The *Haemophilus* Species

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Haemophilus influenzae

polysaccharide capsule
This is a group of small, gram-negative, pleomorphic bacteria that require enriched media, usually containing blood or its derivatives, for isolation. *Haemophilus influenzae* type b is an important human pathogen; *Haemophilus ducreyi*, a sexually transmitted pathogen, causes chancroid; other *Haemophilus* species are among the normal flora of mucous membranes and only occasionally cause disease.
Identification of hemin (X factor) and NAD (V factor) as growth requirements using paper disks. The top strain is only growing around the disk containing both hemin and NAD (black arrow), and is presumptively identified as *H. influenzae*.
Haemophilus influenzae

cultivation 24 hours, 37°C, 5% CO₂
chocolate agar with supplement
(sputum, patient with pneumonia)
Haemophilus influenzae
Epidemiology

Haemophilus species are typically considered to be normal flora in the upper respiratory tract of humans. They are found in the nasopharynx of approximately 75% of healthy individuals. Normal flora H. influenzae are non-encapsulated. Less than 5% of healthy individuals harbour an encapsulated strain of Haemophilus influenzae, which serotypes as type b. Type b (Hib) has historically been considered to be the most common cause of bacterial meningitis in young children ages 3 months to 6 years.
Virulence Factors •
*presence of capsule •
*Non-encapsulated strains may • possess a variety of cell surface proteins, fimbriae and pili which may aid in host cell attachment.
Clinical Manifestations • Non-encapsulated strains may cause the following: • sinusitis • pneumonia • acute exacerbation of COPD • otitis media • Encapsulated strains may cause the following: • meningitis • septic arthritis • pneumonia • conjunctivitis • cellulitis • epiglottitis
Diagnosis

A patient sample must be acquired. It is important to note that Haemophilus spp. are fastidious organisms and particular attention should therefore be paid to the collection, storage and transport of the specimen. This organism can be very susceptible to changes in environmental conditions such as temperature, humidity and atmosphere. If Haemophilus infection is suspected, care should be taken that the proper conditions are provided during recovery and transport, and that the specimen is cultured for growth as soon as possible.

*Specimens should be cultured to chocolate agar plates and incubated in 5-10\% CO\textsubscript{2} at 35-37 °C for 24- 72 hours where they will produce small or medium grey, mucoid (if encapsulated) colonies. A characteristic "mousy" odor is often Investigations may begin with the appearance of pale Gram negative coccobacilli or bacilli on a direct Gram stain. It should be noted that Haemophilus parainfluenzae may be pleomorphic with long filaments but it may also resemble H. influenzae.

Laboratories may use a variety of identification schemes to determine the presence of Haemophilus in a specimen, and many use the fastidious growth requirements of the bacteria as a method of identification. * Haemophilus influenzae requires supplementation with both hemin (X) and NAD (V) for growth, while Haemophilus parainfluenzae requires only NAD (V) supplementation.
One identification scheme involves plating the specimen to a blood agar plate and adding a streak line of *Staphylococcus aureus*, *Streptococcus pneumoniae*, or *Neisseria* species. The plate is then incubated at 5% CO2, 35 °C, for 16-18 hours. *S. aureus* (and the other aforementioned microorganisms) creates NAD (V factor) as a metabolic byproduct. Since blood agar plates contain hemin (X factor), a presumptive identification can be made when bacterial colonies are seen *satelliting* around the S. aureus.* A similar effect may be seen using manufactured discs of X, V and XV factors. If identification using discs is to proceed, the specimen should be planted to a Mueller Hinton, or TSA (trypticase soy agar) plate with the entire surface of the plate being inoculated. X, V, and XV factor discs should be applied, leaving a reasonable amount of space between the discs to prevent diffusion of the factors. Plates are incubated in CO2, and examined after 16-18 hours. Colonies which only surround the XV disc can be identified as *Haemophilus influenzae*, while those surrounding the XV and the V discs are *Haemophilus parainfluenzae*. 
Treatment •
In the past, ampicillin was an effective treatment for *H. influenzae* infection, but many strains are now resistant to beta lactam antibiotics. Identification of *Haemophilus influenzae* therefore warrants testing for the production of beta lactamases. For type b encapsulated strains, **cefuroxime** is recommended for treatment as it has minimal side effects and has the ability to cross the blood brain barrier. For non-encapsulated strains causing upper respiratory infections, amoxicillin coupled with a beta lactamase inhibitor (e.g. **clavulanic acid**) is often employed.
The Bordetellae •

