

Legionella, Bordetella and Haemophilus Gram Negative Rods of the Respiratory Tract

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READING:

Murray, et al.: Medical Microbiology, 6th ed., Chapter 37, *Legionella*, Chapter 35, *Bordetella* and Chapter 34, *Haemophilus*

LEGIONELLAE

Overview:

In 1976, *Legionella pneumophila* was recognized as a newly described pathogen after an outbreak of pneumonia among a group of Legionnaires at a convention. The disease was subsequently referred to as Legionnaires disease. Another flu-like form of the disease is referred to as Pontiac fever. *L. pneumophila* is now recognized as a ubiquitous aquatic saprophyte which causes epidemics and sporadic infections. The organisms are spread via aerosols and no person to person transmission has been reported.

Legionellae are facultative intracellular pathogens, which stain poorly as Gram negative rods. The causative agent was not recognized previously, since it does not grow on conventional agar such as sheep blood agar. Nowadays *L. pneumophila* is cultured on media that contains iron and cysteine vital for growth (e.g. charcoal yeast extract agar). However primary isolation is still difficult from clinical specimens.

Organisms of Clinical Importance:

After recognition of their unique culture characteristics, a large number of other species of *Legionella* were isolated from environmental and clinical samples. These organisms are only occasional causes of human disease and the vast bulk of legionellosis is caused by *Legionella pneumophila* (most are serogroup 1 and 6).

The second most common cause of pneumonia is *Legionella micdadei*. *L. micdadei* also stains weakly acid fast on primary isolation, but loses this property in vitro. This **DOES NOT MEAN** it is anyway related to the Mycobacteria.

Microbiology:

Legionellae are poorly staining Gram negative rods which are identified by growth on buffered charcoal yeast extract (BCYE), and require L-cysteine and iron for growth. The organisms are fairly slow growing requiring 3-7 days at 35⁰ C. Colonies are small with a ground glass appearance.

The Center for Disease Control (CDC) lists 4 tests for the identification of Legionnaires' disease: 1) culture, 2) urine antigen, 3) paired serology, and 4) direct fluorescent antibody stain. PCR tests for *L. pneumophila* in clinical specimens are available; however the CDC does not recommend the routine use of genetic probes or PCR for detection in clinical samples.

Public Health:

Legionella pneumophila is an organism that resides in the environment in pools of stagnant water worldwide. It is found as an intra-cellular agent within protozoa and a component of biofilms. Legionnaire's disease is recognized as a sporadic infection, often associated with travel, an epidemic disease of community-acquired pneumonia and a nosocomial infection. It often infects hot water towers and air conditioning systems. When found in buildings, anti-bacterial treatment of the water supply is recommended. The organism is transmitted in contaminated air but not spread person-person. Legionellosis is listed as one of the Nationally Notifiable Diseases by the Centers for Disease Control.

Clinical Presentation:

Legionellae present as 2 distinct clinical diseases. The first is Legionnaire's disease a typical pneumonia with an incubation period of 2 to 10 days. The mortality rate is as low as 20 % for healthy individuals and as high as 75% for the immune compromised. Legionnaire's disease is treated with erythromycin. The second form of disease presentation is Pontiac Fever. This illness has an incubation period of 1 to 2 days and is self-limiting with flu-like symptoms and no reported mortality.

Pathogenesis:

Pathogenesis of *Legionellae* species requires the organism be phagocytosed into monocytes via complement receptors. Once inside the monocytes the bacteria prevents phagosome lysosome fusion and proceeds to replicate until it lyses the phagosome which leads to apoptosis of the monocyte and release of the bacteria. Humeral immunity has little effect and the sensitized T helper (TH1) cells are required to activate the infected cells. Interferon- γ is also critical to the elimination of *Legionellae*.

BORDETELLA

Overview:

Bordetella organisms are small, gram-negative coccobacilli which are strict aerobes. The three species of this genus vary in motility and certain biochemical characteristics. The most important human pathogen in this genus is *B. pertussis*, the organism which causes **whooping cough**. *Bordetella* occurs worldwide and is strictly a human pathogen. The disease is spread via the respiratory route and the organism is non-invasive.

