Respiratory Tract Bacteria Part 1
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Lecture Objectives (**SLIDE 2**):

- To define the role of capsular polysaccharide in bacterial pathogenesis
- To relate this to three important pathogens *Streptococcus pneumoniae* (pneumococcus), *Haemophilus influenzae* and *Neisseria meningitidis* (meningococcus) that originate in the respiratory tract
- To introduce vaccination based on capsular polysaccharides
- To describe other important organisms (*Pseudomonas aeruginosa, Legionella pneumophila, Bordetella pertussis, Mycoplasma pneumoniae*). These gram-negative pathogens reside in a similar host environment and through different mechanisms cause a different spectrum of disease.

Some characteristics of the human respiratory tract (**SLIDE 3**):

- The upper tract (above the vocal cords) is colonized by an established and extensive microbial flora. Exceptions include the middle ear spaces and sinus cavities, which are sterile. Importantly, the healthy lower respiratory tract is essentially sterile
- Multiple host factors help maintain the sterility of the lung. Mucociliary flow, for example, is important in physically removing aspirated particles. Damage to mucociliary function will increase the risk of lung infection
- Respiratory tract flora can be a source of infection when it spreads beyond its niche in the oropharynx and nasopharynx, where not associated with symptoms, to normally sterile sites, where disease may ensue. The respiratory tract can serve as a site of entry for systemic infections (these will be covered in PART ONE)
- Non-resident organism that do not normally colonize the upper respiratory tract can be acquired by aspiration in hosts with defective clearance mechanisms (these will be covered in PART TWO)

Acute respiratory infection (ARI) is a leading cause of morbidity and mortality-particularly during early childhood (**SLIDE 4**) and in the elderly.
Community acquired pneumonia: Common organisms (**SLIDE 5**)

Streptococcus pneumoniae (30-50%)
Haemophilus influenzae (10%)
Mycoplasma pneumoniae (10%)
Chlamydia pneumoniae (8%?)
influenza virus (7%)

Staphylococcus aureus (2-5%)
Legionella species (3%)
gram negative Enterobacteriaceae (*Klebsiella pneumoniae*) (3%)
Chlamydia psittaci (1%)
Moraxella catarrhalis (0.5%)
other pathogens (8-10%)

1) Acute Respiratory Infection (ARI) is the leading infectious cause of death worldwide. In the US the rate for adults is 1/1000 per year.

2) Pneumonia can be caused by a wide variety of bacterial, viral, fungal, and parasitic agents that require different treatment.

3) Diagnosis remains limited in determining the specific agent involved. In most cases no specific agent is identified.

4) Treatment requires clinical decision making based on an understanding of differences in the presentation of likely agents.

PATHOGENESIS OF ENCAPSULATED BACTERIA:
One of the main host defense mechanisms against bacterial infection involves the complement system, which can be bactericidal through the generation of the membrane attack complex or opsonic (signaling recognition and engulfment by professional phagocyctic cells) when complement components are activated on the bacterial cell surface.

Successful pathogens circumvent complement (**SLIDE 6**).
O-antigen, chains of covalently linked sugar residues extending from the lipopolysaccharide prevents the membrane attack complex from acting in proximity to the cell membrane.
Capsular polysaccharide limits complement activation and antibody binding.
Extracellular pathogens must have mechanisms to evade phagocytosis! (Intracellular pathogens have mechanisms to survive once taken up into host cells.)

Capsular polysaccharides (CPS) are thought to have originally derived from other carbohydrate surface structures (LPS in gram negatives and teichoic acid in gram positives). These are porous, non-covalently attached zones of polysaccharide that completely envelop and protect the cell (**SLIDE 7**). Similar structures are found in yeast like *Cryptococcus neoformans,* which also are capable of invasive infection because of expression of this structure. These polysaccharides do not activate complement efficiently in the absence of specific antibody and, thus, serve to protect underlying bacterial cell-surface components. There are many types of CPS comprised of different sugar constituents or the same array of sugars linked in different ways.
Both O-antigen and CPS tend to be highly immunogenic and are often the dominant antigen on the organism. Because they are the target of selective immune presence in the host, there is often considerable structural and, thus, antigenic variation. Their structures, however, allow for

extracellular bacterial survival in the non-immune host until specific bactericidal and/or opsonic antibody to these polysaccharides is generated and the pathogen is cleared.

