CHAPTER 17

Gram-Positive Rods

CHAPTER CONTENTS

INTRODUCTION

There are five medically important genera of gram-positive rods: *Bacillus, Clostridium, Corynebacterium*, *Listeria*, and *Gardnerella*. *Bacillus* and *Clostridium* form spores, whereas *Corynebacterium*, *Listeria*, and *Gardnerella* do not. Members of the genus *Bacillus* are aerobic, whereas those of the genus *Clostridium* are anaerobic (Table 17–1).

These gram-positive rods can also be distinguished based on their appearance on Gram stain. *Bacillus* and *Clostridium* species are longer and more deeply staining than *Corynebacterium* and *Listeria* species. *Corynebacterium* species are club-shaped (i.e., they are thinner on one end than the other). *Corynebacterium* and *Listeria* species characteristically appear as V- or L-shaped rods. *Gardnerella vaginalis* is a short gram-variable rod.

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.

SPORE-FORMING GRAM-POSITIVE RODS

BACILLUS

There are two medically important *Bacillus* species: *Bacillus anthracis* and *Bacillus cereus*. Important features of pathogenesis by these two *Bacillus* species are described in Table 17–2.

TABLE 17–1 Gram-Positive Rods of Medical Importance

Growth	Anaerobic Growth	Spore Formation	Exotoxins Important in Pathogenesis
Bacillus	–	+	+
Clostridium	+	+	+
Corynebacterium	–	–	+
Listeria	–	–	–
Gardnerella	–	–	–

1. Bacillus anthracis

Disease

Bacillus anthracis causes anthrax (Figure 17–1), which is common in animals but rare in humans. Human disease occurs in three main forms: cutaneous, pulmonary (inhalation), and gastrointestinal. In 2001, an outbreak of both inhalation and cutaneous anthrax occurred in the United States. The outbreak was caused by sending spores of the organism through the mail. There were 18 cases, causing 5 deaths in this outbreak.

Important Properties

Bacillus anthracis is a large gram-positive rod with square ends, frequently found in chains (Figure 17–2). Its antiphagocytic capsule is composed of D-glutamate.

TABLE 17–2 Important Features of Pathogenesis by *Bacillus* Species

Organism	Disease	Transmission/ Predisposing Factor	Action of Toxin	Prevention
B. anthracis	Anthrax	1. Cutaneous anthrax: spores in soil enter wound 2. Pulmonary anthrax: spores are inhaled into lung	Exotoxin has three components: protective antigen binds to cells; edema factor is an adenylate cyclase; lethal factor is a protease that inhibits cell growth resulting in cell death (necrosis)	Vaccine contains protective antigen as the immunogen
B. cereus	Food poisoning	Spores germinate in reheated rice, then bacteria produce exotoxins, which are ingested	Two exotoxins (enterotoxins): 1. Similar to cholera toxin, it increases cyclic AMP 2. Similar to staphylococcal enterotoxin, it is a superantigen	No vaccine

(This is unique—capsules of other bacteria are polysaccharides.) It is nonmotile, whereas other members of the genus are motile. Anthrax toxin is encoded on one plasmid, and the polyglutamate capsule is encoded on a different plasmid.

Transmission

Spores of the organism persist in soil for years. Humans are most often infected cutaneously at the time of trauma to the skin, which allows the **spores on animal products,** such as hides, bristles, and wool, to enter. Spores can also be inhaled into the respiratory tract. Pulmonary (inhalation) anthrax occurs when spores are inhaled into the lungs. Gastrointestinal anthrax occurs when contaminated meat is ingested.

Inhalation anthrax is not communicable from person to person, despite the severity of the infection. After being inhaled into the lung, the organism moves rapidly to the mediastinal lymph nodes, where it causes hemorrhagic mediastinitis. Because it leaves the lung so rapidly, it is not transmitted by the respiratory route to others.

Pathogenesis

Pathogenesis is based primarily on the production of two exotoxins, collectively known as anthrax toxin. The two exotoxins, **edema factor** and **lethal factor,** each consist of two proteins in an A–B subunit configuration. The B, or binding, subunit in each of the two exotoxins is **protective antigen.** The A, or active, subunit has enzymatic activity.

Edema factor, an exotoxin, is an **adenylate cyclase** that causes an increase in the intracellular concentration of cyclic adenosine monophosphate (AMP). This causes an outpouring of fluid from the cell into the extracellular space, which manifests as edema. (Note the similarity of action to that of cholera toxin.)

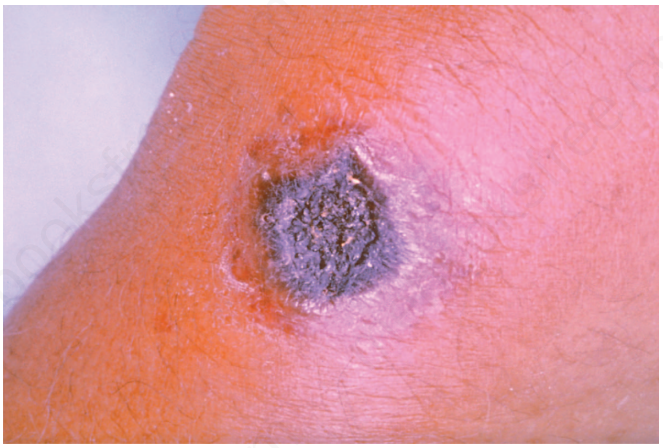

FIGURE 17–1 Skin lesion of anthrax. Note the *black eschar*, a necrotic lesion covered by a crust, caused by lethal factor, an exotoxin produced by *Bacillus anthracis*. Note the area of edema surrounding the eschar, which is caused by another exotoxin called *edema factor*. (Source: Dr. James H. Steele, Centers for Disease Control and Prevention. CDC # 2033.)

