

- **Microscopy:** Gram-negative rods
- **Culture:** Grow easily on common media like blood agar. Colonies have a fruity (grape) odor and may have a metallic sheen due to the production of pigments (blue, yellow or rust). Pyocyanin: blue pigment, Fluorescein: yellow pigment that fluoresces with UV light.
- **Oxidase positive-** differentiates it from *Enterobacteriaceae* which are oxidase negative

Treatment

Pseudomonas are typically resistant to most antibiotics. *Pseudomonas* has been known to harbor a DNA island with as many as 50-genes related to antibiotic resistance. Many of these are beta-lactamases. Using laboratory Antimicrobial Susceptibility Testing is helpful when choosing the appropriate treatment.

- Empiric treatment for pulmonary and invasive infections should be aggressive including broad-spectrum antibiotics.
  - Cephalosporin (ceftazidime/cefepime) Fluoroquinolone (ciprofloxacin/levofloxacin), Anti-pseudomonal Penicillins (carboxypenicillins, ureidopenicillins) Carboxypenicillins (carbenicillin ticarcillin) Ureidopenicillins (piperacillin/tazobactam, ticarcillin/tb) Carbapenems (imipenem/meropenem), With or without: Aminoglycoside (tobramycin/gentamicin)
- Otitis externa can be treated with antibiotic eardrops and drying agents.

Aspiration Pneumonia and lung abscesses: Opportunistic *Enterobacteriaceae*

Aspiration Pneumonia occurs when a large amount of oropharyngeal or gastric contents are aspirated (macroaspiration) into the lungs. Risk factors include alcoholism, diabetes, COPD, altered mental status prolonged supine position, chronic vomiting, enteral tube feeding, and mechanical ventilation. These infections can be caused by bacteria commonly associated with CAP (Staph, Strep. Hib). Members of the gastrointestinal microflora (**anaerobes and Gram-negative rods**) can also cause aspiration pneumonia.

Enterobacteriaceae (Escherichia, Klebsiella,) are extracellular Gram-negative rods commonly found in the gastrointestinal flora. When introduced into the lungs, they cause opportunistic infections like pneumonia and lung abscesses. As gram-negatives these bacteria all express LPS which stimulates the immune response and contributes to their virulence. Symptoms of aspiration pneumonia include: fever, cough, **empyema** and **hemoptysis.**

***Klebsiella pneumoniae* is a common cause of CAP aspiration pneumonia.** It expresses a mucoid polysaccharide capsule that protects it from phagocytosis. The presence of blood and this thick capsule contributes to the **'currant jelly'** appearance of sputum caused by *K. pneumoniae*.

Bordetella pertussis

The genus *Bordetella* contains several pathogenic species that produce a similar illness. *B. pertussis* is the most virulent strain, causing pertussis – Whooping Cough. There are an estimated 50 million cases of pertussis annually worldwide. Humans are the only reservoir for Bordetella. Transmission occurs via respiratory droplets like other bacterial respiratory pathogens. However, *B. pertussis* is

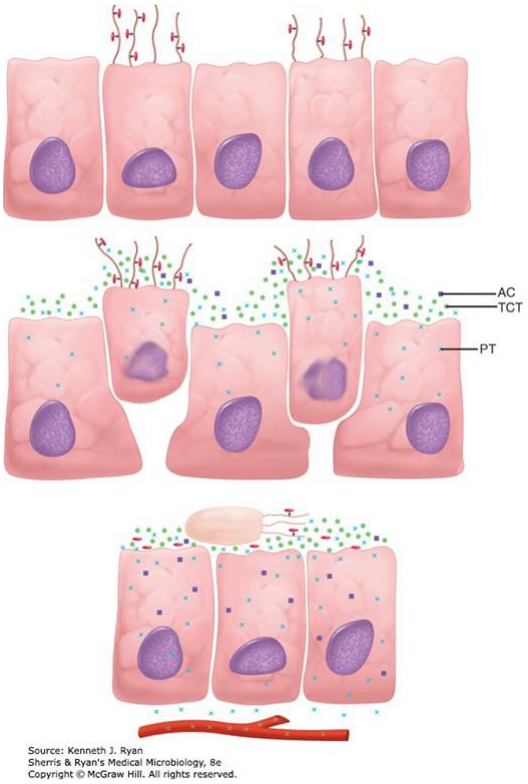
extremely contagious infecting upwards of 90% of exposed individuals. Asympotmatic carriers are a source of outbreaks. Vaccination programs have greatly reduced the occurance of pertussis, but outbreaks still occur every few years.