Expression of CPS is particularly important for an organism to survive for any length of time in the bloodstream (bacteremia or sepsis if overwhelming) where complement is most abundant (**SLIDE 8**).

This is demonstrated by the pathogens that cause meningitis as the ability to pass through the blood-brain barrier generally requires a sustained (rather that transient) bacteremia (SLIDE 9). The major causes of meningitis in newborns and later in life are all encapsulated organisms (**SLIDE 9**). Beyond two months of age all these organisms originate in the nasopharynx and are major causes of infection mortality worldwide (**SLIDE 10 and 11**). (The term meningitis refers to inflammation in the meninges of the brain or spinal cord. Meninges are any of the three membranes that envelop the brain and spinal cord. The disease meningitis is caused by a number of different bacteria and viruses.)

Some CPS structures may be particularly effective. For example, *E. coli* K1 and *N. meningitidis* serogroup B express a CPS made of sialic acid (a polymer of N-acetyl neuraminic acid), which is relatively poorly antigenic, possibly because it is an embryonic 'self-antigen'. The self-antigen nature of the same CPS on meningococcal type B capsule has also presented a problem in developing a vaccine against this organism. Mutagenesis of the genetic locus for CPS biosynthesis leaves these pathogens unable to cause invasive infection (avirulent) in model infection.

Streptococcus pneumoniae **(the pneumococcus) (SLIDE 12)**

Physiology and Structure (**SLIDE 13**):

gram-positive cocci, often as lancet shaped diplococci
α-hemolytic on blood agar
catalase negative, bile salt soluble

A discriminating characteristic used to identify the pneumococcus in the laboratory is it susceptibility to the ethyl hydrocupreine (optochin). (The development of spontaneous resistance to optochin was observed in 1911, 30 years before penicillin, predicting the selection for resistant bacteria.)

Diagnosis:

Sputum Gram's stain.

Culture of organism outside its niche in the upper respiratory tract (blood, CSF). This may be problematic for sputum culture due to contamination as the sample passes through the colonized upper airway.

Detection of antigen secreted in the urine.

Immunity to *Streptococcus pneumoniae*:

Antibody to the CPS is generally protective but requires many days to develop. Because of structural differences among capsules, acquisition of antibody to one type still leaves the host susceptible to the other non-cross reactive types (**SLIDE 14**). Over 90 unique types are currently known. Disease is probably more a function of its ability to trigger an inflammatory response than the expression of toxins.

Key Points: Pathogenesis and Immunity

- Polysaccharide capsule allows for evasion of complement and antibody until 'type' specific antibody develops
- Extensive variation in capsular polysaccharide types (antigenic variation)

Epidemiology of *Streptococcus pneumoniae*:

(**SLIDE 15**) The pneumococcus (which has been called "the captain of the men of death") is a common resident of the mucosal surface of the human nasopharynx (5-10% of healthy adults and 20-40% healthy children in the US; 10-20% of adults and >80% of the young children in the developing world). Humans are probably its only natural host. In the vast majority of instances it is a commensal. Carriage of a strain lasts for weeks to months until there is an antibody response to the specific capsule type and clearance. Person to person spread by colonized individuals through contact with secretions. Disease occurs most commonly in the non-immune <2 yrs or >65 yrs of age (when effective immunity wanes) and may be associated with a predisposing factor such as eustachian tube dysfunction or aspiration of nasopharyngeal contents or antecedent viral (especially influenza) disruption of the nasal mucosa (**SLIDE 16**). Conditions in which antibody production is impaired (such as hypogammaglobulinemia, AIDS) or there is defective clearance of opsonized bacteria by neutrophils and the reticuloendothelial system (such as splenic dysfunction) also predispose to pneumococcal infection