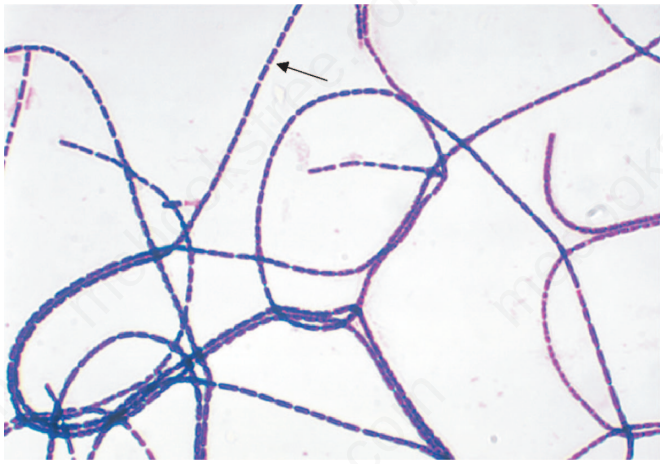

FIGURE 17–2 *Bacillus anthracis*—Gram stain. Arrow points to one large "box car–like" gram-positive rod within a long chain. (Source: Public Health Image Library, Centers for Disease Control and Prevention.)

Lethal factor is a protease that cleaves the phosphokinase that activates the mitogen-activated protein kinase (MAPK) signal transduction pathway. This pathway controls the growth of human cells, and cleavage of the phosphokinase inhibits cell growth. Protective antigen forms pores in the human cell membrane that allows edema factor and lethal factor to enter the cell. The name *protective antigen* refers to the fact that antibody against this protein protects against disease.

Clinical Findings

The typical lesion of cutaneous anthrax is a painless ulcer with a black eschar (crust, scab) (see Figure 17–1). Local edema is striking. The lesion is called a **malignant pustule.** Untreated cases progress to bacteremia and death.

Pulmonary (inhalation) anthrax, also known as "wool-sorter's disease," begins with nonspecific respiratory tract symptoms resembling influenza, especially a dry cough and substernal pressure. This rapidly progresses to hemorrhagic mediastinitis, bloody pleural effusions, septic shock, and death. Although the lungs are infected, the classic features and X-ray picture of pneumonia are not present. Mediastinal widening seen on chest X-ray is an important diagnostic criterion. Hemorrhagic mediastinitis and hemorrhagic meningitis are severe life-threatening complications. The symptoms of gastrointestinal anthrax include vomiting, abdominal pain, and bloody diarrhea.

Laboratory Diagnosis

Smears show large, gram-positive rods in chains (see Figure 17–2). Spores are usually not seen in smears of exudate because spores form when nutrients are insufficient, and nutrients are plentiful in infected tissue. Nonhemolytic colonies form on blood agar aerobically. In case of a bioterror attack, rapid diagnosis can be performed in special laboratories using polymerase chain reaction (PCR)–based assays. Another rapid diagnostic procedure is the direct fluorescent antibody test that detects antigens of the organism in the lesion. Serologic tests, such as an enzyme-linked immunosorbent assay (ELISA) test for antibodies, require acute and convalescent serum samples and can only be used to make a diagnosis retrospectively.

Treatment

Ciprofloxacin is the drug of choice. Doxycycline is an alternative drug. No resistant strains have been isolated clinically.

Prevention

Ciprofloxacin or doxycycline was used as prophylaxis in those exposed during the outbreak in the United States in 2001. People at high risk can be immunized with cell-free vaccine containing purified protective antigen as immunogen. The vaccine is weakly immunogenic, and six doses of vaccine over an 18-month period are given. Annual boosters are also given to maintain protection. Incinerating animals that die of anthrax, rather than burying them, will prevent the soil from becoming contaminated with spores.

2. *Bacillus cereus*

Disease

Bacillus cereus causes food poisoning.

Transmission

Spores on grains such as rice survive steaming and rapid frying. The spores germinate when rice is kept warm for many hours (e.g., **reheated fried rice**). The portal of entry is the gastrointestinal tract.

Pathogenesis

Bacillus cereus produces two enterotoxins. The mode of action of one of the enterotoxins is the same as that of cholera toxin (i.e., it adds adenosine diphosphate ribose, a process called ADP-ribosylation, to a G protein, which stimulates adenylate cyclase and leads to an increased concentration of cyclic AMP within the enterocyte). The mode of action of the other enterotoxin resembles that of staphylococcal enterotoxin (i.e., it is a superantigen).

Clinical Findings

There are two syndromes. (1) One syndrome has a short incubation period (4 hours) and consists primarily of nausea and vomiting, similar to staphylococcal food poisoning. (2) The other has a long incubation period (18 hours) and features watery, nonbloody diarrhea, resembling clostridial gastroenteritis.

Laboratory Diagnosis

This is not usually done.

Treatment

Only symptomatic treatment is given.

Prevention

There is no specific means of prevention. Rice should not be kept warm for long periods.

CLOSTRIDIUM

There are four medically important species: *Clostridium tetani, Clostridium botulinum, Clostridium perfringens* (which causes either gas gangrene or food poisoning), and *Clostridium difficile*. All clostridia are anaerobic,

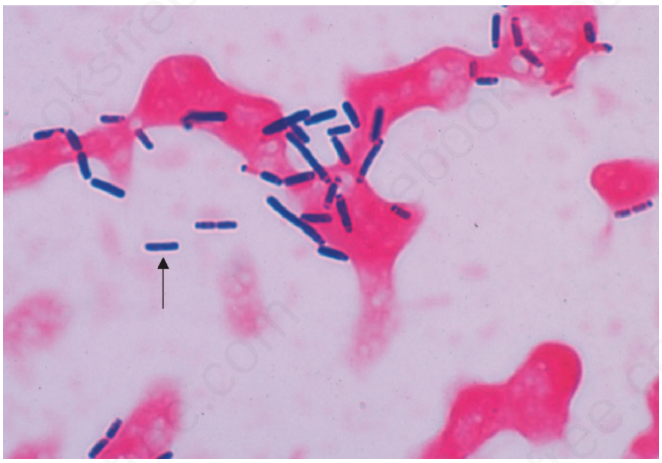

FIGURE 17–3 *Clostridium perfringens*—Gram stain. Arrow points to a large gram-positive rod. (Used with permission from Professor Shirley Lowe, University of California, San Francisco School of Medicine.)

spore-forming, gram-positive rods (Figure 17–3). Important features of pathogenesis and prevention are described in Table 17–3.