There are several species of bordetella. • *Bordetella pertussis*, a highly communicable and important pathogen of humans, causes whooping cough (pertussis). *Bordetella parapertussis* can cause a similar disease. *Bordetella bronchiseptica (Bordetella bronchicanis)* causes diseases in animals. • •. *B. pertussis*, *B. parapertussis*, and *B bronchiseptica* are closely related, with 72–94% DNA homology and very limited differences in multilocus enzyme analysis.
**Bordetella pertussis**  •
**Morphology & Identification**  •
**Typical Organisms**: The organisms are minute gram-negative coccobacilli resembling *H influenzae*. With toluidine blue stain, bipolar metachromatic granules can be demonstrated. A capsule is present.

**Culture**  •
Primary isolation of *B. pertussis* requires enriched media. Bordet-Gengou medium (*potato-blood-glycerol agar*) that contains penicillin G, 0.5 g/mL, can be used; however, a charcoal-containing medium similar to that used for *Legionella pneumophila* is preferable. The plates are incubated at 35–37 °C for 3–7 days in a moist environment (eg, a sealed plastic bag). The small, faintly staining gram-negative rods are identified by immunofluorescence staining. *B. pertussis* is nonmotile. It does not require X and V factors on subculture. Hemolysis of blood-containing medium is associated with virulent *B. pertussis*. 
incubation 5 days, 35°C
Charcoal agar with supplements

short gram-negative rods; nonmotile (x1.000)

Bordetella pertussis
Pathogenesis, & Pathology  *B. pertussis* survives for only brief periods outside the human host. There are no vectors. Transmission is largely by the respiratory route from early cases and possibly via carriers. **The organism adheres to and multiplies rapidly on the epithelial surface of the trachea and bronchi and interferes with ciliary action.** The blood is not invaded. **The bacteria liberate the toxins and substances that irritate surface cells, causing coughing and marked lymphocytosis.** Later, there may be necrosis of parts of the epithelium and polymorphonuclear infiltration, with peribronchial inflammation and interstitial pneumonia. **Secondary invaders** like staphylococci or *H influenzae* may give rise to bacterial pneumonia. Obstruction of the smaller bronchioles by mucous plugs results in atelectasis and diminished oxygenation of the blood. **This probably contributes to the frequency of convulsions in infants with whooping cough.**
This child has pertussis. It is difficult for him to stop coughing and to get air. Coughing spasms with a "whooping" sound that follows the cough are typical. The sound means child is trying to catch his breath before the next round of coughing.
Binding of pertussis toxin to cell membrane is the cause of irretative cough
Diagnostic Laboratory Tests  

Specimens  
A saline nasal wash is the preferred specimen. Nasopharyngeal swabs or cough droplets expelled onto a "cough plate" held in front of the patient's mouth during a paroxysm are sometimes used but are not as good as the saline nasal wash.

Direct Fluorescent Antibody (FA) Test  
The FA reagent can be used to examine nasopharyngeal swab specimens. However, false-positive and false-negative results may occur; the sensitivity is about 50%. The FA test is most useful in identifying *B pertussis* after culture on solid media.

Culture  
The saline nasal wash fluid is cultured on solid medium agar (see above). The antibiotics in the media tend to inhibit other respiratory flora but permit growth of *B pertussis*. Organisms are identified by immunofluorescence staining or by slide agglutination with specific antiserum.

Polymerase Chain Reaction  
PCR is the most sensitive method to diagnosis pertussis. Primers for both *B pertussis* and *B parapertussis* should be included. When available, the PCR test should replace both culture and direct fluorescent antibody tests.

