SHEET NO. 3

Microbiology

Respiratory system

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Bacterial Infections of the Respiratory tract 2

Color code: Text in black is the original slides, Red is what the doctor added!

-In this lecture, we'll talk about 2 organisms:

1) Streptococcus Pneumoniae (Gram +ve)
2) Haemophilus Influenzae (Gram -ve)

-So why are they together in this lecture you might ask? Well, for 2 reasons;

1) They have a similar spectrum of diseases they cause
2) They are both encapsulated!

-These capsules are their main virulence factor. They are Anti-Phagocytic and that gives the bacterium more time to multiply, they're also immunogenic and antigenic.

-Antibodies against the capsule are protective, and that is what forms the base of the vaccines that are available against both organisms.

-The capsules can be detected in blood, they're also the reason these organisms cause invasive diseases!

-Whenever we say "Most common" in this lecture we mean among bacteria only! Remember, upper respiratory tract illnesses are mostly caused by viruses!

Let's start!

-STREPTOCOCCUS PNEUMONIAE

-Pneumococci are gram-positive, lancet-shaped cocci arranged in pairs (diplococci) or short Chains (The term lancet-shaped means that the diplococci are oval with somewhat pointed ends (bullet like) rather than being round.

-All virulent strains have surface capsules, composed of High-molecular-weight polysaccharide polymers.

-On blood agar, they produce α-hemolysis, In contrast to viridans streptococci.

-They're lysed by bile or deoxycholate (Bile sensitive)

-They're also optochin sensitive.

Remember, S.pneumoniae are not applicable to Lancefield classification.

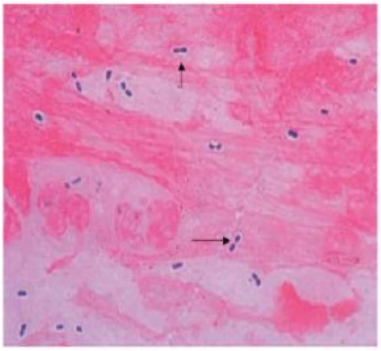


FIGURE 15–15 *Streptococcus pneumoniae*—Gram stain. Arrows point to typical gram-positive diplococci. Note that the clear area around the organism is the capsule. (Used with permission from Professor Shirley Lowe, University of California, San Francisco School of Medicine.)

-Pneumolysin forms pores after release by autolysins (This is the reason for the partial hemolysis they cause on blood agar!)

-Note that pneumolysin is **not** an **exotoxin**! And it's **never** released while the bacterium is still intact. After it undergoes autolysis, pneumolysin is released. (Old cultures of strep.pneumoniae tend to undergo autolysis, only younger cultures give the typical microscopic appearance).

Pathogenesis, virulence factors:

-The most important virulence factor is the capsular polysaccharide, and anticapsular antibody is protective.

-Lipoteichoic acid is a complement activator, it induces inflammatory cytokine production, it contributes to the inflammatory response and to the septic shock syndrome that occurs in some immunocompromised patients (A bit like protein A in LPS of gram -ve's). It helps impeding phagocytosis along with the capsule!

-Pneumolysin, the hemolysin that causes α-hemolysis, may also contribute to pathogenesis.

-Pneumococci produce IgA prote**ase** that enhances the organism's ability to attach and colonize the mucosa of the upper respiratory tract.

-Factors that lower the resistance and predispose persons to pneumococcal infection include: (Factors that reduce mucus clearing/immune reaction)

1) Anything that can depress the cough reflex; **alcohol** or drug **intoxication** or other cerebral impairments (Geriatrics, Cerebral vascular accidents, mental impairment, cerebral palsy), all contribute to an increased "Aspiration" of anything in the pharynx including secretions or the bacteria that resides there! (Thus pneumonia)

2) Abnormality of the respiratory tract (e.g. Viral infections (influenza virus which impairs the barriers (mucociliary function) allowing for superimposed bacterial infection due to lower clearance rates), pooling of mucus, bronchial obstruction, and respiratory tract injury caused by irritants (which disturb the integrity and movement of the mucociliary blanket) all prevent clearing of mucus and predispose to community acquired pneumonia caused by pneumococcus!