1. Clostridium tetani

Disease

Clostridium tetani causes tetanus (Figure 17–4).

Transmission

Spores are widespread in soil. The portal of entry is usually a **wound** site (e.g., where a nail penetrates the foot), but the spores can also be introduced during "skin-popping," a technique used by drug addicts to inject drugs into the skin. Germination of spores is favored by necrotic tissue and poor blood supply in the wound. Neonatal tetanus, in which the organism enters through a contaminated umbilicus or circumcision wound, is a major problem in some developing countries.

Pathogenesis

Tetanus toxin (tetanospasmin) is an exotoxin produced by vegetative cells at the wound site. This polypeptide toxin is carried intra-axonally (retrograde) to the central nervous system, where it binds to ganglioside receptors and blocks release of inhibitory mediators (e.g., glycine and γ-aminobutyric acid [GABA]) at spinal synapses.

Tetanus toxin and botulinum toxin (see later) are among the most toxic substances known. They are both proteases that cleave the proteins involved in mediator release from the neurons.

Tetanus toxin has one antigenic type, unlike botulinum toxin, which has eight. There is therefore only one antigenic type of tetanus toxoid in the vaccine against tetanus.

Clinical Findings

Tetanus is characterized by strong muscle spasms (spastic paralysis, tetany). Specific clinical features include **lockjaw** (trismus) due to rigid contraction of the jaw muscles, which prevents the mouth from opening; a characteristic grimace known as **risus sardonicus;** and exaggerated reflexes. **Opisthotonos,** a pronounced arching of the back due to spasm of the strong extensor muscles of the back, is often seen (see Figure 17–4). Respiratory failure ensues. A high mortality rate is associated with this disease. Note that in tetanus, **spastic paralysis** (strong muscle contractions) occurs, whereas in botulism, **flaccid paralysis** (weak or absent muscle contractions) occurs.

Laboratory Diagnosis

There is no microbiologic or serologic diagnosis. Organisms are rarely isolated from the wound site. *Clostridium tetani* produces a **terminal spore** (i.e., a spore at the end of the rod). This gives the organism the characteristic appearance of a "tennis racket."

Treatment

Tetanus immune globulin (tetanus antitoxin) is used to neutralize the toxin. The role of antibiotics is uncertain. If antibiotics are used, either metronidazole or penicillin G can be given. An adequate airway must be maintained and respiratory support given. Benzodiazepines (e.g., diazepam [Valium]) should be given to prevent spasms.

TABLE 17–3 Important Features of Pathogenesis by *Clostridium* Species

Organism	Disease	Transmission/Predisposing Factor	Action of Toxin	Prevention
C. tetani	Tetanus	Spores in soil enter wound	Blocks release of inhibitory transmitters (e.g., glycine)	Toxoid vaccine
C. botulinum	Botulism	Exotoxin in food is ingested	Blocks release of acetylcholine	Proper canning; cook food
C. perfringens	1. Gas gangrene	Spores in soil enter wound	Lecithinase	Debride wounds
	2. Food poisoning	Exotoxin in food is ingested	Superantigen	Cook food
C. difficile	Pseudomembranous colitis	Antibiotics suppress normal flora	Cytotoxin damages colon mucosa	Appropriate use of antibiotics

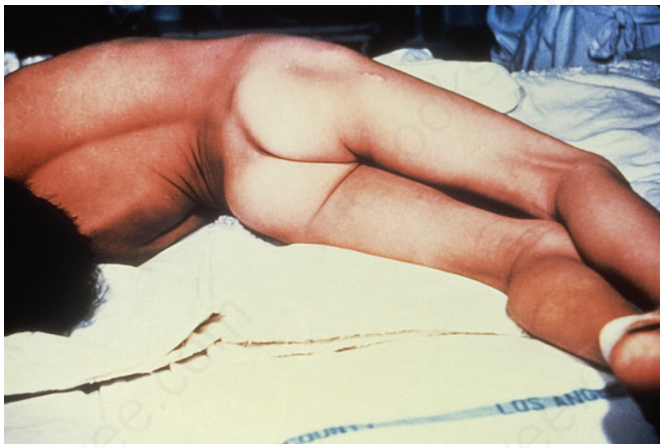

FIGURE 17–4 Tetanus. Note the marked hyperextension of the back, a position called *opisthotonos*, caused by tetanus toxin, an exotoxin that inhibits the release of mediators of the inhibitory neurons in the spinal cord. (Source: Centers for Disease Control and Prevention. CDC # 6373.)

Prevention

Tetanus is prevented by immunization with tetanus **toxoid** (formaldehyde-treated toxin) in childhood and every 10 years thereafter. Tetanus toxoid is usually given to children in combination with diphtheria toxoid and the acellular pertussis vaccine (DTaP).

When trauma occurs, the wound should be cleaned and debrided, and tetanus toxoid booster should be given. If the wound is grossly contaminated, **tetanus immune globulin,** as well as the toxoid booster, should be given and penicillin administered. Half of the immune globulins should be infiltrated into the wound and the other half given IM at a site separate from the tetanus toxoid.

Tetanus immune globulin (tetanus antitoxin) is made in humans to avoid serum sickness reactions that occur when antitoxin made in horses is used. The administration of both immune globulins and tetanus toxoid (at different sites in the body) is an example of **passive–active immunity.**

2. Clostridium botulinum

Disease

Clostridium botulinum causes botulism.