Serology  
Serologic tests on patients are of little diagnostic help because a rise in agglutinating or precipitating antibodies does not occur until the third week of illness. A single serum with high titer antibodies may be helpful in diagnosing the cause of a long-term cough, one of several weeks' duration.
Treatment • Administration of erythromycin during the catarrhal stage of disease promotes elimination of the organisms and may have prophylactic value. Treatment after onset of the paroxysmal phase rarely alters the clinical course. Oxygen inhalation and sedation may prevent anoxic damage to the brain.

Prevention • Pertussis vaccine is usually administered in combination with toxoids of diphtheria and tetanus (DTP).

Prophylactic administration of erythromycin for 5 days may also benefit unimmunized infants or heavily exposed adults.
**Bordetella parapertussis**

This organism may produce a disease similar to whooping cough, but it is generally less severe. The infection is often subclinical. *Bordetella parapertussis* grows more rapidly than typical *B pertussis* and produces larger colonies. It also grows on blood agar. *B parapertussis* has a silent copy of the pertussis toxin gene.
Legionellae the motile aerobic rod-shaped Gram-negative bacterium that thrives in central heating and air conditioning systems and can cause Legionnaires' disease.

**Typical Organisms:**

Legionellae are fastidious, aerobic gram-negative bacteria that are 0.5–1 m wide and 2–50 m long. They often stain poorly by Gram's method and are not seen in stains of clinical specimens. Gram-stained smears should be made for suspect legionella growth on agar media. Basic fuchsin (0.1%) should be used as the counter stain, because safranin stains the bacteria very poorly.
Legionella bacteria found at Boffa Hospital
DFA technique to detect the Legionella antigen directly in patient specimens. Respiratory tract specimens are spread on a glass slide. A monoclonal antibody to Legionella that is tagged with a fluorescein dye is added to the slide. If the antigen is present, the antibody will bind and the outline of the bacilli can be detected by microscopy under UV light.
Culture: 

Legionellae can be grown on complex media such as buffered charcoal-yeast extract (BCYE) agar with -ketoglutarate, at pH 6.9, temperature 35 °C, and 90% humidity. Antibiotics can be added to make the medium selective for legionella. A biphasic BCYE medium can be used for blood cultures. 

Legionellae grow slowly; visible colonies are usually present after 3 days of incubation. Colonies that appear after overnight incubation are not legionella. Colonies are round or flat with entire edges. They vary in color from colorless to iridescent pink or blue and are translucent or speckled. Variation in colony morphology is common, and the colonies may rapidly lose their color and speckles. Many other genera of bacteria grow on BCYE medium and must be differentiated from legionella by Gram staining and other tests. 

Legionellae in blood cultures usually require 2 weeks or more to grow. Colonies can be seen on the agar surface of the biphasic medium.

Growth Characteristics: 

The legionellae are catalase-positive. *L pneumophila* is oxidase-positive; the other legionellae are variable in oxidase activity. *L pneumophila* hydrolyzes hippurate; the other legionellae do not. Most legionellae produce gelatinase and -lactamase; *L micdadei* produces neither gelatinase nor -lactamase.
Legionella agglutination test

Bacteria streak tsa

bacteria colonies

Gram –ve rods

coliform
Legionellae are found in warm moist environments. They are found in lakes, streams, and other bodies of water. They can multiply in free-living amebas and can coexist with them in biofilms. Infection of debilitated or immunocompromised humans commonly follows inhalation of the bacteria from aerosols generated from contaminated air-conditioning systems, shower heads, and similar sources. *L. pneumophila* usually produces a lobar, segmental, or patchy pulmonary infiltration. Histologically, the appearance is similar to that produced by many other bacterial pathogens. Acute purulent pneumonia involving the alveoli is present with a dense intra-alveolar exudate of macrophages, polymorphonuclear leukocytes, red blood cells, and proteinaceous material. Most of the legionellae in the lesions are within phagocytic cells. There is little interstitial infiltration and little or no inflammation of the bronchioles and upper airways. *L. pneumophila* readily enters and grows within human alveolar macrophages and monocytes and is not effectively killed by polymorphonuclear leukocytes. The bacteria multiply within the vacuoles until they are numerous, the cells are destroyed, the bacteria are released, and infection of other macrophages then occurs. The presence of iron (transferrin-iron) is essential for the process of intracellular growth of the bacteria, but other factors important to the processes of growth, cell destruction, and tissue damage are not well understood.
Clinical Findings

Asymptomatic infection is common in all age groups.

