CHAPTER 19

Gram-Negative Rods Related to the Respiratory Tract

CHAPTER CONTENTS

INTRODUCTION

There are four medically important gram-negative rods typically associated with the respiratory tract, namely, *Haemophilus influenzae, Bordetella pertussis, Legionella pneumophila, and Acinetobacter baumannii* (Table 19–1). *Hemophilus influenzae* and *B. pertussis* are found only in humans, whereas *L. pneumophila* is found primarily in environmental water sources. *Acinetobacter baumannii* is found in environmental water sources but also colonizes the skin and upper respiratory tract.

Additional information regarding the clinical aspects of infections caused by the organisms in this chapter is provided in Part IX entitled Infectious Diseases beginning on page 593.

HAEMOPHILUS

Diseases

Hemophilus influenzae used to be the leading cause of meningitis in young children, but the use of the highly effective "conjugate" vaccine has greatly reduced the incidence of meningitis caused by this organism. It is still an important cause of upper respiratory tract infections (otitis media, sinusitis, conjunctivitis, and epiglottitis) and sepsis in children. It also causes pneumonia in adults, particularly in those with chronic obstructive lung disease. *Haemophilus ducreyi*, the agent of chancroid, is discussed in Chapter 27.

Important Properties

Hemophilus influenzae is a small gram-negative rod (coccobacillary rod)) with a polysaccharide capsule (Figure 19–1). It is one of the three important **encapsulated pyogens,** along with the pneumococcus and the meningococcus. Serologic typing is based on the antigenicity of the capsular polysaccharide. Of the six serotypes (a–f), **type b** is the most important. Type b used to cause most of the severe, invasive diseases, such as meningitis and sepsis, but the widespread use of the vaccine containing the type b capsular polysaccharide as the immunogen, has greatly reduced

TABLE 19–1 Gram-Negative Rods Associated with the Respiratory Tract

Species	Major Diseases	Laboratory Diagnosis	Factors X and V Required for Growth	Vaccine Available	Prophylaxis for Contacts
H. influenzae	Meningitis[1]; otitis media, sinusitis, pneumonia, epiglottitis	Culture; capsular polysaccharide in serum or spinal fluid	+	+	Rifampin
B. pertussis	Whooping cough (pertussis)	Fluorescent antibody on secretions; culture	–	+	Azithromycin
L. pneumophila	Pneumonia	Serology; urinary antigen; culture	–	–	None
A. baumannii	Ventilator-associated pneumonia	Culture	–	–	None

[1] In countries where the *H. influenzae* b conjugate vaccine has been deployed, the vaccine has greatly reduced the incidence of meningitis caused by this organism.

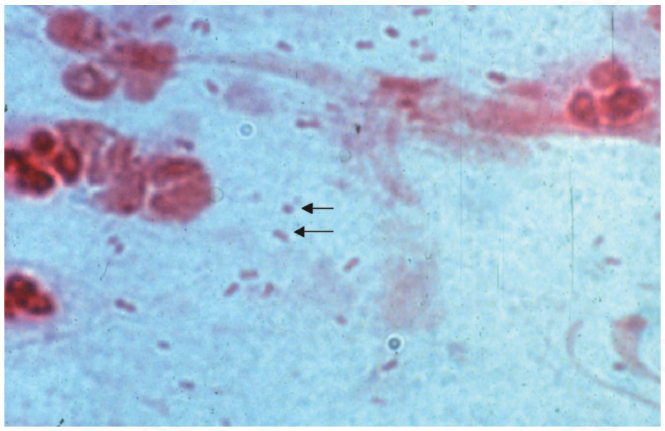

FIGURE 19–1 *Haemophilus influenzae*—Gram stain. Arrows point to two small "coccobacillary" gram-negative rods. (Used with permission from Professor Shirley Lowe, University of California, San Francisco School of Medicine.)

the incidence of invasive disease caused by this type. The type b capsule is composed of polyribitol phosphate.