Transmission

Spores, widespread in soil, contaminate vegetables and meats. When these foods are canned or vacuum-packed without adequate sterilization, spores survive and germinate in the anaerobic environment. Toxin is produced within the canned food and **ingested preformed.** The highest-risk foods are (1) alkaline vegetables such as green beans, peppers, and mushrooms and (2) smoked fish. The toxin is relatively heat-labile; it is inactivated by boiling for several minutes. Thus, disease can be prevented by sufficient cooking.

Pathogenesis

Botulinum toxin is absorbed from the gut and carried via the blood to peripheral nerve synapses, where it **blocks release of acetylcholine.** It is a protease that cleaves the proteins involved in acetylcholine release. The toxin is a polypeptide encoded by a lysogenic phage. Along with tetanus toxin, it is among the most toxic substances known. There are eight immunologic types of toxin; types A, B, and E are the most common in human illness. Botox is a commercial preparation of exotoxin A used to remove wrinkles on the face. Minute amounts of the toxin are effective in the treatment of certain spasmodic muscle disorders such as torticollis, "writer's cramp," and blepharospasm.

Clinical Findings

Descending weakness and paralysis, including diplopia, dysphagia, and respiratory muscle failure, are seen. No fever is present. In contrast, Guillain-Barré syndrome is an ascending paralysis (see Chapter 66).

Two special clinical forms occur: (1) wound botulism, in which spores contaminate a wound, germinate, and produce toxin at the site; and (2) infant botulism, in which the organisms grow in the gut and produce the toxin there. Ingestion of honey containing the organism is implicated in transmission of infant botulism. Affected infants develop weakness or paralysis and may need respiratory support but usually recover spontaneously. In the United States, infant botulism accounts for about half of the cases of botulism, and wound botulism is associated with drug abuse, especially skin-popping with black tar heroin.

Laboratory Diagnosis

The organism is usually not cultured. Botulinum toxin is demonstrable in uneaten food and the patient's serum by mouse protection tests. Mice are inoculated with a sample of the clinical specimen and will die unless protected by antitoxin. Enzyme-linked immunoassay (EIA) tests are also used to detect the toxin and polymerase-chain reaction (PCR) tests are being developed.

Treatment

The heptavalent antitoxin containing all seven types (A to G) is preferred to the trivalent antitoxin containing types A, B, and E. Respiratory support is provided as well. The antitoxin is made in horses and serum sickness may occur. A bivalent antitoxin (types A and B) purified from the plasma of humans immunized with botulinum toxoid is available for the treatment of infant botulism.

Prevention

Proper sterilization of all canned and vacuum-packed foods is essential. Food must be adequately cooked to inactivate the toxin. Swollen cans must be discarded (clostridial proteolytic enzymes form gas, which swells cans).

3. Clostridium perfringens

Clostridium perfringens causes two distinct diseases, gas gangrene and food poisoning, depending on the route of entry into the body.

Disease: Gas Gangrene

Gas gangrene (myonecrosis, necrotizing fasciitis) is one of the two diseases caused by *C. perfringens* (Figure 17–5). Gas gangrene is also caused by other histotoxic clostridia such as *Clostridium histolyticum, Clostridium septicum, Clostridium novyi*, and *Clostridium sordellii*. (*C. sordellii* also causes toxic shock syndrome in postpartum and postabortion women.)

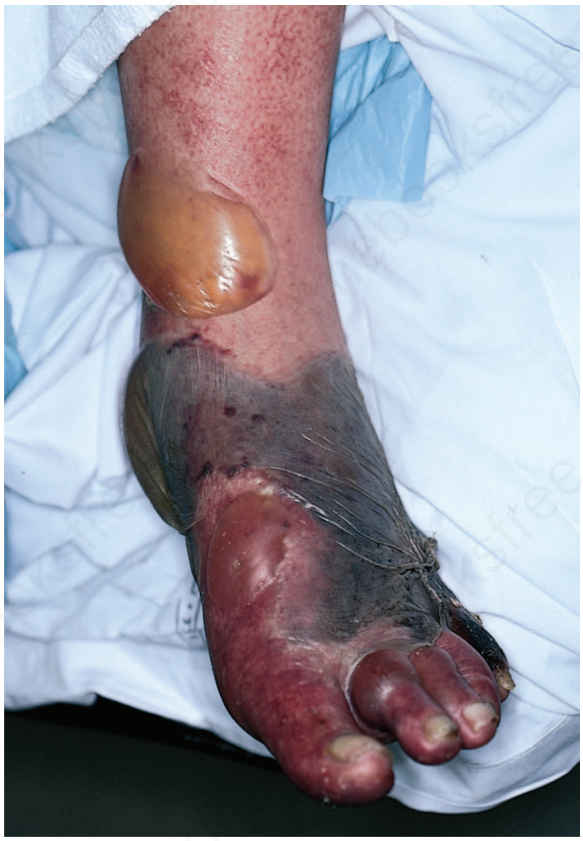

FIGURE 17–5 Gas gangrene. Note large area of necrosis on lateral aspect of foot. Necrosis is mainly caused by lecithinase produced by *Clostridium perfringens*. Gas in tissue is a feature of gangrene produced by these anaerobic bacteria. A large gas- and fluid-filled bulla is seen near the ankle. (Used with permission from David Kaplan, MD.)

Transmission

Spores are located in the soil; vegetative cells are members of the **normal flora of the colon and vagina.** Gas gangrene is associated with war wounds, automobile and motorcycle accidents, and septic abortions (endometritis).

Pathogenesis

Organisms grow in traumatized tissue (especially muscle) and produce a variety of toxins. The most important is **alpha toxin** (lecithinase), which damages cell membranes, including those of erythrocytes, resulting in hemolysis. Degradative enzymes produce gas in tissues.

Clinical Findings

Pain, edema, cellulitis, and gangrene (necrosis) occur in the wound area (see Figure 17–5). If crepitus is palpated in the affected tissue, it indicates gas in the tissue. This gas is typically hydrogen produced by the anaerobic bacteria. Hemolysis and jaundice are common, as are blood-tinged exudates. Shock and death can ensue. Mortality rates are high.