Organisms of Clinical Importance:

After *B. pertussis* 2 other species of *Bordetella* are also clinically relevant. *B. parapertussis* which can cause a mild pharyngitis and *Bordetella bronchiseptica* which is an animal pathogen (i. e. kennel cough in dogs). *B. bronchiseptica* rarely causes human disease, but can cause broncho-pulmonary symptoms in severely immunosuppressed individuals.

***Bordetella pertussis*-Microbiology**

Morphology and physiology:

It is an extremely small; slow growing, strictly aerobic, Gram negative, capsulated, **non-motile** cocobacillus (short rod). Compared to other Bordetella species, *B. pertussis* **does not grow on common laboratory media (sheep blood agar and MacConkey)**. Selected media include **Bordet-Gengou medium**.

	Growth on common lab media	Urease	Oxidase	Motility
<i>B. pertussis</i>	-	-	+	-
<i>B. parapertussis</i>	+	+	-	-
<i>B. bronchiseptica</i>	+	+	+	+

Diagnosis:

Symptoms are characteristic. Laboratory diagnosis is made by obtaining nasopharyngeal secretions. Primary cultures are obtained on Bordet-Gengou medium with incubation for 10-14 days. The organism grows as small transparent hemolytic colonies on blood agar. The slow growth rate makes direct fluorescent antibody testing on nasopharyngeal specimens a good diagnostic tool. PCR if available is highly sensitive and specific. Slide agglutination with specific antibodies is also used. Serologically *B. pertussis* can be distinguished from *B. parapertussis* and *B. bronchiseptica*.

Public Health:

Most of the patients with whooping cough are less than a year old, although older children may also get the disease and the incidence among adults has increased disproportionately in recent years. There were 199 cases of whooping cough in SC in 2006, 15, 632 in the entire U.S.

Clinical Presentation:

The organism, contained in aerosol droplets, gains access via inhalation and colonizes the cilia of the mammalian respiratory epithelium. The incubation period is 7 to 10 days followed by mild symptoms of rhinitis, mild cough and sneezing occur (catarrhal stage) which last 1-2 weeks. During the catarrhal stage, the propagation of the organism increasingly compromises ciliary function resulting in the increased frequency and intensity of symptoms. At this stage the organism can be recovered in large numbers from pharyngeal cultures, and the severity and duration of the disease can be reduced by antimicrobial treatment. The patient is highly contagious and not very ill.

After 2 weeks the disease progresses to the paroxysmal stage, characterized by gradually increasing prolonged and paroxysmal coughing that often ends in a characteristic inspiratory gasp (whoop). The cough recurs at variable intervals; often every few minutes and interferes with oral intake. The swallowed mucus may induce vomiting, resulting in severe dehydration and weight loss. Hypoxia during prolonged attacks may lead to seizure, hypoxic encephalopathy or coma.

During the paroxysmal stage, *B. pertussis* can rarely be recovered, and antimicrobial agents have no effect on the progress of the disease. This stage of the disease is mediated by a variety of soluble toxins.

The cough episodes gradually decrease after 2 to 4 weeks and the patient recovery can take 3-16 weeks (convalescent stage). Recovery is followed by immunity. Complications include pneumonia (due to other bacterial pathogens; rarely due to *B. pertussis*), otitis media, rectal prolapse and meningo-encephalitis.