3) Abnormal circulatory dynamics (e.g. pulmonary congestion and heart failure) will congest the blood in the lungs (more stasis), increasing pulmonary secretions → pneumococcus

4) Splenectomy (Both **Complete splenectomy** (spleen is the most commonly bleeding organ after car accidents). **Functional Asplenia**, these people have auto splenectomy usually after infarcts in the spleen in people with **sickle cell disease**)

These people have reduced immunity especially against capsulated organisms; they're more susceptible to both organisms of today's lecture! For 2 reasons:
1) Macrophages that can remove encapsulated organisms are only found in the spleen, and they filter the blood, so with no spleen these encapsulated bacteria can seed anywhere in the body!
2) Impaired opsonization, a lot of opsonins are produced in the spleen!

And certain chronic diseases such as **nephrosis**!

5) Trauma to the head which causes leakage of spinal fluid through the nose predispose to pneumococcal meningitis. (If CSF was able to leak OUT, strep.pneumoniae can leak IN).

Transmission

-Humans are the natural hosts for pneumococci; there is no animal reservoir.

-Because a proportion of (5%-50%) of the healthy population harbors virulent organisms in the oropharynx, pneumococcal infections are not considered highly communicable (It often happens from your own flora!)

-Strep pneumonia is part of normal flora, nasopharyngeal/oropharyngeal swaps give a range of carriage rate between 5-50%, so 5-50% of the population has strep pneumonia and they don't feel ill! This variation in the carriage rate is due to **3 reasons; Age, season, outbreaks**; so, **children** have higher carriage rates compared to adults. Also sampling in **winter** will give higher rates than in summer. Swaps **during epidemics/outbreaks** yield much higher results compared to when it's only sporadic. So, 50% might be between children in winter and during an outbreak!

-This leads us to wonder whether the cause of the infection is inhalation of our own flora that resides in the pharynx or through transmission of exogenous strains from outside of your body? this is debatable, just remember that strep. pneumoniae is NOT THAT CONTAGIOUS! especially the pneumonia part (usually inhalation of flora) unlike otitis media/sinusitis in children which is usually through exchange of exogenous strains.

-Resistance is high in health young people, and disease results most often when predisposing factors are present.

-Transmission is through respiratory secretions (droplets/aerosols) (when exchanging new strain exogenous strains)

Diseases of strep. pneumoniae

-Streptococcus pneumoniae (pneumococcus) causes:
1) Pneumonia
2) Bacteremia – probably sepsis
3) Meningitis – (Bacterial septic meningitis)
4) URTI (Upper respiratory tract infections) – mainly otitis media and sinusitis

-Pneumococci are the most common (Bacterial) cause of **community-acquired pneumonia**, **meningitis**, **sepsis in splenectomized individuals**, **otitis media** and **sinusitis**

-Types of pneumonia are:
1) Community-acquired
2) Hospital-acquired (nosocomial)
3) Ventilator-acquired (patients on ventilators)

-They are a common cause of conjunctivitis.

Pneumonia

-Strep.pneumoniae is the **most frequent cause of pneumonia** with an estimated annual incidence of 1-3 per 1000 of the population, **with a 5% case fatality rate**.

-Pneumococcal pneumonia usually follows aspiration (!) with subsequent migration of through the bronchial mucosa to involve the surrounding lymphatics. (trachea → bronchi → alveolar sacs).

-The inflammatory reaction is **focused primarily within the alveolus of a single lobule or love, although multilobar disease can also occur.**