Unencapsulated strains can also cause disease, especially mucosal diseases of the upper respiratory tract such as sinusitis and otitis media, but are usually noninvasive. Growth of the organism on laboratory media requires the addition of two components, **heme (factor X)** and **NAD (factor V),** for adequate energy production.

Pathogenesis & Epidemiology

Hemophilus influenzae infects only humans; there is no animal reservoir. It enters the body by the inhalation of airborne droplets into the **respiratory tract,** resulting in either asymptomatic colonization or infections such as otitis media, sinusitis, or pneumonia. The organism produces an IgA protease that degrades secretory IgA, thus facilitating attachment to the respiratory mucosa. After becoming established in the upper respiratory tract, the organism can enter the bloodstream (bacteremia) and spread to the meninges. Meningitis is caused primarily by the encapsulated strains, but nonencapsulated strains are frequently involved in otitis media, sinusitis, and pneumonia. Note that the incidence of meningitis caused by capsular type b has been greatly reduced because the vaccine contains the type b polysaccharide as the immunogen. Pathogenesis of *H. influenzae* involves its antiphagocytic capsule and endotoxin; no exotoxin is produced.

Most infections occur in children between the ages of 6 months and 6 years, with a peak in the age group from 6 months to 1 year. This age distribution is attributed to a decline in maternal IgG in the child coupled with the inability of the child to generate sufficient antibody against the polysaccharide capsular antigen until the age of approximately 2 years.

Clinical Findings

Meningitis caused by *H. influenzae* cannot be distinguished on clinical grounds from that caused by other bacterial pathogens (e.g., pneumococci or meningococci). The rapid onset of fever, headache, and stiff neck, along with drowsiness, is typical. Sinusitis and otitis media cause pain in the affected area, opacification of the infected sinus, and redness with bulging of the tympanic membrane. *Hemophilus influenzae* is second only to the pneumococcus as a cause of these two infections.

Other serious infections caused by this organism include septic arthritis, cellulitis, and sepsis, the latter occurring especially in splenectomized patients. Rarely, **epiglottitis,** which can obstruct the airway, occurs. A swollen "cherry-red" epiglottis is seen. This life-threatening disease of young children is caused almost exclusively by *H. influenzae*. Pneumonia in elderly adults, especially those with chronic respiratory disease, can be caused by untypeable strains of *H. influenzae*.

Laboratory Diagnosis

Laboratory diagnosis depends on isolation of the organism on heated-blood ("chocolate") agar enriched with two growth factors required for bacterial respiration, namely, factor X (a heme compound) and factor V (NAD). The blood used in chocolate agar is heated to inactivate nonspecific inhibitors of *H. influenzae* growth.

An organism that grows only in the presence of both growth factors is presumptively identified as *H. influenzae;* other species of *Haemophilus*, such as *Haemophilus parainfluenzae*, do not require both factors. Definitive identification can be made with either biochemical tests or the capsular swelling (quellung) reaction. Additional means of identifying encapsulated strains include fluorescent-antibody staining of the organism and counterimmunoelectrophoresis or latex agglutination tests, which detect the capsular polysaccharide.

Treatment

The treatment of choice for meningitis or other serious systemic infections caused by *H. influenzae* is ceftriaxone. 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 (e.g., subdural empyema) is high. Untreated *H. influenzae* meningitis has a fatality rate of approximately 90%. *H. influenzae* upper respiratory tract infections, such as otitis media and sinusitis, are treated with either amoxicillin-clavulanate or trimethoprim-sulfamethoxazole.

Prevention

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.

BORDETELLA

Disease

Bordetella pertussis causes whooping cough (pertussis).

Important Properties

Bordetella pertussis is a small, coccobacillary, encapsulated gram-negative rod.

Pathogenesis & Epidemiology

Bordetella pertussis, a pathogen **only for humans,** is transmitted by **airborne droplets** produced during the severe coughing episodes. The organisms attach to the ciliated epithelium of the upper respiratory tract but do not invade the underlying tissue. Decreased cilia activity and subsequent death of the ciliated epithelial cells are important aspects of pathogenesis.