The incidence of clinically significant disease is highest in immunocompromized patients.

Infection may result in nondescript febrile illness of short duration or in a severe, rapidly progressive illness with high fever, chills, malaise, nonproductive cough, hypoxia, diarrhea, and delirium. Chest x-rays reveal patchy, often multilobar consolidation. There may be leukocytosis, hyponatremia, hematuria (and even renal failure), or abnormal liver function. During some outbreaks, the mortality rate has reached 10%. The diagnosis is based on the clinical picture and exclusion of other causes of pneumonia by laboratory tests. Demonstration of legionella in clinical specimens can rapidly yield a specific diagnosis. The diagnosis can also be made by culture for legionella or by serologic tests, but results of these tests are often delayed beyond the time when specific therapy must be started.

*L pneumophila* also produces a disease called "Pontiac fever," after the clinical syndrome that occurred in an outbreak in Michigan. The syndrome is characterized by fever and chills, myalgia, malaise, and headache that develop over 6–12 hours. Dizziness, photophobia, neck stiffness, and confusion also occur. Respiratory symptoms are much less prominent in Pontiac fever than in Legionnaire's disease and include mild cough and sore throat.
Diagnostic Laboratory Tests  •
Specimens  •
In human infections, the organisms can be recovered from bronchial washings, pleural fluid, lung biopsy specimens, or blood. Isolation of legionella from sputum is more difficult because of the predominance of bacteria of the normal flora. Legionella is rarely recovered from other anatomic sites.

Smears  •
Legionellae are not demonstrable in Gram-stained smears of clinical specimens. Direct fluorescent antibody tests of specimens can be diagnostic, but the test has low sensitivity compared with culture. Silver stains are sometimes used on tissue specimens.

Culture  •
Specimens are cultured on BCYE agar (buffered charcoal-yeast extract). Cultured organisms can be rapidly identified by immunofluorescence staining. BCYE agar containing antibiotics can be used to culture contaminated specimens.

Specific Tests  •
Sometimes legionella antigens can be demonstrated in the patient's urine by immunologic methods. The urine antigen test is specific for *L pneumophila* serotype 1.

Serologic Tests  •
Levels of antibodies to legionellae rise slowly during the illness. Serologic tests have a sensitivity of 60–80% and a specificity of 95–99%. Serologic tests are most useful in obtaining a retrospective diagnosis in outbreaks of legionella infections.
Treatment

Legionellae are susceptible to erythromycin and some other drugs. The treatment of choice is erythromycin, which has been effective even in immunocompromised patients. Rifampin, 10–20 mg/kg/d, has been used in patients whose response to treatment was delayed. Assisted ventilation may be necessary, and management of shock is essential.

Epidemiology & Control

The natural habitats for legionellae are lakes, streams, rivers, and especially thermally heated bodies of water and soil. Legionellae grow best in warm water in the presence of amebas and water bacteria. They proliferate in amebas much as they do in pulmonary macrophages in the lung. When harsh environmental conditions occur and the amebas encyst, the amebas and legionellae both survive until better growth conditions occur, allowing excystment. The legionellae, amebas, and other microorganisms exist in biofilms; the legionellae go into a sessile state. The legionellae survive water treatment processes, and small numbers enter the water distribution systems where they proliferate. Cooling towers and evaporative condensers can be heavily contaminated with *L pneumophila*. Presumably, aerosols exiting such towers or condensers spread the organisms to susceptible persons. Similarly, there are links between contamination of residential water systems and community-acquired Legionnaires' disease and between contamination of hospital water systems and nosocomial *L pneumophila* infection. Hyperchlorination and superheating of water can help control the multiplication of legionellae in water and in air-conditioning systems.