General Characteristics

- Small Gram-negative coccobacilli growing in singles or pairs
- Aerobic with fastidious growth requirements
- Oxidase positive

Virulence and Pathogenesis

Once inside the respiratory tract *B. pertussis* adheres to ciliated bronchial epithelial cells and colonizes. It produces several toxins (described below) that damage the epithelial layer and impair immune function. Eventually ciliated epithelial cells are destroyed impairing clearance of respiratory secretions. *B. pertussis* is non-invasive remains localized to the epithelial cells of the respiratory tract. Only the toxins are invasive and cause symptoms of disease.



- **Filamentous hemaglutinin (FHA):** Rod-like structures on surface the of *B. pertussis* named for their ability to agglutinate RBC. FHA contain an amino acid sequence that recognize host receptors such as host integrins, fibronectin and extracellular matrix proteins allowing the bacteria to attach to host ciliated epithelial cells. FHA also mediates attachment to complement receptors on macrophages.
- **Pertussis toxin (PT): Major virulence factor. A/B subunit toxin that ADP-ribosylates GTP-binding (G) proteins that alter inhibitory regulation adenylate cyclase activity.** This results in many effects including, inhibition of monocyte, NK and lymphocyte migration (causes lymphocytosis) and impaired immune cell function modifying several distinct G-proteins which are required for their intercellular signaling mechanisms. PT released from the site of infection can have systemic effects on other systems such as altered insulin secretion.
- **Adenylate cyclase toxin (AC, hemolysin):** Present on the surface of *B. pertussis* acting as a contact toxin and effecting only cells that touch the bacterium. Once activated inside a host cell it Converts ATP to cAMP, producing huge amounts of cAMP. It interferes with cellular signaling, chemotaxis, superoxide generation, and function of immune cells, including PMNs, lymphocytes, macrophages, and dendritic cells. It impairs macrophage function and induces apoptosis or programed death.
- **Tracheal cytotoxin (TCT):** Consists of fragments of cell wall peptidoglycan that are released by *B. pertussis* cells. Together TCT and LPS induce nitric oxide production which kills ciliated epithelial cells. Studies indicate that TCT is also directly toxic to epithelial cells.

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Clinical Presentation

Clinical Disease- Three stages (5-10 days)

1. **Prodromal (catarrhal**): Mild upper respiratory infection (1-2 weeks)
   - Symptoms resemble “Common Cold” with non-specific symptoms -fever, sneezing, rhinorrhea.
   - At this stage there are many bacteria attached to ciliated epithelial cells of bronchi, which are released in nasal secretions and respiratory droplets. This is the most contagious stage of the illness. Ideally, antibiotic treatment in this stage would be most effective at clearing the pathogen and preventing complications such as secondary bacterial pneumonia and neurological sequelae. However, most people do not seek treatment because they think it is just a cold.

2. **Paroxysmal:** Paroxysmal cough first appears (2-3 weeks)
   - This stage reflects killing of ciliated epithelial cells by TCT and Endotoxin
   - Patients experience episodes of paroxysmal coughing that can occur many times/day. Following a series of coughs there is a characteristic whoop that occurs when patients rapidly draw in air through the narrowed glottis.
   - Coughing can create anoxia in infants leading to neural damage.
   - Lymphocytosis and/or hypoglycemia can also occur
   - The paroxysmal cough with characteristic "whoop“ usually leads to a diagnosis in this stage when treatment is less effective b/c the toxins have already damaged the airways.

3. **Convalescent:** Bacteria are gone, cough gradually diminishes (may last for months)

Diagnostic Tests

- **Culture:** Definitive diagnosis depends on cultural isolation of *B. pertussis.* The best specimens are 2 nasopharyngeal swabs taken during the 1st or early 2nd stage. Cultured immediately on **Bordet-Gengou or Charcoal Horse Blood Agar:** provides nicotinamide
- **PCR** can also be used to identify *B. pertussis* DNA from nasopharygeal secretions

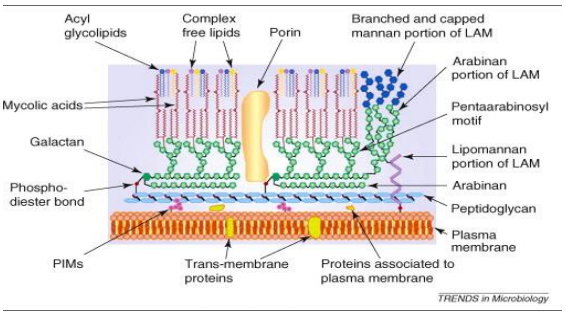
Treatment and Prevention

Macrolide (Azithromycin and clarithromycin): Must be given in prodromal stage to be effective. In the prodromal stage and beyond, supportive care (preventing dehydrations,suction to remove mucus secretions, oxygen therapy -especially in young infants) is the most effective treatment.