Pertussis is a highly contagious disease that occurs primarily in infants and young children and has a worldwide distribution. The number of cases has declined in the United States because use of the vaccine is widespread. However, outbreaks of pertussis during the years 2005, 2010, and 2012 has led to concern about waning immunity to the vaccine and to the recommendation that an additional booster immunization be given (see "Prevention").

Several factors play a role in the pathogenesis:

(1) Attachment of the organism to the cilia of the epithelial cells is mediated by a protein on the pili called filamentous hemagglutinin. Antibody against the filamentous hemagglutinin inhibits attachment and protects against disease.

(2) **Pertussis toxin** stimulates adenylate cyclase by catalyzing the addition of adenosine diphosphate ribose—a process called ADP-ribosylation—to the inhibitory subunit of the G protein complex (G_i protein). This results in prolonged stimulation of adenylate cyclase and a consequent rise in cyclic adenosine monophosphate (AMP) and in cyclic AMP–dependent protein kinase activity. This results in edema of the respiratory mucosa that contributes to the severe cough of pertussis. The toxin also has a domain that mediates its binding to receptors on the surface of respiratory tract epithelial cells. It is an A-B subunit toxin.

Pertussis toxin also causes a striking **lymphocytosis** in the blood of patients with pertussis. The toxin inhibits signal transduction by chemokine receptors, resulting in a failure of lymphocytes to enter lymphoid tissue such as the spleen and lymph nodes. Because the lymphocytes do not enter lymphoid tissue, there is an increase in their number in the blood (see the discussion of chemokines in Chapter 58). The inhibition of signal transduction by chemokine receptors is also caused by ADP-ribosylation of the G_i protein.

(3) The organisms also synthesize and export adenylate cyclase. This enzyme, when taken up by phagocytic cells (e.g., neutrophils), can inhibit their bactericidal activity. Bacterial mutants that lack cyclase activity are avirulent.

(4) Tracheal cytotoxin is a fragment of the bacterial peptidoglycan that damages ciliated cells of the respiratory tract. Tracheal cytotoxin appears to act in concert with endotoxin to induce nitric oxide, which kills the ciliated epithelial cells.

Clinical Findings

Whooping cough is an acute tracheobronchitis that begins with mild upper respiratory tract symptoms followed by a severe paroxysmal cough, which lasts from 1 to 4 weeks. The paroxysmal pattern is characterized by a series of hacking coughs, accompanied by production of copious amounts of mucus, that end with an inspiratory "whoop" as air rushes past the narrowed glottis. Despite the severity of the symptoms, the organism is restricted to the respiratory tract and blood cultures are negative. A pronounced leukocytosis with up to 70% lymphocytes is seen. Although central nervous system anoxia and exhaustion can occur as a result of the severe coughing, death is due mainly to pneumonia.

The classic picture of whooping cough described above occurs primarily in young children. In adults, *B. pertussis* infection often manifests as a paroxysmal cough of varying severity lasting weeks. The characteristic whoop is often absent, leading to difficulty in recognizing the cough as caused by this organism. In the correct clinical setting, adults with a cough lasting several weeks (often called the 100-day cough) should be evaluated for infection with *B. pertussis*.

Laboratory Diagnosis

The organism can be isolated from nasopharyngeal swabs taken during the paroxysmal stage. Bordet-Gengou[1] medium used for this purpose contains a high percentage of blood (20%–30%) to inactivate inhibitors in the agar.

Identification of the isolated organism can be made by agglutination with specific antiserum or by fluorescent-antibody staining. However, the organism grows very slowly in culture, so direct fluorescent-antibody staining of the nasopharyngeal specimens can be used for diagnosis. Polymerase chain reaction–based tests are highly specific and sensitive and should be used if available.

Isolation of the organism in patients with a prolonged cough is often difficult. Serologic tests that detect antibody in the patient's serum can be used for diagnosis in those patients.