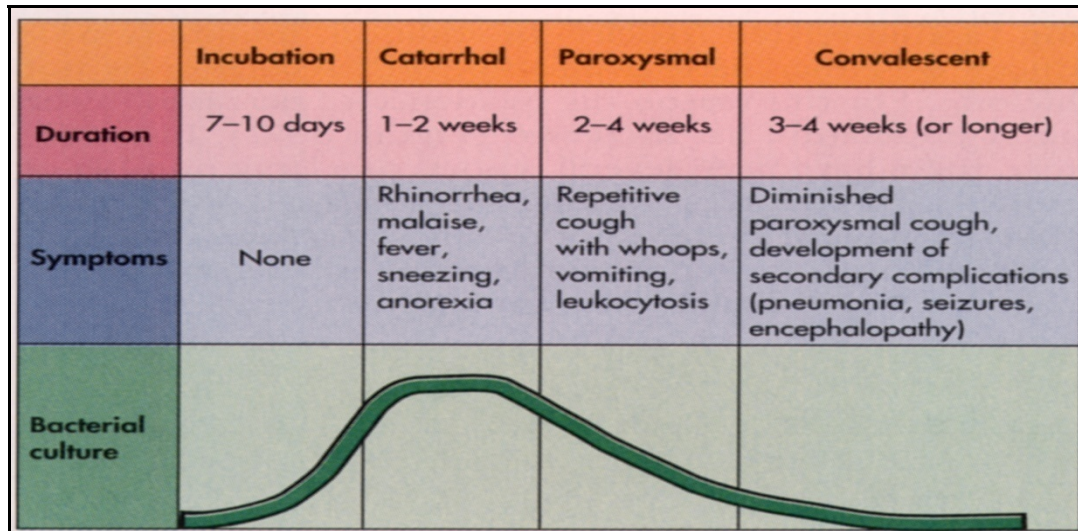


Figure 3: Disease Progression

Pathogenesis:

The symptoms following the infection are due to many factors. In addition to the attachment to and growth on ciliated cells, the organism produces a number of exotoxins which contribute to these symptoms. Virulence is regulated by a central locus, *bvs* which responds to environmental stimuli.

Colonization is mediated by filamentous hemagglutinin, and the pertussis toxin.

1. Filamentous hemagglutinins: These molecules are not exotoxins. They are filament associated lipo-oligo-saccharides which are involved in the binding of the organism to ciliated epithelial cells.
2. Pertussis toxin (pertussigen): It is an AB-type exotoxins with 6 subunits (A: subunit 1; B: subunit 2-5 complex) which is the major cause of the abnormal cough. The toxin is both secreted into the extracellular fluid and cell bound. **Subunits S2 and S3 function as adhesins**, and bind the bacteria to host cells.

The S1 subunit of pertussis toxin is an ADP-ribosyltransferase. The ADP-ribosyltransferase acts by the covalent addition of ADP-ribose to the GTP-binding Gi-protein and in so doing prevents the deactivation of adenylate cyclase. This results in the accumulation of large amounts of cAMP and consequently increased mucus secretion and the disruption of many cellular functions. Systemically it causes lymphocytosis, enhanced insulin secretions (hypoglycemia),

increased IgE synthesis, increased histamine production and endotoxin sensitivity. The toxin inhibits mitogenicity for T lymphocytes and inhibits chemotaxis, phagocytosis and respiratory burst as well as impairing NK cell killing.

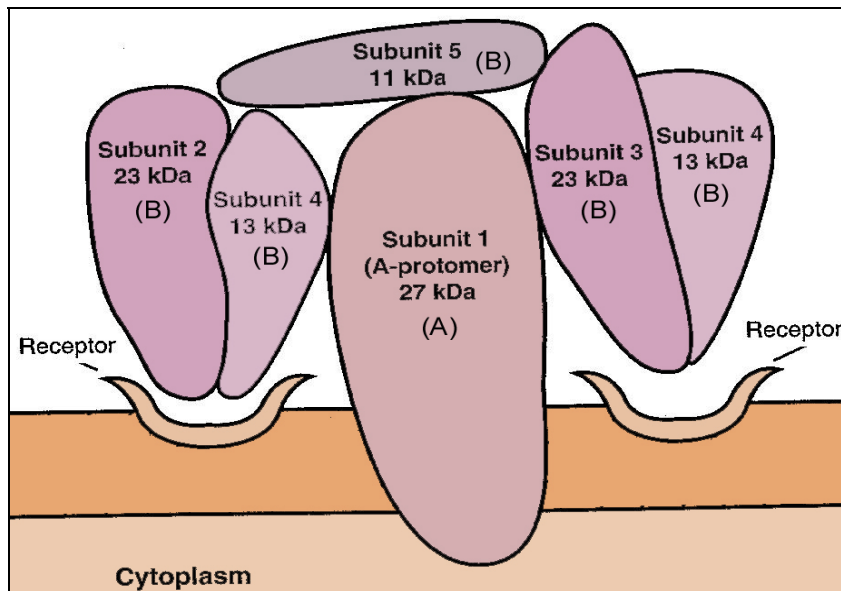


Figure 4: Binding of pertussis toxin to the cell membrane

Pertussis is primarily a toxin mediated disease. Localized damage and systemic symptoms are caused by the production of various toxins.