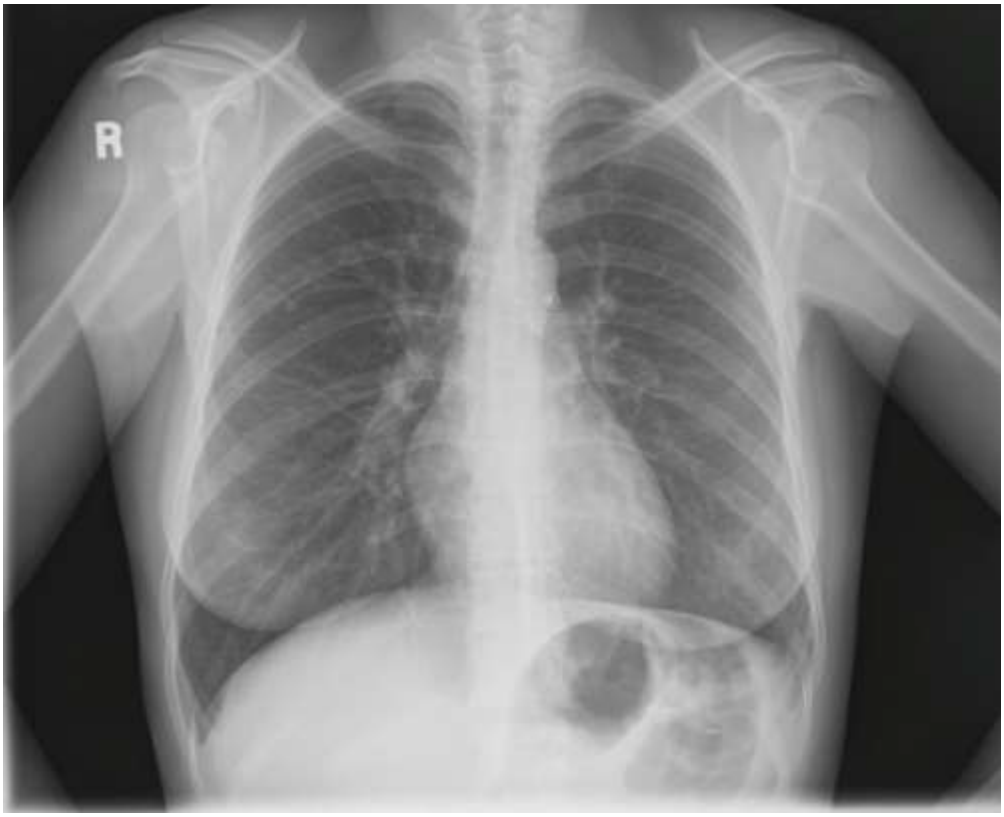
-They tend to consolidate in a certain lobe/lobule unlike viral pneumonia.

-The inflammatory reaction is where we have the multiplying strep.pneumoniae+PMN+edema.

-Contagious spread commonly results in inflammatory involvement of the pleura; this may progress to empyema. (Pus in space of the pleura)

-Pericarditis in an uncommon but well recognized complication.

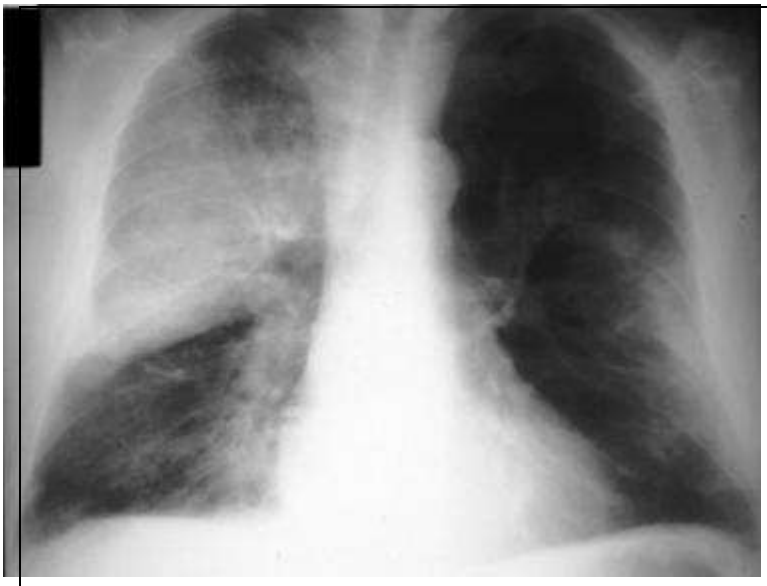
-This is the normal chest xray:

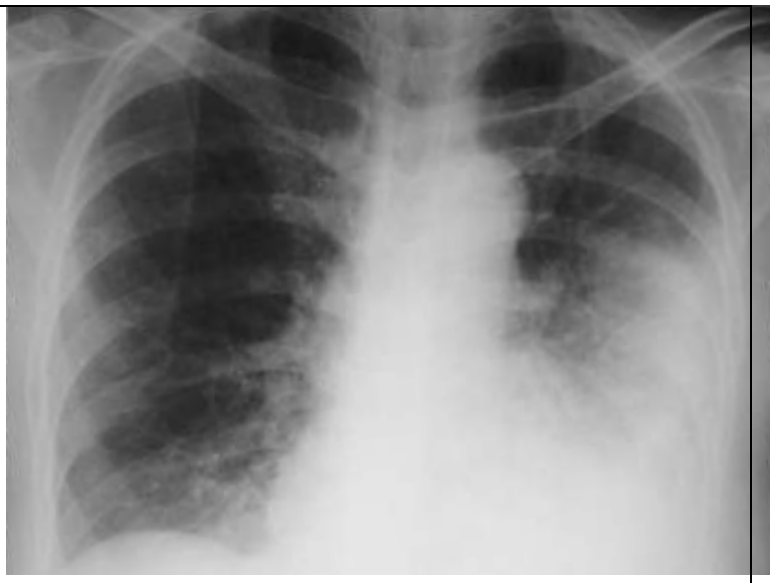


While these 2 below are bacterial pneumoniae Description include: (**the opacification is confined to certain lobule**

and they have **well demarcated lung fissure**, with **Air bronchogram (bronchi are spared of**

pathogenesis)





Right middle lobe Left lower lobe

-Note that the majority of these people will spontaneously recover, and they'll retain the normal Lungs (There will be no formation of dead spaces, no volume loss) we only have atelectasis (collapse of a lobe) occurring and after recovery everything will go back to normal.

-Occasionally, lung necrosis and intrapulmonary abscess formation occur with more virulent pneumococcal serotypes.

-Bacteremia may complicate pneumococcal pneumonia in up to 15% of patients.

-This can result in **metastatic involvement** of the meninges, joints and rarely the endocardium.

-The mortality rate from pneumococcal pneumonia in those admitted to hospital in the UK is approximately 15%.

-It is increased by age, underlying disease, bloodstream involvement, metastatic infection and certain types of pneumococci with large capsules (e.g. serotype 3). This serotype 3, among the 90 available, has high morbidity.

Otitis media and sinusitis

-Otitis media is also known as hot ear disease, It's a middle ear infection that affects approximately half of all children between the ages of 6 months and 3 years; approximately one-third of cases are caused by s.pneumoniae. (Tympanic membrane is red and appears bulging)

-Disease occurs after **acquisition of a new strain** to which there is no pre-existing immunity.(not your own flora)

-The prevalence is highest among children attending kindergarten or primary school. Where there is constant exchange of pneumococcal strains.

-Other less common causes of otitis media and sinusitis include Haemophilus influenzae and then Moraxella Catarrhalis

Meningitis

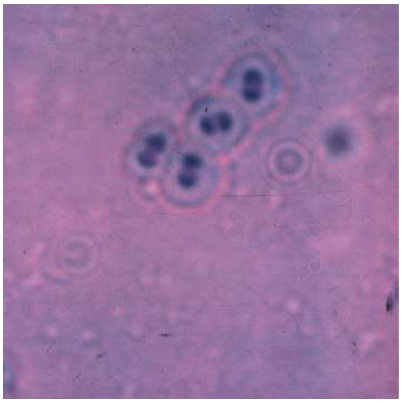
-Strep.pneumoniae is among the three leading causes of bacterial meningitis. It is assumed that invasion arises from the pharynx to the meninges via the blood-stream, as bacteremia usually coexists.
Meningitis may occasionally complicate pneumococcal infection at other sites, such as lung and middle ear.
So meningitis can be a complication over already formed pneumonia or direct access of the normal flora to the blood without an illness before.
-The incidence of pneumococcal meningitis is bimodal and affects children less that 3 years of age and adults of 45 years and above.
-The fatality rates are 20% (<3years)and 30%(>45years), respectively, considerably higher than those associated with other types of bacterial meningitis.