Clinical Diseases caused by *Streptococcus pneumoniae*:

Streptococcus pneumoniae is a prominent cause of lobar pneumonia (**SLIDE 17**)in which consolidation of the entire lobe has occurred. (Lobar infiltrate results from spread with in airspaces until tissue barriers are reached).This is the most common pattern for pneumococcal pneumonia. A closer view of the lobar pneumonia demonstrates the distinct difference between the upper lobe and the consolidated lower lobe.

Radiographically, areas of consolidation appear as infiltrates (Right middle lobe in **SLIDE 17**).

Histology of pneumococcal pneumonia (**SLIDE 19**)

A) Patchy area of alveoli that are filled with acute inflammatory cells. The alveolar structure is still maintained, which is why a pneumonia often resolves with minimal residual destruction or damage to the lung.

B) At high magnification, the alveolar exudate of mainly neutrophils is seen. The surrounding alveolar walls have capillaries that are dilated and filled with RBC's. This exudate gives rise to the productive cough of purulent yellow or rust colored sputum.

Treatment of *Streptococcus pneumonia* (**SLIDE 20 and 21**):
Until recently all strains were highly susceptible to penicillin. There has been a rapid and widespread acquisition of intermediate and high level resistance due to decreased affinity of penicillin binding proteins (Pbp's). Altered target (pbp genes) were obtained from other oral streptococcal species through the natural transformability of the pneumococcus. Pbp's with reduced affinity for penicillin also confer various degrees of resistance to modified forms of penicillin and cephalosporins. This was the first time that problematic antibiotic resistance occurred in a community-acquired organism. Current rates of penicillin resistance are proportional to local rates of antibiotic usage and generally range from widely from 10-40% non-susceptibility (intermediate or high-level resistance). Penicillin resistance frequently occurs in strains already resistant to other commonly used oral antibodies, leaving few treatment alternatives. β-lactams may still be useful against most pneumococci if administration achieves a concentration above the organisms MIC (minimum inhibitory concentration).

Resistance to beta-lactam antibiotics, as well as other classes, increased among pneumococci in the US in the 1990s (**SLIDE 22**). Although the rates of resistance fell briefly following the introduction of routine childhood vaccination for *S. pneumoniae* in 2000 (because many of the serotypes with the highest rates of resistance were among those targeted by the vaccine), antibiotic resistance rates are now rising again.

Key Points: Epidemiology, Clinical Diseases, and Treatment

- Colonizes the upper respiratory tract
- Carriage especially common early in childhood.
- Disease most common in infants and elderly (often in the setting of recent influenza)
- Important cause of infection in normally sterile parts of respiratory tract (pneumonia, Acute otitis media) or beyond (sepsis, meningitis)
- A leading cause of community acquired pneumonia-typically pneumococcal pneumonia is lobar
- Resistance to commonly prescribed oral antibiotics, including β-lactams, now common

Haemophilus influenzae

Physiology, Structure and Laboratory Identification (**SLIDE 23**):
A small, nonmotile gram-negative pleomorphic coccobacillus. Identification is by culture and its unique growth requirements. Aerobic growth requires two supplements, hemin (hence the "blood loving" designation) and nicotinamide adenine dinucleotide (NAD) also known as factor X and V, respectively.

Epidemiology and Clinical Diseases (**SLIDE 24-25**):
Next to the pneumococcus, probably the most common cause of respiratory tract infection. When first described in 1892 by Pfeiffer, *H. influenzae* was thought to be the etiologic agent of influenza (hence the name). The organism resides only in the human nasopharynx and carriage has been reported to be as high as 80% of the population. While type b disease is controlled by immunization with type b CPS, non-typeable *H. influenzae* (NTHi) is a leading cause of acute otitis media and conjunctivitis in children and exacerbations of chronic bronchitis (COPD) in adults.