Prevention: There are currently two vaccines available in the US for pertussis. **DTap and Tdap.** Both vaccines protect against tetanus (*Clostridium tetani*), diphtheria (*Corynebacterium diphtheria*), and pertussis. Both are **acellular vaccines** and contain multiple pertussis antigens (PT, FHA, and others), diphtheria and tetanus toxoids. The current acellular vaccines have reduced side effects compared to earlier preparations that contained whole dead bacterial cells. However, the immunity produced by these vaccines is not as long-lasting as previous vaccine preparations. Thus, people need to receive multiple doses of the vaccine and boosters to maintain adequate immunity throughout life. This reduced immunity is thought to be contributing to increased outbreaks of pertussis, because immunity from the vaccine wanes after about 10 years. Adults who have been vaccinated previously usually have a milder form of the disease. However, infants who have never had the vaccine or maternal antibodies experience severe disease.

- Infants and children under 7 receive DTap: 2 months, 4 months, 6 months, 15 through 18 months, and 4 through 6 years.
- Adolescents: Tdap at 11-12 yo.
- Adults should receive a Tdap booster every 10yrs
- Women should also receive Tdap during every pregnancy to boost maternal antibodies that that provide early protection for the infant until they can be vaccinated.

Mycobacterium tuberculosis (Mtb)



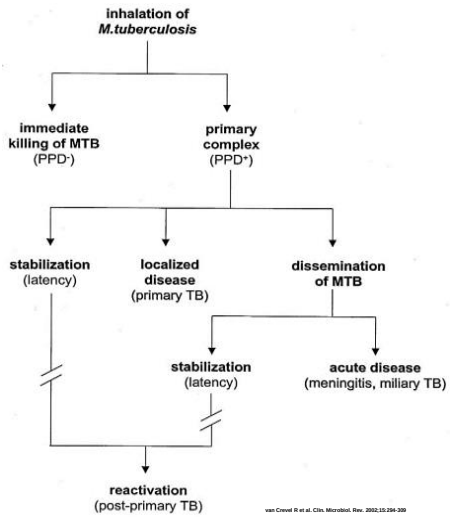
Note Below is a summary of *Mycobacterium tuberculosis*. Refer to Dr. Hayman's Endocarditis session for a more detailed description of this pathogen.

General Characteristics

- Facultative Intracellular Non-motile, Aerobic Rods, Branched filaments,
- Acid-Fast (Cording)
- Niacin Positive
- Slow growers -18-24hr generation time, Non-Pigmented
- **Lipid-rich *Mycobacteria*l cell wall → lipoarabinomannan (LAM; the molecule is related to LPS), arabinogalactin and MANY long chain (70-90 Carbons) fatty acids called mycolic acids** *The* mycobacterial *cell wall proteins are extracted and purified to make purified protein derivatives (PPD).*

Risk Factors for TB infection/Disease
Close/Prolonged contact with infectious people (especially infants)
Living or extensive travel to countries with high incidence of TB disease.
Immunocompromised Individuals HIV, organ transplants, immunosuppressive therapy, diabetes
Drug and alcohol abusers
Residents and employees of high-risk institutions (prisons, homeless shelters, etc.)
Medical Workers
Homeless
Age (old and young)

Epidemiology - Overall number of cases of TB in the US has declined in the last decade with only 8,916 cases reported to the CDC in 2019. However, the World Health Organization (WHO) estimates that 13 million people worldwide develop TB and 1.5 million dies from the disease each year, making it one of the top 10 causes of death: Highest incidence: Africa, Asian - China and India. **Cases of TB in the US decreasing # of infections. Most reported cases are in in Foreign-born individuals**



Virulence and Pathogenesis

Mtb are transmitted via inhalation of Airborne particles called **droplet nuclei** - **cough, sneeze, shout or sing**. Risk of developing active disease is 10% over the course of an immunocompetent person's lifetime. The risk increases with immunocompromised patients (HIV, diabetes). *Mycobacteria* are **phagocytized by alveolar macrophages.** They prevent death by **blocking phagosome/lysosome** and escape in to the cytosol. **(ESX-1 Mycobacterial Type VII**