Treatment

Azithromycin is the drug of choice. Note that azithromycin reduces the number of organisms in the throat and decreases the risk of secondary complications but has little effect on the course of the disease at the "prolonged cough" stage because the toxins have already damaged the respiratory mucosa. Supportive care (e.g., oxygen therapy and suction of mucus) during the paroxysmal stage is important, especially in infants.

Prevention

There are two types of vaccines: an acellular vaccine containing purified proteins from the organism and a killed vaccine containing inactivated *B. pertussis* organisms. The **acellular vaccine** contains five antigens purified from the organism. It is the vaccine currently used in the United States. The main immunogen in this vaccine is inactivated pertussis toxin (pertussis toxoid). The toxoid in the vaccine is pertussis toxin that has been inactivated genetically by introducing two amino acid changes, which eliminates its ADP-ribosylating activity but retains its antigenicity. It is the first vaccine to contain a genetically inactivated toxoid. The other pertussis antigens in the vaccine are filamentous hemagglutinin, pertactin, and fimbriae types 2 and 3. The acellular vaccine has fewer side effects than the killed vaccine but has a shorter duration of immunity.

The pertussis vaccine is usually given combined with diphtheria and tetanus toxoids (DTaP) in three doses beginning at 2 months of age. A booster at 12 to 15 months of age and another at the time of entering school are recommended. Because outbreaks of pertussis have occurred among teenagers, a booster for those between 10 and 18 years old is recommended. This vaccine, called Boostrix, contains diphtheria and tetanus toxoids also. Another vaccine called Adacel also contains diphtheria and tetanus toxoids. A pertussis booster dose is recommended for adults as well. To protect newborns, pregnant women should receive pertussis vaccine. Antipertussis IgG will pass the placenta and protect the newborn.

[1] The French scientists who first isolated the organism in 1906.

The killed vaccine is no longer used in the United States because it is suspected of causing various side effects, including postvaccine encephalopathy at a rate of about one case per million doses administered. The killed vaccine is in use in many countries other than the United States.

Azithromycin is useful in prevention of disease in exposed, unimmunized individuals. It should also be given to immunized children younger than 4 years who have been exposed because vaccine-induced immunity is not completely protective.

LEGIONELLA

Disease

Legionella pneumophila (and other legionellae) causes pneumonia, both in the community and in hospitalized immunocompromised patients. The genus is named after the famous outbreak of pneumonia among people attending the American Legion convention in Philadelphia in 1976 (Legionnaires' disease).

Important Properties

Legionellae are gram-negative rods that **stain faintly with the standard Gram stain.** They do, however, have a gram-negative type of cell wall, and increasing the time of the safranin counterstain enhances visibility. Legionellae in lung biopsy sections do not stain by the standard hematoxylin-and-eosin (H&E) procedure; therefore, special methods, such as the Dieterle silver impregnation stain, are used to visualize the organisms.

During the 1976 outbreak, initial attempts to grow the organisms on ordinary culture media failed. This is because the organism requires a high concentration of iron and cysteine. Culture media supplemented with these nutrients will support growth.

Legionella pneumophila causes approximately 90% of pneumonia attributed to legionellae. There are 16 serogroups of *L. pneumophila*, with most cases caused by serogroup 1 organisms. There are about 30 other *Legionella* species that cause pneumonia, but most of the remaining 10% of cases are caused by two species, *Legionella micdadei* and *Legionella bozemanii*.

Pathogenesis & Epidemiology

Legionellae are associated chiefly with **environmental water sources** such as air conditioners and water-cooling towers. Outbreaks of pneumonia in hospitals have been attributed to the presence of the organism in water taps, sinks, and showers. Legionellae can replicate to large numbers in **free-living amebas** in these water sources. The amebas also enhance the survival of Legionellae. Under adverse environmental conditions, the amebas encyst, ensuring both their own survival and the survival of the intracellular Legionellae as well.