Middle ear infection (**SLIDE 26**) is among the most common diseases of childhood (and the most common diagnosis resulting in antibiotic use and abuse). Poor drainage through the eustachian tube causes the accumulation of fluid in the middle ear space. This becomes infected with organisms residing in the upper airway that can transit up the eustachian tube. This process results in a painful ear with fever and evidence of inflammation on examination of the tympanic membrane.

COPD ranks fifth among causes of death in developed countries and is rapidly approaching similar levels in developing countries.

Pathogenesis, Epidemiology of *Haemophilus influenzae* type b (**SLIDE 27**):
Among capsulated strains, those with type b CPS—comprised of polyribose phosphate—are the most virulent. In the pre-vaccination era 'Hib' was a common cause of life-threatening pneumonia, bacteremia, epiglottitis and meningitis in young children >2 mo and <4 years of age. Beyond this age most people had naturally-acquired antibody to PRP and were protected. Immunity is based on antibody to CPS (typeable strains).

Treatment of *Haemophilus infuenzae*:
About 70-80% remain sensitive to penicillins. Resistance is mediated by the expression of beta-lactamase and so many cephalosporins remain effective. Resistance to other common oral agents is now common.

Key Points: Haemophilus influenza

- Gram negative coccobacillus that requires hemin and NAD for growth
- Commonly colonizes the upper respiratory tract
- Encapsulated strains, especially with type b capsular polysaccharide, able to cause invasive infection (but vaccination to the type b capsular polysaccharide has controlled this problem).
- Non-typeable strain are unencapsulated and cause infection mostly limited to the respiratory tract (pneumonia, acute otitis media)

Neisseria meningitidis

(**SLIDE 28**) The meningococcus, a Gram-negative, bean-shaped diplococcus, is identical in its staining and morphological characteristics to *Neisseria gonorrhoeae*. However, at the ultrastructural level, *N. meningitidis* has a prominent antiphagocytic polysaccharide capsule.

Structure and Classification

Meningococcal capsular polysaccharides provide the basis for grouping the organism. Twelve serogroups have been identified (A, B, C, H, I, K, L, X, Y, Z, 29E, and W-135). The most important serogroups associated with disease in humans are A, B, C, Y, and W-135.

Epidemiology of *Neisseria meningitidis*:

The organism tends to colonize the posterior nasopharynx of humans, and humans are the only known host. Between 5 and 20% of normal individuals are carriers at any given time, yet few develop meningococcal disease. Most individuals in close contact with a case of meningococcal meningitis become carriers of the organism.

Carriage rates are highest in older children and young adults in conditions that promote transmission (dorms, military camps) with the highest incidence during late winter and early spring. Disease tends to occur soon after acquisition of colonization, which has important implications for prevention of secondary cases. Meningococcal meningitis occurs both sporadically or in small clusters (mainly groups B, C and Y meningococci, each responsible for about 1/3 of cases in the US) and in epidemics (including yearly outbreaks in the sub-Saharan "meningitis belt", mainly group A but also W-135) (**SLIDE 29**). Note that it resides in the respiratory tract but doesn't cause disease there!

Clinical Diseases by *Neisseria meningitidis*:

Infection with *N. meningitidis* has two presentations, meningococcemia, characterized by skin lesions (Petechiae—minute hemorrhagic spots in the skin, or purpura—larger hemorrhages into the skin) (**SLIDE 30 and 31**) and acute bacterial meningitis. Meningococcemia (with or without meningitis) is characterized fulminant sepsis with rapidly progressive multisystem involvement and high mortality. This is thought to be mediated by endotoxin, although unknown host factors contribute to susceptibility. Meningococcal meningitis is also a complication of bacteremic spread but may lack the features of overwhelming sepsis and is thereby more prolonged and treatable. The manifestations of meningococcal meningitis are similar to acute bacterial meningitis caused by *Streptococcus pneumoniae* and *Haemophilus influenzae* including fever, altered mental status, headache, nausea, vomiting, and photophobia.