1. Adenylate cyclase toxin: This exotoxin acts locally to inhibit phagocyte and NK cell functions. It also helps the organism initiate infection. Adenylate cyclase toxin is only active in the presence of calmodulin and catalyzes the conversion of ATP to cAMP. This exotoxin was originally identified as a hemolysin since it lyses red blood cells. However, the cAMP increase caused by this exotoxin, in contrast with pertussigen, is short-lived.
2. Dermonecrotic (heat-labile) toxin: This toxin causes inflammation and local necrosis adjacent to sites where *B. pertussis* is located. It is also a very strong vasoconstrictor and causes extravasation of leukocytes. In association with tracheal cytotoxin, it causes necrosis of the tracheal tissue.
3. Tracheal cytotoxin: It is a peptidoglycan-like molecule which binds to ciliated epithelial cells, preventing the ciliated cells from beating (ciliostasis). This virulence factor is not regulated by *bvg*. It also causes extrusion and destruction of ciliated epithelial cells. The destruction of these cells contributes to the symptoms of pertussis. The extrusion of ciliated cells leads to mucous plugs resulting in pulmonary obstructions and atelectasis.
4. Lipopolysaccharide (LPS): Like LPS (endotoxin) of other Gram-negative bacteria, it is pyrogenic, mitogenic, and can activate and induce tumor necrosis factor production in macrophages.

Prevention and treatment:

Erythromycin is the antibiotic of choice. Antibiotic treatment during the catarrhal stage may ameliorate the disease. However, antibiotic treatment once the paroxysmal stage has begun may have no apparent effect on the course of the disease.

The pertussis vaccine used in the U. S. is an acellular vaccine consisting of filamentous hemagglutinins and detoxified pertussigen. These components are combined with diphtheria and tetanus toxoids in the DTaP vaccine.

HAEMOPHILUS

Overview:

Members of the genus *Haemophilus* are small, nonmotile Gram-negative bacteria. The genus contains many species but *H. influenzae* is the most common pathogen. *Haemophilus* are present in the normal flora of the human mouth and respiratory tract. *H. influenzae* **type b polysaccharide capsule** is the most virulent, although some non-encapsulated (non typeable) strains are also pathogenic.

Organisms of Clinical Importance:

Other species of *Haemophilus* that are normal flora and rarely cause disease are *H. aegyptius* (pink eye [purulent conjunctivitis]), *H. influenzae aegyptius* (Brazilian purpuric fever), *H. parainfluenzae* (pneumonia and endocarditis), and *H. aphrophilus* (pneumonia, endocarditis). *H. ducreyi* is the causative agent of chancroid, a sexually transmitted disease.

<i>Organism</i>	<i>Hemolysis</i>	<i>Growth factor</i>	
		<i>X</i>	<i>V</i>
<i>H. influenzae</i>	-	+	+
<i>H. aegyptius</i>	-	+	+
<i>H. ducreyi</i>	-	+	-
<i>H. parainfluenzae</i>	+	-	+
<i>H. aphrophilus</i>	-	-	-

***Haemophilus influenzae*-Microbiology**

Morphology and physiology:

The organism is a small Gram negative rod which can be grown on chocolate agar (supplemented with IsoVitalex) and requires hemin (factor X) and nicotinamide-adenine-dinucleotide (factor V) for growth. Growth with 5% CO2 is enhanced. It does not grow on normal blood agar. The factor V and factor X requirement can be used to distinguish between *H. influenzae* which requires both, *H. parainfluenzae* which requires factor V only and *H. ducreyi* which requires factor X only. *H. influenzae* will only grow on sheep blood agar around colonies of *Staphylococcus aureus* (satellite phenomenon).

H. influenzae strains are divided on the basis of capsular polysaccharides (a-f) or the absence of a capsule (nontypeable). The type b capsule is composed of polyribose-ribitol phosphate (PRP).

Diagnosis:

Presumptive diagnosis is based on history, physical examination and symptoms. Cultures should be obtained from CSF (meningitis symptoms), blood, pleural fluids (pneumonia), and middle ear aspirates. The organism grows well in culture on the appropriate media (chocolate agar). Blood cultures may be delayed since commercially prepared blood culture broth does not contain X and V factors. Gram staining can facilitate a presumptive diagnosis. All invasive disease should be serotyped.