Clinical findings

-**Pneumonia:**
-Sudden chill, fever, Productive (contains sputum) cough, and pleuritic pain (Chest pain that increases with chest movement or breathing).
-Sputum is red or brown "**rusty**" color - (sputum color is an important indicator that can help in diagnosis of different infections; green in psudomonas, red in klebsiella (extra)).
-Bacteremia occurs in 15-25% of cases.
-Spontaneous recovery may begin in 5 to 10 days and is accompanied by development of anticapsular antibodies.
-pneumococci are a prominent cause of otitis media, sinusitis, mastoiditis, conjunctivitis, purulent bronchitis, pericarditis, bacterial meningitis, and sepsis.
-pneumococci are the **leading cause of sepsis in patients without a functional spleen.**

Laboratory Diagnosis

-In sputum: Lancet-shaped gram +ve diplococci in gram-stained smears.
-Can be detected using the Quellung reaction with multitype antiserum
Quellung reaction is a test where we add "omni serum" which contains 91 antibodies against 91 capsular antigens, we add this serum, this will cause the bacteria to clump together due to cross linkage of antibodies with halo around the clumps Look below for the pic
-On blood agar, pneumococci form small α-hemolytic colonies.
-The colonies are bile-soluble (lysed by bile), and growth is inhibited by optochin (optochin sensitive).
-Blood cultures are positive in 15-25% of pneumococcal infections



Quellung reaciton
Look how they clump together.
Observe the halo around each clump

On blood culture, we get α-hemolysis, optochin sensitive, bile soluble

Left Side

S. mitis

Resistant to optochin

Right Side

S. pneumoniae

Susceptible to optochin

Treatment

-Most pneumococci are susceptible to penicillins and erythromycin, although significant resistance to penicillins has emerged
-In severe pneumococcal infections, penicillin G is the drug of choice, whereas in mild pneumococcal infections, oral penicillin V can be used.
-A fluoroquinolone with good antipneumococcal activity, such as levofloxacin, can also be used.
-In penicillin-allergic patients, erythromycin or one of its long-acting derivatives (e.g., azithromycin) can be used.
-An increasing percentage of isolates, ranging from 15% to 35% depending on location, show high-level resistance, **which is attributed to multiple changes in penicillin binding proteins Remember this because it's an important difference compared to H.Influenzae**
-They do not produce B-lactamase. Vancomycin is the drug of choice for the penicillin-resistant pneumococci, especially for severely ill patients.
-; Ceftriaxone (3rd gen cephalosporin) or levofloxacin (Fluroquinolone) can be used for less severely ill patients.
So again, the mechanism of resistance here is changes in Penicillin binding proteins.

Prevention

-Despite the efficacy of antimicrobial drug treatment, the mortality rate of pneumococcal infections is high in immunocompromised (especially splenectomized) patients and children under the age of 5 years Such persons should be immunized with the 13-valent pneumococcal conjugate vaccine (Prevnar 13) (must be given booster doses every 5 years).
In the past, they used PCV-7 (before 2006), which is an abbreviation for pneumococcal conjugated vaccine, it contained 7 most common serotypes, now we use PCV13 against 13 serotypes!

-(Conjugated vaccines because polysaccharides are T-Independent so they conjugate it to get T dependent response which provides long protection)
-The immunogen in this vaccine is the pneumococcal polysaccharide of the 13 most prevalent serotypes conjugated (Coupled) to a carrier protein (Diphteria toxoid). The **unconjugated 23-valent pneumococcal vaccine (pneumovax 23)** should be given to healthy individuals aged 50 years or older (Booster doses at 65)
-These vaccines are safe and effective and provide long-lasting (at least 5 years protection).
-Under the age of **5** we give Prevnar 13 (5 doses at 2 months old, 4, 6, 14 ,18)
Over the age of **50** we give unconjugated pneumovax 23
Immunity of each vaccine lasts for **5** years

H.Influenzae

-H.Influenzae Gram -ve ROD encapsulated with a polysaccharide capsule.
-One of the three important encapsulated pyogens (Pneumococcus and the meningococcus)
-Using serologic methods against the antigen of the polysaccharide capsule, six serotypes are detected, with serotype B (group B) being the most significant one.
-These are in the 2nd position (The second most common when it comes to bacterial infections of the upper respiratory tract). They also cause a similar spectrum of diseases!
-Gram -ve bacilli (In older cultures) but we call them "Coccobacilli" so they're not either coccus or bacillus In younger cultures.
-Also, their main virulence factor is the capsule, with 6 serotypes depending on the capsule, but there is also