Laboratory Diagnosis

Smears of tissue and exudate samples show large gram-positive rods. Spores are not usually seen because they are formed primarily under nutritionally deficient conditions. The organisms are cultured anaerobically and then identified by sugar fermentation reactions and organic acid production. *Clostridium perfringens* colonies exhibit a double zone of hemolysis on blood agar. The colonies also produce a precipitate in egg yolk agar caused by the action of its lecithinase. Serologic tests are not useful.

Treatment

Penicillin G is the antibiotic of choice. Wounds should be debrided.

Prevention

Wounds should be cleansed and debrided. Penicillin may be given for prophylaxis. There is no vaccine.

Disease: Food Poisoning

Food poisoning is the second disease caused by *C. perfringens*.

Transmission

Spores are located in **soil** and can contaminate **food.** The heat-resistant spores survive cooking and germinate. The organisms grow to large numbers in reheated foods, especially meat dishes.

Pathogenesis

Clostridium perfringens is a member of the normal flora in the colon but not in the small bowel, where the enterotoxin

acts to cause diarrhea. The mode of action of the enterotoxin is the same as that of the enterotoxin of *Staphylococcus aureus* (i.e., it acts as a superantigen).

Clinical Findings

The disease has an 8- to 16-hour incubation period and is characterized by watery diarrhea with cramps and little vomiting. It resolves in 24 hours.

Laboratory Diagnosis

This is not usually done. There is no assay for the toxin. Large numbers of the organisms can be isolated from uneaten food.

Treatment

Symptomatic treatment is given; no antimicrobial drugs are administered.

Prevention

There are no specific preventive measures. Food should be adequately cooked to kill the organism.

4. Clostridium difficile

Disease

Clostridium difficile causes antibiotic-associated pseudomembranous colitis (Figure 17–6). *Clostridium difficile* is the most common nosocomial (hospital-acquired) cause of diarrhea. It is the leading infectious cause of gastrointestinal-associated deaths in the United States.

Transmission

The organism colonizes the **large intestine** of approximately 3% of the general population and up to 30% of hospitalized patients. Note that most people are not colonized, which explains why most people who take antibiotics do not get pseudomembranous colitis. It is transmitted by the fecal–oral route. Either the spores or the bacterial organism itself can be transmitted.

The majority of cases occur in hospitalized patients but about one-third of cases are community-acquired. The hands of hospital personnel are important intermediaries.

Pathogenesis

Antibiotics suppress drug-sensitive members of the normal flora of the colon, allowing *C. difficile* to multiply and produce **exotoxins A and B**. Both exotoxin A and exotoxin B are glucosyltransferases (i.e., enzymes that glucosylate [add glucose to] a G protein called Rho GTPase). The main effect of exotoxin B in particular is to cause depolymerization of actin, resulting in a loss of cytoskeletal integrity, apoptosis, and death of the enterocytes.

Clindamycin was the first antibiotic to be recognized as a cause of pseudomembranous colitis, but many antibiotics are known to cause this disease. At present, third-generation cephalosporins are the most common cause because they are so frequently used. Ampicillin and fluoroquinolones are also commonly implicated. In addition to antibiotics, **cancer chemotherapy** also predisposes to pseudomembranous colitis. *Clostridium difficile* rarely invades the intestinal mucosa.

Clinical Findings

Clostridium difficile causes diarrhea associated with **pseudomembranes** (yellow-white plaques) on the colonic mucosa (see Figure 17–6). (The term *pseudomembrane* is defined in Chapter 7 on page 39). The diarrhea is usually not bloody, and neutrophils are found in the stool in about half of the cases. Fever and abdominal pain often occur. The organism rarely enters the blood stream and rarely causes metastatic infection.

The pseudomembranes are visualized by sigmoidoscopy. Toxic megacolon can occur, and surgical resection of the colon may be necessary. Pseudomembranous colitis can be distinguished from the transient diarrhea that occurs as a side effect of many oral antibiotics by testing for the presence of the toxin in the stool. Even with adequate treatment, the organism may not be eradicated from the colon and recurrences occur at a rate of approximately 15% to 20%.

In 2005, a new, more virulent strain of *C. difficile* emerged. This *hypervirulent* strain causes more severe disease, is more likely to cause recurrences, and responds less

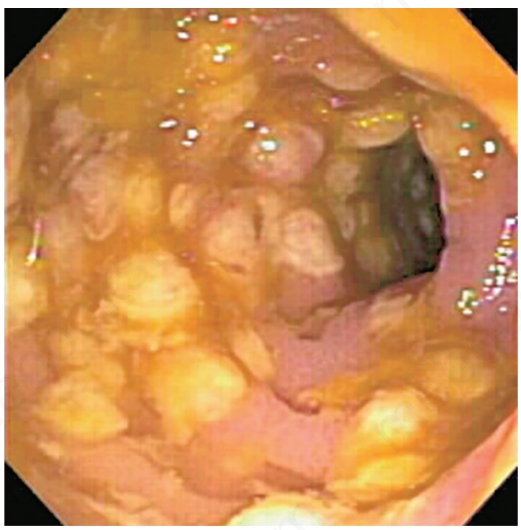

FIGURE 17–6 Pseudomembranous colitis. Note yellowish plaquelike lesions in colon. Caused by an exotoxin produced by *Clostridium difficile* that inhibits a signal transduction protein, leading to death of enterocytes. (Reproduced with permission from Fauci AS et al (eds): *Harrison's Principles of Internal Medicine*. 17th ed. New York: McGraw-Hill, 2008, pg 1837. Copyright © 2008 by The McGraw-Hill Companies, Inc.)

well to metronidazole than the previous strain. The strain is also characterized by resistance to quinolones. It is thought that the widespread use of quinolones for diarrheal disease may have selected for this new strain.