Immunity

The role of bactericidal antibodies in prevention of invasive disease explains why high attack rates are seen in infants from 6 to 9 months old, the time at which maternal antibodies are being lost. Individuals with complement deficiencies (C5, C6, C7, or C8) may develop meningococcemia despite protective antibody and are at high risk for disease.

Diagnosis

Isolation by culture on chocolate agar (hemolyzed blood) from normally sterile sites such as blood or cerebrospinal fluid. Neisseria possess an oxidative enzyme that turns purple with 'oxidase reagent'. It is differentiated from other Neisseria by oxidation of glucose and maltose but not sucrose or lactose.

Treatment

Penicillin or a third-generation cephalosporin remains the drug of choice to treat meningococcemia and meningococcal meningitis. Meningococcal disease is contracted through association with infected individuals, as evidenced by the 500- to 800-fold greater attack rate among household or close contacts than among the general population. Because such individuals are at high risk, they require prompt chemoprophylaxis.

Key Points: Meningococcus

- Gram negative diplococcus
- Colonizes the upper respiratory tract but not a major cause of respiratory tract disease
- Ability to cause invasive infection due to expression of capsule, of which there are several antigenically distinct forms comprising serogroups
- Cause meningitis and a fulminant disease meningococcemia.
- Disease often occurs in small outbreaks in individuals in close contact with an index case
- Prevention is important and includes vaccine (but none yet to serogroup b) and antibiotic prophylaxis in outbreaks

Prevention of Infection by Encapsulated Pathogens (**SLIDE 32**)

Because these are acute infections with virulent organisms that occur in the non-immune host (those with antibody to CPS are protected), treatment is often too late (example: meningococcemia). The morbidity and mortality in meningitis remains high even with treatment. In the elderly, pneumococcal pneumonia is often rapidly fatal. The only effective strategy is prevention and this requires generation of antibody to the protective antigen, the CPS.

In the case of the pneumococcus this is problematic for two reasons.
a) There are over 90 distinct CPSs with little cross-reactivity
b) Those at highest risk (the young and the old) have the poorest response to immunization based on CPS. The elderly make antibody but it is often of lower quality. Children under 2 years do not respond to oligosaccharide antigens.

Capsular polysaccharide-based vaccines

In the 1970's a 14- then 23-valent pneumococcal vaccine made from the 23 most common types of oligosaccharide (covering more than 90% of infections) was developed through the efforts of Dr. Robert Austrian (Univ. of Penn) and others (**SLIDE 33**). The vaccine is now recommended for high risk groups and all persons >65 years. Despite the availability this vaccine, rates of disease remain unacceptably high because it is underutilized.

Because this vaccine elicits a thymus-independent antibody response (**SLIDE 34 and 35**), it has several limitations:
1) No memory response
2) Lack of affinity maturation and impaired immunoglobulin class switching

3) Not immunogenic in children < 2 years of age.

Conjugate vaccines (SLIDE 36):

- ***Haemophilus influenzae* type b (Hib)**
- ***Streptococcus pneumoniae* (7-valent initially, now 13-valent)**
- ***Neisseria meningitidis* groups A, C, Y and W-135 (not B)**

In the 1980's a polysaccharide vaccine was developed for the type b CPS of *H. influenzae.* This was improved in the early 90's by covalently linking the PRP to a protein carrier (like diphtheria toxoid). This made the polysaccharide portion immunogenic in the highest risk group, young children, by converting the PRP carbohydrate component from a T-cell independent to a T-cell dependent antigen.