Nontypeable H.Influenzae, **no capsule**, **non invasive diseases**; **otitis media and sinusitis,** VERY IMPORTANT**,** they cause **acute exacerbation of COPD** and have **70-80% carriage rate as normal flora.** this rate is much higher that both strep pneumoniae and typeable H.Influenzae, why?
VACCINES! Lower carriage rates of typeable serotypes are due to vaccination (They're part of the national program now)
-**Typeable H.Influenzae** cause invasive diseases, typeable means that they have a capsule, they're typed depending on that capsule, Capsular antigens are called PRB, polyribitol polysaccharide capsule, we have 6 of these, (A,B,C,D,E,F) but 90% of invasive infections caused by typeable H.Influenzae are caused by **Type B**
-Serotype B is the one most responsible for the more serious illnesses (meningitis, epiglottitis, sepsis)
-The type B capsule is composed of polyribitol phosphate, promotes anti-phagocytosis and invasiveness.
-Unencapsulated strains are less invasive but can cause disease usually limited to the upper respiratory tract (sinusitis and otitis media).
-Growth of the organism on laboratory media requires the addition of two components, **heme (factor X)** and **NAD (factor V)**, for adequate energy production. "Fastidious bacteria" - selective cultures (staph aureus can provide the NAD)

Satellite phenomena

-Diseases H. influenzae used to be the leading cause of meningitis in young children
-Note we have 1 representative from each Gram reaction and shape that is a respiratory organism, the three capsulated ones are causative of meningitis and have vaccines made against the capsule:
-Pneumococcus G+ve coccus = capsulated respiratory organism causes mengitis and URTI
-Meningiococcus (Neisseria) G-ve coccus also capsulated which can colonize the respiratory epithelium
-and now the Gram negative ROD, Haemophilus is also a respiratory capsulated organism that is the third most common cause of meningitis.

Under gram stain, they're very small, coccobacilli.

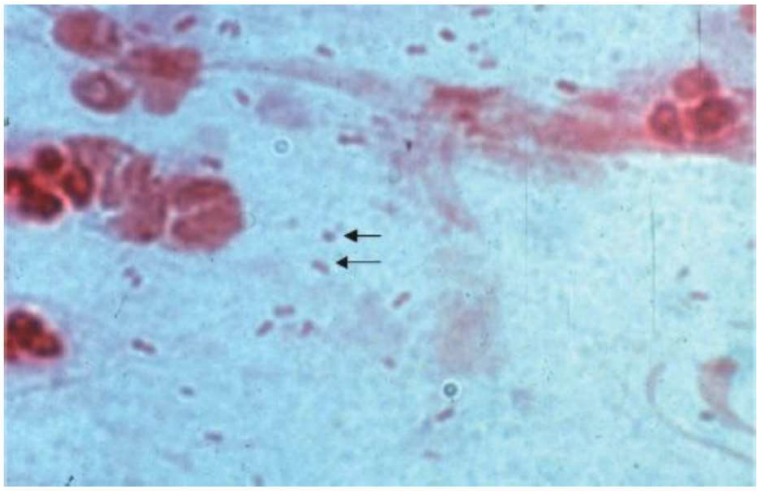
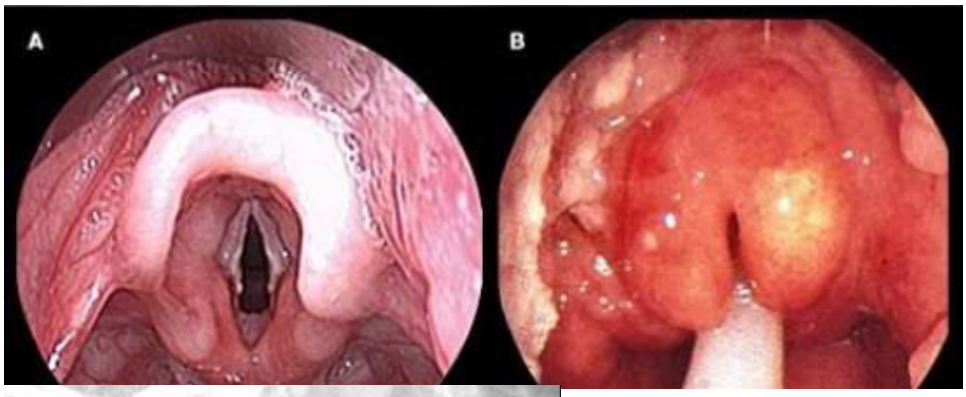