Laboratory Diagnosis

The presence of exotoxins in the filtrate of a patient's stool specimen is the basis of the laboratory diagnosis. It is insufficient to culture the stool for the presence of *C. difficile* because people can be colonized by the organism and not have disease.

There are two types of tests used to make the laboratory diagnosis. One detects the exotoxin itself and the other detects the genes that encode the exotoxin. To detect the *exotoxin itself*, an ELISA test employing antibody to the exotoxin is used. To detect the *genes that encode the exotoxin*, a PCR assay to determine the presence of the *toxin gene DNA* is used. The DNA-based test has greater sensitivity and specificity than the ELISA test. However, these NAAT tests (nucleic acid amplification tests) should be interpreted with caution because a person may only be colonized by *C. difficile* and be recorded as positive when in fact, *C. difficile* is not the cause of the patient's disease.

Treatment

The causative antibiotic should be withdrawn. Oral metronidazole or vancomycin should be given and fluids replaced. Metronidazole is preferred because using vancomycin may select for vancomycin-resistant enterococci. However, in life-threatening cases, vancomycin should be used because it is more effective than metronidazole. Also in life-threatening cases, surgical removal of the colon may be required.

In many patients, treatment does not eradicate the carrier state, and recurrent episodes of colitis can occur. Fidaxomicin (Dificid) is used both in the treatment of pseudomembranous colitis and in preventing relapses of this disease. It is effective in life-threatening cases.

Fecal transplantation is another possible therapeutic approach. It involves administering bowel flora from a normal individual either by enema or by nasoduodenal tube to the patient with pseudomembranous colitis. This approach is based on the concept of bacterial interference (i.e., to replace the *C. difficile* with normal bowel flora). Very high cure rates are claimed for this technique, but aesthetic considerations have limited its acceptance.

Prevention

There are no preventive vaccines or drugs. Because antibiotics are an important predisposing factor for pseudomembranous colitis, they should be prescribed only when necessary. In the hospital, strict infection control procedures, including rigorous handwashing, are important. Probiotics, such as the yeast *Saccharomyces*, may be useful to prevent pseudomembranous colitis.

NON–SPORE-FORMING GRAM-POSITIVE RODS

There are three important pathogens in this group: *Corynebacterium diphtheriae*, *Listeria monocytogenes*, and *Gardnerella vaginalis*. Important features of pathogenesis and prevention of *C. diphtheriae* and *L. monocytogenes* are described in Table 17–4.

CORYNEBACTERIUM DIPHTHERIAE

Disease

Corynebacterium diphtheriae causes diphtheria (Figure 17–7). Other *Corynebacterium* species (diphtheroids) are implicated in opportunistic infections.

Important Properties

Corynebacteria are gram-positive rods that appear **club-shaped** (wider at one end) and are arranged in palisades or in V- or L-shaped formations (Figure 17–8). The rods have a beaded appearance. The beads consist of granules of highly polymerized polyphosphate—a storage mechanism for high-energy phosphate bonds. The granules stain **metachromatically** (i.e., a dye that stains the rest of the cell blue will stain the granules red).

Transmission

Humans are the only natural host of *C. diphtheriae*. Both toxigenic and nontoxigenic organisms reside in the upper

TABLE 17–4 Important Features of Pathogenesis by *Corynebacterium diphtheriae* and *Listeria monocytogenes*

Organism	Type of Pathogenesis	Typical Disease	Predisposing Factor	Mode of Prevention
C. diphtheriae	Toxigenic	Diphtheria	Failure to immunize	Toxoid vaccine
L. monocytogenes	Pyogenic	Meningitis; sepsis	Neonate; immunosuppression	No vaccine; pasteurize milk products

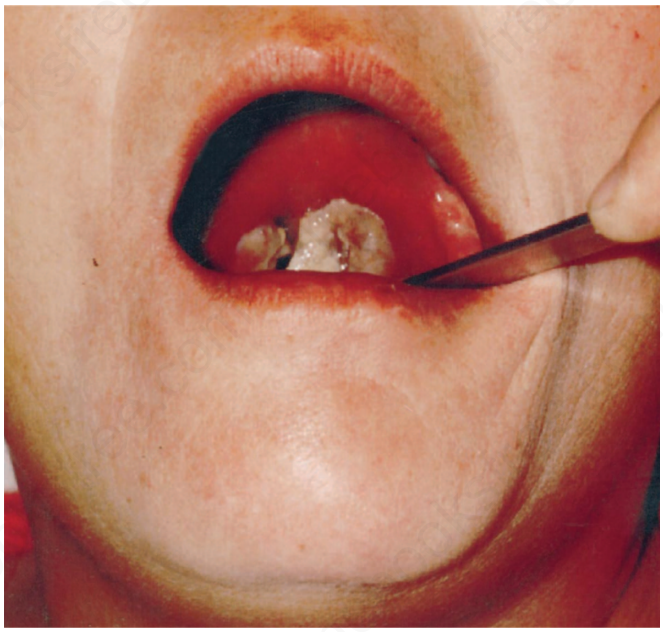

FIGURE 17–7 Diphtheria. Note whitish-gray pseudomembrane covering posterior pharynx and marked inflammation of palate and pharynx. Caused by diphtheria toxin, an exotoxin that inhibits protein synthesis by inhibiting elongation factor-2. (Used with permission from Dr. Peter Strebel.)

respiratory tract and are transmitted by **airborne droplets.** The organism can also infect the skin at the site of a preexisting skin lesion. This occurs primarily in the tropics but can occur worldwide in indigent persons with poor skin hygiene.

Pathogenesis

Although exotoxin production is essential for pathogenesis, invasiveness is also necessary because the organism must first establish and maintain itself in the throat. Diphtheria toxin inhibits protein synthesis by **ADP-ribosylation of elongation factor-2** (EF-2). The toxin affects all eukaryotic cells regardless of tissue type but has no effect on the analogous factor in prokaryotic cells.