Public Health:

H. influenzae nonencapsulated (nontypeable) organisms are part of normal flora of the respiratory tract. The carrier rate for nontypeable strains is 50-80% of individuals. The carrier rate for *H. influenzae* type b is 2-4% with 95% of the invasive disease caused by this strain.

There were 2436 cases of all types of *H. influenzae* in the U. S. in 2006, 40 of those cases were in South Carolina, up 5 cases from 2005. The availability of the *H influenzae* type-b (Hib) vaccine has significantly reduced the cases of Hib meningitis in children between 5 months and 5 years of age, as well as older children, adolescents and adults. Before the availability of the vaccine *H. influenzae* was the most common cause of meningitis in children ages 5 months to 5 years with mortality rates of 90% in untreated cases.

Hib infection initially causes a mild upper respiratory disease (runny nose, low grade fever and headache) followed within 1-3 days by meningitis. This **invasive** organism enters the circulation then crosses the blood-brain barrier. The resulting meningitis is rapidly progressing and can result in death. Timely treatment may prevent complications and death. Complications include deafness, epilepsy and mental retardation. Hib may also cause septic arthritis, cellulitis, pneumonia, and epiglottitis, the latter results in the obstruction of the upper airway and suffocation.

Diseases caused by *H. influenzae* since the introduction of the Hib vaccine include: septic arthritis, osteomyelitis, cellulitis, pericarditis, pneumonia (most frequent is serotype f), otitis media (*S pneumoniae* and then nontypeable Hf are the most common), sinusitis, chronic bronchitis and purulent bacterial conjunctivitis

Pathogenesis:

The presence of a capsule, which is anti-phagocytic, is a major factor in virulence. *H. influenzae* **does not produce any exotoxins**. Type-b *H. influenzae* are more invasive and pathogenic than other strains. The lipopolysaccharide is responsible for the inflammatory process. All virulent strains produce neuraminidase and an IgA protease which may aid their mucosal colonization.

Treatment and prevention:

Unless prompt treatment is initiated, *H. influenzae*-b meningitis and epiglottitis have a high mortality rate. Antibiotics of choice are cefotaxime sodium, ceftriaxone sodium or ampicillin in combination with chloramphenicol. Three Hib conjugate vaccines are available in the U. S. Each vaccine consists of capsular PRP conjugated to a protein carrier and is a part of the recommended routine vaccination schedule.

Non *H. influenzae* species

H. ducreyi is an extracellular pathogen which is the major cause of human genital ulcer disease (chancroid) in developing countries (Asia, Africa, and Latin America) but, less commonly in the United States. A significant concern is that the genital ulcers of chancroid have been epidemiologically associated with sexual transmission of HIV virus.

Chancroid presents with single or multiple painful necrotizing ulcers at the site of infection, frequently accompanied by painful swelling and suppuration of regional lymph nodes. In males, most ulcers are found on the prepuce near the frenulum or in the coronal sulcus. The infection is generally asymptomatic in women, but most lesions are found at the entrance of the vagina. Incubation is generally 3 to 14 days after exposure followed by a tender papule that becomes pustular then ulcerates over the course of 2 days.

H. ducreyi is a fastidious organism and laboratory diagnosis is made by isolation of *H. ducreyi* from lesion exudates and serology. The organism can be isolated from the ulcerated chancroid exudate and a stained smear shows Gram-negative short rods in parallel rows of small rods in chains.

The incidence of chancroid in the U. S. for 2005 is 17 cases. The pathogenic factors include a peptidoglycan-associated lipoprotein, adhesive pili, and cytolethal distending toxin. Antibiotic treatment with azithromycin, ceftriaxone, ciprofloxacin, or erythromycin will cure the disease.

H. influenzae* biogroup *aegyptius is a non-encapsulated invasive organism which causes a fulminant pediatric disease Brazilian purpuric fever. Symptoms include high fever, hemorrhagic skin lesions, septicemia, vascular collapse, hypotensive shock and death usually within 48 hours of onset.

H. aegyptius, also known as the Koch-Weeks bacillus is associated with an acute purulent and contagious form of conjunctivitis (pink eye).

H. aphrophilus is a part of the normal flora of the oral and respiratory tract. This organism can cause endocarditis and pneumonia.