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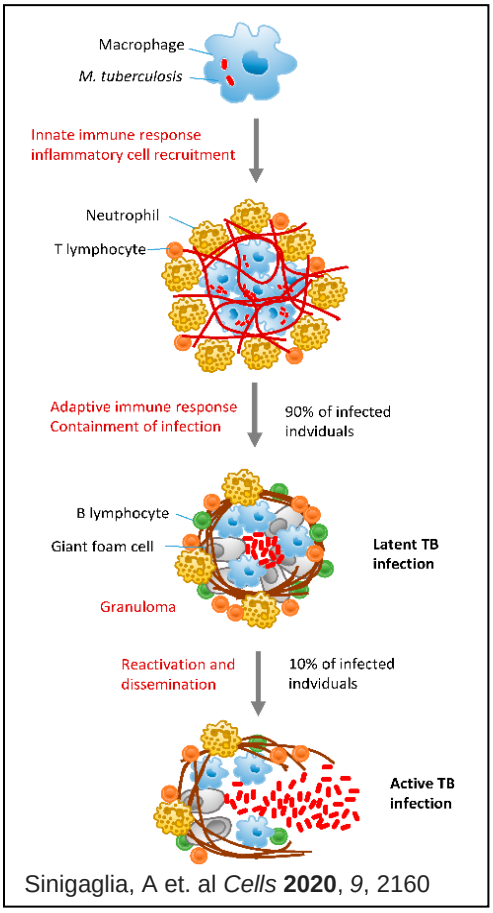
Secretion System effectors, ESAT-6, cell wall and cord factor). The infected macrophages are activated → TNF-α and IL-12 → recruits neutrophils →cascade of cytokines calls uninfected macrophages, dendritic cells, NK cells, $CD4^+$ $CD8^+$ T-cells and B-cells to the site → **Granuloma Formation**

* **Ghon Complex – Initial lesion + local hilar lymph node involvement**
* **GRANULOMA (TUBERCLE) -** Hallmark of *Mycobacteria* Infection

Infected macrophages, multi-nucleated giant cells (Langhans cells) and foamy macrophages are commonly observed inside the granuloma surrounded by layers of lymphocytes. Over time a fibrous capsule will form around the mass of infected/uninfected immune cells effectively 'walling-off' the infection. Some granulomas will calcify.



Sinigaglia, A et. al *Cells* **2020**, *9*, 2160

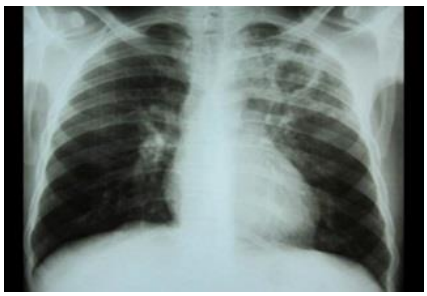
- ***Mycobacteria*l cell wall components (lipids, trehalose dimyocolate [cord factor], lipoarabinomannan, arabinomannan,** etc) are stimulators of the immune system. **Nitric Oxide (NO)** released from the activated immune cells (stimulated by TNF-α) kills or inhibits growth of bacteria. Hypoxic conditions inside the granuloma inhibit growth and stimulate dormancy response.
- **Latent TB infections (granuloma)** exist in the body for years until the immune system is impaired [age, AIDS, stress, diabetes, immunosuppressive medications (organ transplant patients, cancer patients etc)]. The latent infection can then transition to **Active Disease**. Mtb is able to overcome the immune response and actively grow in the tissue. The core of the granuloma becomes **necrotic and caseum accumulates in the center. The granuloma will rupture spilling the bacteria laden caseum into the airways and forming a cavity in the lung tissue.** This material is coughed up and the bacteria can be transmitted to the new host.

Latent TB Infection (LTBI)	TB Disease (in the lungs)
Inactive, contained tubercle bacilli in the body	**Active**, multiplying tubercle bacilli in the body
TST or blood test results usually positive	TST or blood test results usually positive
Chest x-ray usually **normal**	Chest x-ray usually **abnormal**
Sputum smears and cultures **negative**	Sputum smears and cultures may be **positive**
No symptoms	**Symptoms** such as cough, fever, weight loss
Not infectious	**Often infectious** before treatment
Not a case of TB	**A case** of TB

CDC: TB Learning Module 1

Clinical Presentations

Pulmonary tuberculosis Symptoms Cough with Hemoptysis, Fever, Unintentional, Weight Loss, Night Sweats and Fatigue