The toxin is a single polypeptide with two functional domains. The binding (B) domain mediates binding of the toxin to glycoprotein receptors on the cell membrane. The active (A) domain possesses enzymatic activity that cleaves nicotinamide from nicotinamide adenine dinucleotide (NAD) and transfers the remaining ADP-ribose to EF-2, thereby inactivating it. Other organisms whose exotoxins act by ADP-ribosylation are described in Tables 7–10 and 7–11.

The DNA that codes for diphtheria toxin is part of the DNA of a temperate bacteriophage called beta phage. During the lysogenic phase of viral growth, the DNA of this virus integrates into the bacterial chromosome and the toxin is synthesized. *Corynebacterium diphtheriae* cells that are not lysogenized by this phage do not produce exotoxin and are nonpathogenic.

The host response to *C. diphtheriae* consists of the following:

(1) A local inflammation in the throat, with a fibrinous exudate that forms the tough, adherent, gray **pseudomembrane** characteristic of the disease.

(2) Antibody that can neutralize exotoxin activity by blocking the interaction of the binding domain with the receptors, thereby preventing entry into the cell. The immune status of a person can be assessed by Schick's test. The test is performed by intradermal injection of 0.1 mL of purified standardized toxin. If the patient has no antitoxin, the toxin will cause inflammation at the site 4 to 7 days later. If no inflammation occurs, antitoxin is present and the patient is immune. The test is rarely performed in the United States except under special epidemiologic circumstances.

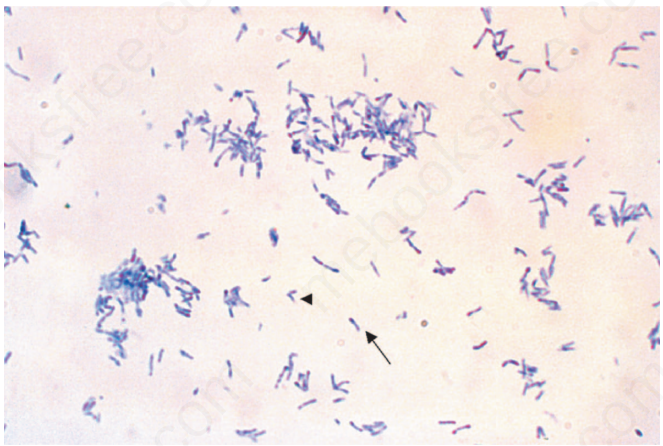

FIGURE 17–8 *Corynebacterium diphtheriae*—Gram stain. Arrow points to a "club-shaped" gram-positive rod. Arrowhead points to typical V- or L-shaped corynebacteria. (Source: Public Health Image Library, Centers for Disease Control and Prevention.)

Clinical Findings

Although diphtheria is rare in the United States, physicians should be aware of its most prominent sign, the thick, gray, adherent **pseudomembrane** over the tonsils and throat (see Figure 17–7). (The term *pseudomembrane* is defined in Chapter 7 on page 39.) The other aspects are nonspecific: fever, sore throat, and cervical adenopathy. There are three prominent complications:

(1) Extension of the membrane into the larynx and trachea, causing airway obstruction.

(2) Myocarditis accompanied by arrhythmias and circulatory collapse.

(3) Nerve weakness or paralysis, especially of the cranial nerves. Paralysis of the muscles of the soft palate and pharynx can lead to regurgitation of fluids through the nose. Peripheral neuritis affecting the muscles of the extremities also occurs.

Cutaneous diphtheria causes ulcerating skin lesions covered by a gray membrane. These lesions are often indolent and often do not invade surrounding tissue. Systemic symptoms rarely occur. In the United States, cutaneous diphtheria occurs primarily in the indigent.

Laboratory Diagnosis

Laboratory diagnosis involves both isolating the organism and demonstrating toxin production. It should be emphasized that the decision to treat with antitoxin is a clinical one and cannot wait for the laboratory results. A throat swab should be cultured on Loeffler's medium, a **tellurite plate,** and a blood agar plate. The tellurite plate contains a tellurium salt that is reduced to elemental tellurium within the organism. The typical gray-black color of tellurium in the colony is a telltale diagnostic criterion. If *C. diphtheriae* is recovered from the cultures, either animal inoculation or an antibody-based gel diffusion precipitin test is performed to document toxin production. A PCR assay for the presence of the toxin gene in the organism isolated from the patient can also be used.

Smears of the throat swab should be stained with both Gram stain and methylene blue. Although the diagnosis of diphtheria cannot be made by examination of the smear, the finding of many tapered, pleomorphic gram-positive rods can be suggestive. The methylene blue stain is excellent for revealing the typical metachromatic granules.

Treatment

The treatment of choice is **antitoxin,** which should be given immediately on the basis of clinical impression because there is a delay in laboratory diagnostic procedures. The toxin binds rapidly and irreversibly to cells and, once bound, cannot be neutralized by antitoxin. The function of antitoxin is therefore to neutralize unbound toxin in the blood. Because the antiserum is made in horses, the patient must be tested for hypersensitivity, and medications for the treatment of anaphylaxis must be available. Serum sickness (see Chapter 65) may occur after administration of antiserum made in horses.

Treatment with penicillin G or erythromycin is also recommended, but neither is a substitute for antitoxin. Antibiotics inhibit growth of the organism, reduce toxin production, and decrease the incidence of chronic carriers.

Prevention

Diphtheria is very rare in the United States because children are immunized with **diphtheria toxoid** (usually given as a combination of diphtheria toxoid, tetanus toxoid, and acellular pertussis vaccine, often abbreviated as DTaP). Diphtheria toxoid is prepared by treating the exotoxin with formaldehyde. This treatment inactivates the toxic effect but leaves the antigenicity intact. Immunization consists of three doses given at 2, 4, and 6 months of age, with boosters at 1 and 6 years of age. Because immunity wanes, a booster every 10 years is recommended. Immunization does not prevent nasopharyngeal carriage of the organism.