The portal of entry is the respiratory tract, and pathologic changes occur primarily in the lung. However, in severe cases, bacteremia occurs, accompanied by damage to the vascular endothelium in multiple organs, especially the brain and kidneys. The major virulence factor of the organism is lipopolysaccharide (endotoxin). No exotoxins are produced.

The typical candidate for Legionnaires' disease is an older man who smokes and consumes substantial amounts of alcohol. Patients with acquired immunodeficiency syndrome (AIDS), cancer, or transplants (especially renal transplants) or patients being treated with corticosteroids are predisposed to *Legionella* pneumonia, which indicates that **cell-mediated immunity** is the most important defense mechanism. Despite airborne transmission of the organism, person-to-person spread does *not* occur, as shown by the failure of secondary cases to occur in close contacts of patients.

Clinical Findings

The clinical picture can vary from a mild influenzalike illness to a severe pneumonia accompanied by mental confusion, nonbloody diarrhea, proteinuria, and microscopic hematuria. Although cough is a prominent symptom, sputum is frequently scanty and nonpurulent. Hyponatremia (serum sodium ≤130 mEq/L) is an important laboratory finding that occurs more often in *Legionella* pneumonia than in pneumonia caused by other bacteria. Most cases resolve spontaneously in 7 to 10 days, but in older or immunocompromised patients, the infection can be fatal.

Legionellosis is an **atypical pneumonia**[2] and must be distinguished from other similar pneumonias such as *Mycoplasma* pneumonia, viral pneumonia, psittacosis, and Q fever.

Pontiac fever is a mild, flulike form of *Legionella* infection that does not result in pneumonia. The name "Pontiac" is derived from the city in Michigan that was the site of an outbreak in 1968.

Laboratory Diagnosis

Sputum Gram stains reveal many neutrophils but no bacteria. The organism **fails to grow on ordinary media** in a culture of sputum or blood, but it will grow on charcoal-yeast agar, a special medium supplemented with iron and cysteine. Diagnosis usually depends on a significant increase in antibody titer in convalescent-phase serum by the indirect immunofluorescence assay. Detection of *L. pneumophila* antigens in the urine is a rapid means of making a diagnosis. The urinary antigen test is available only for serogroup 1 organisms. If tissue is available, it is possible to demonstrate *Legionella* antigens in infected lung tissue by using fluorescent-antibody staining. The cold-agglutinin titer does not rise in *Legionella* pneumonia, in contrast to pneumonia caused by *Mycoplasma*.

[2] A pneumonia is atypical when its causative agent cannot be isolated on ordinary laboratory media or when its clinical picture does not resemble that of typical pneumococcal pneumonia.

Treatment

Azithromycin or erythromycin (with or without rifampin) is the treatment of choice. Certain fluoroquinolones, such as levofloxacin and trovafloxacin, are also drugs of choice. These drugs are effective not only against *L. pneumophila*, but also against *Mycoplasma pneumoniae* and *Streptococcus pneumoniae*. The organism frequently produces β-lactamase, and so penicillins and cephalosporins are less effective.

Prevention

Prevention involves reducing cigarette and alcohol consumption, eliminating aerosols from water sources, and reducing the incidence of *Legionella* in hospital water supplies by using high temperatures and hyperchlorination. There is no vaccine.

ACINETOBACTER

Acinetobacter species are small coccobacillary gram-negative rods found commonly in soil and water, but they also colonize the skin and upper respiratory tract. They are opportunistic pathogens that readily colonize patients with compromised host defenses. *Acinetobacter baumannii*, the species usually involved in human infection, causes disease chiefly in a hospital setting usually associated with respiratory therapy equipment (ventilator-associated pneumonia) and indwelling catheters. Pneumonia and urinary tract infections are the most frequent manifestations. Laboratory diagnosis is made by culturing the organism. *Acinetobacter baumannii* is remarkably antibiotic resistant, and some isolates are resistant to all known antibiotics. Imipenem is the drug of choice for infections caused by susceptible strains. Colistin is useful in carbapenem-resistant strains. There is no vaccine. Previous genus names for this organism include *Herellea* and *Mima*.