FIGURE 19–1 *Haemophilus influenzae*—Gram stain. Arrows point to two small "coccobacillary" gram-negative rods. (Used with permission from Professor Shirley Lowe, University of California, San Francisco School of Medicine.)

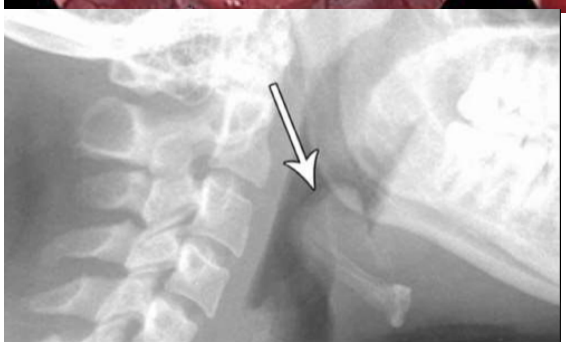
Pathogenesis is very similar to strep.pneumoniae, the new infection here is **Epiglottitis**
-H. influenzae infects only humans with no animal reservoir.
-Similar to other respiratory pathogens, it is transmitted by the inhalation of airborne droplets into the respiratory tract, this can result in a symptomatic colonization or infection (otitis media,sinusitis, pneumonia).
-Also like all respiratory pathogens, to be able to survive in this environment, the organism produces an IgA protease that degrades secretory IgA which would otherwise inhibit its attachment to the mucosa.
-After becoming established in the upper respiratory tract, the organism can enter the bloodstream (bacteremia) and spread to the meninges
-As mentioned, capsulated strains cause meningitis (they have to have antiphagocytic capability to survive the trip through the blood to reach the meninges, this is true for Pneumococcus and (Meningiococcus)
-meningitis caused by capsular type b has been greatly reduced by vaccine contains the type b polysaccharide as the immunogen.

-Similar to pneumococcus and meningococcus, the pathogenesis of H. infleunzae is pyogenic with **no exotoxin** production (capsule and endotoxin based)
-In epiglottitis, the epiglottis gets inflammed (called cherry-red epiglottis) with edematous surrounding tissue = airway obstruction! This is typically found in children, along with fever, drooling, stridor (A respiratory sound). We need to secure the airway in these patients, usually by tracheostomy or endotracheal intubation. So Epiglottitis is an emergency that we need to be careful while dealing with, it's rare but more common after vaccination!

Normal



Cherry-red epiglottis! **DON'T TOUCH IT** Or **laryngospasm occurs**



Lateral X-Ray of the neck, where we see **"Thumb sign"**

-**Epiglottitis** →rare, but can obstruct the airway and **CAN BE FATAL**. Upon inspection, a swollen "cherry-red" epiglottis is seen. This life-threatening disease of young children is caused almost exclusively by H. influenzae. Symptoms include, drooling, stridor (high pitched breathing noise) and comfort on sitting up
-Meningitis caused by H. influenzae produces a clinical picture that is almost identical pneumococcal or meningococcal meningitis.
-Meningitis → The rapid onset of fever, headache, **stiff neck**, (neurological symptoms; drowsiness), is typical.
-URTI → Sinusitis and otitis media cause pain in the affected area, opacification of the infected sinus, and redness with bulging of the tympanic membrane.
-H. influenzae is second only to the pneumococcus as a cause of these two infections.
-Other serious infections : septic arthritis, cellulitis, and sepsis(more in asplenic patients, due to the fact that this is a capsulated organism).
-Pneumonia in elderly adults, especially those with chronic respiratory disease, can be caused by untypeable strains of H. influenzae

Laboratory Diagnosis

Definitive diagnosis/ Gold standard: **Culture on chocolate agar with factor x and v (selective)**

-Need to isolate the organism to make the Dx, inactivated blood must be used (chocolate agar, to remove inhibitors of growth in the blood) enriched with two growth factors required for bacterial respiration (chocolate agar +factor x and factor V).