Immunity from conjugate vaccines decreases carriage and, thus, adds to efficacy because of 'herd immunity'. The dramatic success with Hib conjugate vaccine, which almost eliminated this serious source of disease, has spurred the development of other conjugate type vaccines that could be used to protect children <2 years of age (**SLIDE 37**). In 2000 a conjugate vaccine for the pneumococcus became available for children as young as 2 months of age. This contains the seven most common type CPSs conjugated to the diphtheria toxoid and is highly effective in reducing bacteremia and meningitis and to a lesser extent otitis media and pneumonia (**SLIDE 38**). The long-term problem may be serotype replacement by non-vaccine types because of selective pressure on colonization by the organism (**SLIDE 39**). Non-vaccine serotypes of pneumococcus, particularly 19A, have increased in frequency since vaccination began. In 2010, an expanded 13-valent pneumococcal conjugate vaccine replaced the 7-valent preparation in the US, but utilization in the developing world, where most needed, has lagged due to its high price. In the decade since pneumococcal conjugate vaccine was introduced, there have been an average of 168,000 fewer hospitalizations for pneumonia in the US (see http://www.nejm.org/doi/full/10.1056/NEJMoa1209165) – this appears to be due to decreased transmission from children to adults (protection of the unvaccinated population or herd immunity).
A quadravalent meningococcal conjugate is recommended at age 11-12 for protection during teen-age years.

PART II (SLIDE 40)

Bordetella pertussis

Physiology and Structure (**SLIDE 41**):

- Small, aerobic, Gram-negative coccobacilli.
- Localized infection of the ciliated surfaces of the upper respiratory tract following person to person transmission.

Pathogenesis.

A. Pertussis senses environmental signals such as temperature or divalent cations to effect specific changes in gene regulation (two-component signal transduction systems). This allows for detection of the host environment allowing for the production of pertussis toxin and other colonization factors and repression of other gene products not needed on the mucosal surface.

B. Organism produces several exotoxins (pertussis toxin, adenylate cyclase toxin and tracheal cytotoxin).

C. Pertussis toxin (**SLIDE 42**) has 6 subunits: 5 subunits are for receptor binding (B subunits), while 1 subunit (A) has ADP-ribosylating activity acting on guanine nucleotide-binding proteins (G proteins) which interferes with cell signaling. Similar to cholera toxin, inhibition of adenylate cyclase leads to overproduction of cAMP (also an effect of a separate toxin, adenylate cyclase toxin, made by *B. pertussis*), which act to promote production of respiratory secretions and interfere with neutrophil functions involved in clearance.

D. Pertussis toxin also acts to mediate binding to epithelial cells and block activity of immune effector cells. The toxin causes marked lymphocytosis, a hallmark of the disease.

E. Another product, tracheal cytotoxin, inhibits ciliary beating, which inhibits clearance by the mucociliary elevator.

Bordetella pertussis Epidemiology:

Highly contagious (person to person spread) and causes outbreaks, including outbreaks in hospitals.

Bordetella pertussis Clinical Disease (**SLIDE 43 and 44**):

Severe cough is the prominent symptom.
Three stages of infection:
1. Catarrhal stage—indistinguishable from viral upper respiratory infection or URI
2. Paroxysmal stage—episodic coughing, may or may not have inspiratory whoop following paroxysms of coughing.
3. Convalescent stage—may require weeks ("the 100-day cough"). Frequent complications in infants, who may develop pneumonia or apnea (failure to breath).

High morbidity and mortality in first six months of life. Also occurs in adults, especially with waning vaccine induced immunity, but seldom diagnosed. Thus, older children or adults can be the source of spread in a population. May be the cause of 5-10% of cough >3weeks duration in adults.