SELF-ASSESSMENT QUESTIONS

1. Your patient is a 75-year-old man who has smoked cigarettes (two packs a day for more than 50 years) and consumed alcoholic drinks (a six pack of beer each day) for most of his adult life. He now has the signs and symptoms of pneumonia. Gram stain of the sputum reveals neutrophils but no bacteria. Colonies appear on buffered charcoal yeast (BYCE) agar but not on blood agar. Which one of the following bacteria is most likely to be the cause of his pneumonia?

(A) *Bordetella pertussis*
(B) *Haemophilus influenzae*
(C) *Klebsiella pneumoniae*
(D) *Legionella pneumophila*
(E) *Pseudomonas aeruginosa*

2. Regarding the patient in Question 1, which one of the following is the best antibiotic to treat the infection?

(A) Azithromycin
(B) Ceftriaxone
(C) Gentamicin
(D) Metronidazole
(E) Piperacillin/tazobactam

3. Your patient is a 6-year-old boy who is complaining that his ear hurts. His mother says this began yesterday and that he has a fever of 103°F. On physical exam, you see a perforated ear drum that is exuding a small amount of pus. Using a swab, you obtain a sample of the pus and do a Gram stain and culture. The Gram stain reveals small coccobacillary rods. There is no growth on a blood agar plate, but a chocolate agar plate supplemented with X and V factors grows small grey colonies. Which one of the following bacteria is the most likely cause of his otitis media?

(A) *Bordetella pertussis*
(B) *Haemophilus influenzae*
(C) *Klebsiella pneumoniae*
(D) *Legionella pneumophila*
(E) *Pseudomonas aeruginosa*

4. It's time to play "What's my name?" I am a small gram-negative rod that causes an important respiratory tract disease. I produce an exotoxin that ADP-ribosylates a G protein. One remarkable feature of my disease is a great increase in lymphocytes. I don't cause disease commonly in the United States now because of the widespread use of the vaccine that induces antibodies against five of my proteins, one of which is the exotoxin. The identity of the mystery organism is mostly likely which of the following?

(A) *Bordetella pertussis*
(B) *Haemophilus influenzae*
(C) *Klebsiella pneumoniae*
(D) *Legionella pneumophila*
(E) *Pseudomonas aeruginosa*

5. Your patient is a 75-year-old woman with a 110 pack-year history of cigarette smoking who now has a fever of 39°C and a cough productive of yellowish sputum. Gram stain of the sputum shows small gram-negative rods. There is no growth on blood agar, but colonies do grow on chocolate agar supplemented with hemin and NAD. Which one of the following bacteria is the most likely cause of her pneumonia?

(A) *Bordetella pertussis*
(B) *Haemophilus influenzae*
(C) *Klebsiella pneumoniae*
(D) *Legionella pneumophila*
(E) *Pseudomonas aeruginosa*

6. Your patient is a 5-year-old boy with a high fever and signs of respiratory tract obstruction. Visualization of the epiglottis shows inflammation characterized by marked swelling and "cherry-red" appearance. Which one of the following is the best antibiotic to treat the infection?

(A) Ampicillin
(B) Ceftriaxone
(C) Doxycycline
(D) Gentamicin
(E) Metronidazole

ANSWERS

(1) (**D**)
(2) (**A**)
(3) (**B**)
(4) (**A**)
(5) (**B**)
(6) (**B**)

SUMMARIES OF ORGANISMS

Brief summaries of the organisms described in this chapter begin on page 662. Please consult these summaries for a rapid review of the essential material.

PRACTICE QUESTIONS: USMLE & COURSE EXAMINATIONS

Questions on the topics discussed in this chapter can be found in the Clinical Bacteriology section of Part XIII: USMLE (National Board) Practice Questions starting on page 713. Also see Part XIV: USMLE (National Board) Practice Examination starting on page 751.