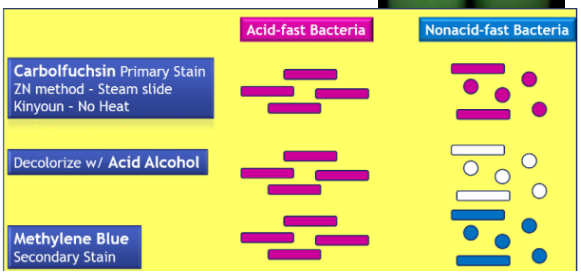


* **Extrapulmonary *Tuberculosis* - Pulmonary Infection may also be Present with extrapulmonary disease!**
  * **Meningitis** –Headache, Confusion, Stiff neck, Fever
  * **Scrofula** – Cervical lymphadenitis
  * **Pott's Disease** – TB infection in spinal column

    * Back pain -osteomyelitis and arthritis that usually involves more than 1 vertebra.
    * Vertebral collapse cause deformities, Paralysis
* **Genitourinary tract** – Flank pain, Dysuria, Blood in urine, Frequency of Urination
    * epididymitis (men), symptoms of PID (women)
* **Millary Tuberculosis –** Disseminated granulomas in any organ system
    * Children Under 5yo and Immunocompromised-AIDS
* **Carditis**

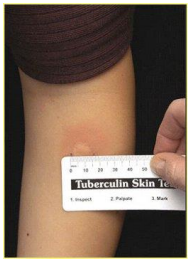
Diagnostic Tests

Laboratory



* Microscopy: Sputum Stain: Acid-Fast Bacilli
    * Kinyoun and Ziel-Neelsen stains
* Culture -Lowenstein-Jensen Agar – Weeks, Middlebrook Broth -Days
* Nucleic acid Amplification Tests (NAATs)
* Biochemical tests – Niacin positive
* Antibiotic Susceptibility Testing

Mantoux Tuberculin Skin Test (TST)



* Diagnosis and Screening
* PPD (tuberculin) is injected intradermally, Results 48-72hr
* A reaction site diameter of >15mm is Postitive for people with no known risk factors for TB. Those with significant risk factors or recent known exposure are considered positive if they have smaller (5-10mm) reaction site diameters.
* Delayed Type Hypersensitivity Reaction
* Positive after ~2-8 wks post exposure

IFNγ Release Assay

* Measures IFNγ released from T-cells sensitized by M. *tuberculosis* antigens

- **Booster phenomenon** exposed to TB a long time ago →immune response to TB antigens has waned. TST test is negative. Retested later→TST becomes positive (no exposure to Mtb) Initial test 'boosted' the patient's immune response
- **Anergy** occurs in patients with weakened immune systems who have lost the ability to react to the antigens.

TST	IGRA
Requires **two or more patient visits** to conduct the test	Requires one patient visit to conduct the test
Results are available 48 to 72 hours	Results can be available in 24 hours
Can cause **booster phenomenon**	Does not cause booster phenomenon
Reading by HCW may be subjective	Laboratory test not affected by HCW perception or bias
BCG vaccination can cause **false-positive** result	BCG vaccination does not cause false-positive result and infection with most nontuberculous mycobacteria does not cause false-positive result
A negative reaction to the test does not exclude the diagnosis of LTBI or TB disease -**Annergy**	A negative reaction to the test does not exclude the diagnosis of LTBI or TB disease

Treatment and Prevention

4-9 Month Drug Regimens -Daily and Weekly

Multiple drugs are included in the regimens to prevents resistance from developing

* **First line - Isoniazid, Rifampin, Ethambutol, and Pyrazinamide.**
* **Second Line -** Cycloserine, Ethionamide, Streptomycin, Amikacin/Kanamycin, Capreomycin, p-Aminosalicylic acid (PAS), Fluoroquinolones (Levofloxacin), Ribabutin and Rifapentine (may also be considered first line drugs)

Drug resistant *M. tuberculosis* is a significant problem. In 2012, 84 countries had reported at least one case of XDR-TB (including the US).

* **Multidrug-resistant TB (MDR TB)**
    * resistant to at least isoniazid and rifampin
* **Extensively drug-resistant TB (XDR TB)**
    * resistant to isoniazid and rifampin, plus any fluoroquinolone and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin)

BCG Vaccine -Bacille Calmette-Guerin – made from *Mycobacteria bovis.* Not routinely given in US. Can cause a false positive reaction on TST.