Bordetella pertussis Diagnosis and Treatment:

Diagnosis by fluorescent antibody (FA) testing or culture from nasopharyngeal swab.
Culture requires non-routine medium such as Bordet-Gengou medium (so not identified on routine cultures). PCR assays are more sensitive that culture and should be the preferred method of diagnosis when available.
Diagnosis often overlooked until late in course if illness, at which time bacteria are mostly gone and treatment does little except limit transmission.
Treatment is erythromycin or other macrolides, but usually is too late as toxin-mediated damage to the respiratory epithelium has already occurred. Treatment is important in limiting spread as pertussis is highly contagious. Treat close contacts.
Immunization is effective at prevention. An acellular vaccine (DTaP), which includes pertussis toxin and other factors, has a low rate of side effects and is now in use in the US.
Despite high rates of childhood immunization, pertussis remains a problem. Boosting immunity in teenagers and adults (with Tdap vaccination—a modified form of the booster vaccine used against diphtheria and tetanus) is now recommended to help control this problem (**SLIDE 45**).

Key Points: Pertussis

- Gram-negative coccobacillus that requires special media (so not identified in routine culture
- Toxin-mediated damage to respiratory epithelium results in prolonged illness characterized by paroxysmal cough
- High mortality in infants due to respiratory compromise
- Highly contagious
- Prevention by toxoid vaccine

Mycoplasma pneumonia (**SLIDE 46 and 47**)
This extracellular pathogen is unusual in lacking a cell wall, and therefore is not affected by antibiotics such as penicillin and beta-lactams that interfere with cell wall synthesis. This organism causes an "atypical" pneumonia characterized by insidious onset of fever, headache, malaise and cough. Cough is relatively non-productive of sputum. Fever and chills are generally less severe than with pneumonia from pneumococcus. Chest x-ray generally shows an interstitial infiltrate that is often patchy and involves multiple lobes. May be described as "walking pneumonia" due to lesser degree of severity.

Pseudomonas aeruginosa (**SLIDE 48**)

Physiology and Structure:

- Gram negative rod
- Non-fermentative, aerobic (oxygen or nitrate as electron acceptor)
- Minimal growth requirements so it can live in many environments
- Motile with polar flagella
- Some form mucoid colonies on culture plates
- Produces pigments
- Easily grown in the lab for diagnosis and characterization of antibiotic resistance patterns

Epidemiology (**SLIDE 49**):

Ubiquitous, opportunistic pathogen, able to infect animals, plants, invertebrates
Common in the environment, not common as host flora.
Readily takes advantage in many scenerios where there is host compromise (**SLIDE 50**),
Examples include chronic lung infection in cystic fibrosis (**SLIDE 51**), burn victims, corneal damage, ventilator associated pneumonia.

Widely distributed in moist, well aerated environments (damaged lung)

Intrinsically resistant to many antibiotics, antiseptics

Common in hospital sinks, basins, toilets (wet surfaces), respirators. Ability to form 'biofilms' makes eradication difficult(**SLIDE 52**) –think of dental plaque. This explains ability to infect intraveneuos catheters. Quorum sensing in response to production of autoinducers (derivatives of homoserine lactone) allow for coordinated activity such as construction of 'biofilms' that may allow for populating catheters.

Person to person spread unusual.

Diagnosis and Treatment (**described in SLIDE 53 and 54**)
(Added new SLIDE #55)

Pseudomonas aeruginosa Pathogenesis (additional features):

- Typical extracellular pathogen with mechanisms to bind to and damage host tissue and avoid clearance.
- Neutrophils are important in host defense. Individuals with poor neutrophil function or low numbers of neutrophils (e.g. chemotherapy) are at greatly increased risk.
- The pathogen also limits neutrophil mediated clearance by contact-dependent injection of toxins. For example, ExoA, an ADP-ribosylating enzyme with the same activity as diphtheria toxin, causes inactivation of elongation factor required for host protein synthesis.
- Airways of CF patients become chronically infected with *Pseudomonas aeruginosa*. These are typically mucoid strains that express alginate—a polysaccharide that blocks effective clearance of the organism.