-Note; Chocolate agar is the same as blood agar, but the RBC's are lysed thus appears brown.

-An organism that grows on Chocolate+Factors X and V is assumed to be H.influenzae;other species of Haemophilus, such as Haemophilus parainfluenzae, do not require both factors.

-Quelling reaction (Antibody against the capsule which shows swelling of the capsule if contained the antigen for the provided antibody) can be used. Also, biochemical tests.

-Additional means of identifying encapsulated strains include **fluorescentantibody** staining of the organism and counter **immunoelectrophoresis** or **latex agglutination** tests, which detect the capsular polysaccharide. These ways work for both strep.pneumoniae and H.Influenzae

Treatment

Drug of choice: 3rd gen cephalosporins - Ceftriaxone

Ampicillins can be effective here but the **incidence of resistance is very high and it's due to** B-Lactam**ases** and **not** penicillin binding proteins!

-For meningitis and serious systemic infections (remember these are more invasive and aggressive) caused by H. influenzae the treatment of choice is ceftriaxone (3rd gen).

-From 20% to 30% of H. influenzae type b isolates produce a β-lactamase that degrades penicillinase-sensitive β-lactams such as ampicillin but not ceftriaxone.

-It is important to institute antibiotic treatment promptly, because the incidence of neurologic sequelae **(subdural empyema)** is high. (with meningitis)

-Untreated H. influenzae meningitis has a fatality rate of approximately 90%

Untreated fatality is very high! unlike strep.pneumoniae

-H. influenzae upper respiratory tract infections (such strains as mentioned are less aggressive and less invasive), that cause otitis media and sinusitis, are treated with either amoxicillin-clavulanate or trimethoprim-sulfamethoxazole.

Prevention

-Capsule= vaccine, so the vaccine contains the capsular polysaccharide of H. influenzae type b conjugated to diphtheria toxoid or other carrier protein.

-Depending on the carrier protein, it is given some time between the ages of 2 and 15 months.

-This vaccine is much more effective in young children than the unconjugated vaccine and has reduced the incidence of meningitis caused by this organism by approximately 90% in immunized children.

-Meningitis in close contacts of the patient can be prevented by rifampin.

-Rifampin is used because it is secreted in the saliva to a greater extent than ampicillin. Rifampin decreases respiratory carriage of the organism, thereby reducing transmission

-Household contacts especially in children are prone to develop the whole spectrum of H.Influenzae, so we need to protect them by giving Rifampin (Prophylactically).

Note: Rifampin has high concentration in respiratory secretions; highly potent against it, And a side effect is that it causes a red colored body secretions!

-It's part of the hexa vaccine program; (Diphteria, Tetanus, Cellular pertussis, Hepatitis B, Poliomyelitis and Haemophilus Influenzae type B).

The END! (But below, past questions + a table summary)! GOOD LUCK!

	Strep. pneumonae	H.Influenzae
Resistance development mechanism	Alteration of penicillin binding proteins	B-Lactamase production
Communicability	Not that communicable (Infections are often from your normal flora)	Highly communicable, prophylactic Rifampin for contacts!
Untreated Fatality Rate of meningitis	Low, spontaneous healing is common	Very high, up to 90%

An 18-month-old boy has been playing with a child who develops Haemophilus influenzae meningitis. The boy's parents consult his pediatrician, who says she is comfortable that the child will be fine because he has been fully immunized with the polyribitol ribose phosphate (PRP) – protein conjugate vaccine. For what reason is it necessary to immunize infants of 2 months to 2 years of age with polysaccharide – protein conjugate vaccines?

A) The conjugate protein is diphtheria toxoid, and the goal is for the infant to develop simultaneous immunity to diphtheria.
B) Infants 2 months to 2 years of age do not immunologically respond to polysaccharide vaccines that are not conjugated to a protein.
C) The conjugate vaccine is designed for older children and adults as well as infants.
D) Maternal (transplacental) antibodies against Haemophilus influenzae are gone from the infant's circulation by 2 months of age.
E) None of the above

Answer: B