Other difficult to treat opportunistic 'respiratory' gram-negative rods to be aware of:
(Added new SLIDE #56)

Burkholderia cepacia (formerly *P. cepacia)* is increasing in incidence, particularly in CF (in some CF clinics, *B. cepacia* is cultured from < 10% of pt., other clinics may be 40%.). Also distinguished from *P. aeruginosa* in CF by increased severity and rapidity of disease, increased transmissibility, and increased resistance to antibiotics. Antibiotic therapy as with *P. aeruginosa*, guided by susceptibility testing.

Stenotrophomonas maltophilia Broad spectrum of nosocomial infections, particularly pneumonia, with risk factors including underlying malignancy, central venous catheters and broad spectrum antibiotic therapy. Resistant to most β-lactam and aminoglycoside antibiotics but usually susceptible to sulfa-trimethoprim (In older texts and articles, this organism was called *Xanthomonas maltophila* or *Pseudomonas maltophilia.)*

Acinetobacter species Numerous species (e.g., *A. baumannii*) frequently found in nature & hospital environment. Emerging pathogen causing nosocomial pneumonia (particularly ventilator-associated pneumonia, in one study, 45% of tracheostomy sites had *Acinetobacter*), bacteremia associated with i.v. catheters, wound & skin infections, UTI, burns, eye infections; can be highly resistant to nearly all commercially available antibiotics but most strains are susceptible to doripenem. Resistance is increasingly becoming a problem and for doripenem-resistant strains, use combination therapy with aminoglycosides (gentamicin, amikacin) and polymyxin B. (Current problems with multiple drug resistant *Acinetobacter* in U.S. military hospitals due to injured soldiers infected in Iraq.)

Key Points: *Pseudomonas aeruginosa*

- Easily cultured gram-negative rod found in many environments
- An important source of infection in compromised patients
- A major challenge for treatment due to intrinsic and acquired resistance

Legionela pneumophila:

Structure and Physiology (**SLIDE 55**):

- Gram negative motile (flagella), small coccobacilli
- Nutritionally fastidious—does not grow on routine bacteriologic media
- Non-fermentative, energy from amino acids, unusual metabolism requires special medium to support growth
- Distinct phospholipid content in cell envelope—poorly stained by Gram stain

Pathogenesis (**SLIDE 56**)*:*

- Parasite of aquatic protozoa.
- Amoebae are thought to be the natural host in the environment.
- Bacteria falsely recognizing macrophages in human for its natural host (amoeba)?
- Able to survive in macrophages in an intracellular vacuole, multiply and spread. This contrasts with other intracellular pathogens such as listeria and salmonella, which escape from the phagolysosome.
- Dot/icm genes are required for intracellular growth – regulate host phagosome biogenesis and transport to modify the phagolysosome to suit the organism.
- When nutrients become limiting the bacteria become motile and seek a new protozoan host.

Epidemiology (**SLIDE 57**)*:*

Common in natural bodies of water, water distribution systems
(cooling towers). Outbreaks due to common source of contaminated water

Elderly (decreased immunity, pulmonary function) are at higher risk of disease

Correlation with heavy tobacco/alcohol use, men 2-3x more frequently infected than women. First reported outbreak in 1976 among legionnaires at a convention in Philadelphia resulted in 182 cases and 29 deaths from pneumonia.

Clinical Diseases (**SLIDE 58**)
Infections common but unreported (often asymptomatic or flu-like illness referred to as Pontiac fever). More severe disease results in pneumonia and extra-pulmonary complications

Defects in cellular immunity a risk factor (Antibody not thought to be important for this intracellular pathogen)

No person to person spread

Most likely source of infection – potable water, aerosol promotes transmission
Acquisition into respiratory tract by aspiration

Diagnosis and Treatment (**SLIDE 59 and 60**):
Requires antibiotics such as macrolides or fluoroquinolones that penetrate into host cells.
Urine antigen test useful as not routinely cultured

Key points: Legionella

- Gram-negative coccobacillus that does not grow in routine culture
- Intracellular pathogen obtained from aerosolized contaminated water sources
- Primarily causes pneumonia in compromised hosts