LISTERIA MONOCYTOGENES

Diseases

Listeria monocytogenes causes meningitis and sepsis in newborns, pregnant women, and immunosuppressed adults. It also causes outbreaks of febrile gastroenteritis. It is a major cause of concern for the food industry.

Important Properties

Listeria monocytogenes is a small gram-positive rod arranged in V- or L-shaped formations similar to corynebacteria. The organism exhibits an unusual **tumbling** movement that distinguishes it from the corynebacteria, which are nonmotile. Colonies on a blood agar plate produce a narrow zone of β-hemolysis that resembles the hemolysis of some streptococci.

Listeria grows well at cold temperatures, so storage of contaminated food in the refrigerator can increase the risk of gastroenteritis. This paradoxical growth in the cold is called "cold enhancement."

Pathogenesis

Listeria infections occur primarily in two clinical settings: (1) in the fetus or in a newborn as a result of transmission **across the placenta** or **during delivery;** and (2) in pregnant women and immunosuppressed adults, especially renal transplant patients. (Note that pregnant women have reduced cell-mediated immunity during the third trimester.)

The organism is distributed worldwide in animals, plants, and soil. From these reservoirs, it is transmitted to humans primarily by ingestion of unpasteurized milk products, undercooked meat, and raw vegetables. Contact with domestic farm animals and their feces is also an important source. In the United States, listeriosis is primarily a foodborne disease associated with eating unpasteurized cheese and delicatessen meats. Following ingestion, the bacteria appear in the colon and then can colonize the female genital tract. From this location, they can infect the fetus if membranes rupture or infect the neonate during passage through the birth canal.

The pathogenesis of *Listeria* depends on the organism's ability to invade and survive within cells. Invasion of cells is mediated by internalin made by *Listeria* and E-cadherin on the surface of human cells. The ability of *Listeria* to pass the placenta, enter the meninges, and invade the gastrointestinal tract depends on the interaction of internalin and E-cadherin on those tissues.

Upon entering the cell, the organism produces **listeriolysin,** which allows it to escape from the phagosome into the cytoplasm, thereby escaping destruction in the phagosome. Because *Listeria* preferentially grows intracellularly, cell-mediated immunity is a more important host defense than humoral immunity. Suppression of **cell-mediated immunity** predisposes to *Listeria* infections.

Listeria monocytogenes can move from cell to cell by means of **actin rockets**—filaments of actin polymerize and propel the bacteria through the membrane of one human cell and into another.

Clinical Findings

Infection during pregnancy can cause abortion, premature delivery, or sepsis during the peripartum period. Newborns infected at the time of delivery can have acute meningitis 1 to 4 weeks later. The bacteria reach the meninges via the bloodstream (bacteremia). The infected mother either is asymptomatic or has an influenzalike illness. *Listeria monocytogenes* infections in immunocompromised adults can be either sepsis or meningitis.

Gastroenteritis caused by *L. monocytogenes* is characterized by watery diarrhea, fever, headache, myalgias, and abdominal cramps but little vomiting. Outbreaks are usually caused by contaminated dairy products, but undercooked meats such as chicken and hot dogs and ready-to-eat foods such as coleslaw have also been involved.

Laboratory Diagnosis

Laboratory diagnosis is made primarily by Gram stain and culture. The appearance of gram-positive rods resembling **diphtheroids** and the formation of small, gray colonies with a narrow zone of β-hemolysis on a blood agar plate suggest the presence of *Listeria*. The isolation of *Listeria* is confirmed by the presence of motile organisms, which differentiate them from the nonmotile corynebacteria. Identification of the organism as *L. monocytogenes* is made by sugar fermentation tests.

Treatment

Treatment of invasive disease, such as meningitis and sepsis, consists of trimethoprim-sulfamethoxazole. Combinations, such as ampicillin and gentamicin or ampicillin and trimethoprim-sulfamethoxazole, can also be used. Resistant strains are rare. *Listeria* gastroenteritis typically does not require treatment.

Prevention

Prevention is difficult because there is no immunization. Limiting the exposure of pregnant women and immunosuppressed patients to potential sources such as farm animals, unpasteurized milk products, and raw vegetables is recommended. Trimethoprim-sulfamethoxazole given to immunocompromised patients to prevent *Pneumocystis* pneumonia can also prevent listeriosis.

GARDNERELLA VAGINALIS

Disease

Gardnerella vaginalis is the main organism associated with bacterial vaginosis. This disease is the most common vaginal infection of sexually active women.

Important Properties

Gardnerella vaginalis is a small, facultative **gram-variable rod**. The term "gram-variable" refers to the observation that some organisms are purple while others are pink in a Gram-stained specimen. Structurally, it has a gram-positive cell wall but the wall is thin and older organisms tend to lose the purple color.

Pathogenesis

The pathogenesis of bacterial vaginosis is uncertain. *Gardnerella vaginalis* is often found in association with anaerobes such as *Mobiluncus* (see Chapter 27) and together they cause the symptoms of this disease. It is *not* considered to be a sexually transmitted infection.

Clinical Findings

Bacterial vaginosis is characterized by a malodorous, white or gray-colored vaginal discharge. The discharge has a characteristic "fishy" odor. Inflammatory changes are typically absent which is why it is called a "vaginosis" rather than a "vaginitis." Mild itching may occur. Women with bacterial vaginosis have a higher incidence of preterm deliveries and, consequently, a higher incidence of morbidity and mortality occurs in their newborn children.

Laboratory Diagnosis

Clue cells, which are vaginal epithelial cells covered with bacteria, are an important laboratory finding seen in a microscopic examination of the vaginal discharge (Figure 17-9). In addition, the **"whiff" test**, which consists of treating the vaginal discharge with 10% KOH and smelling a pungent, "fishy" odor, is often positive. However, trichomoniasis, which can also cause a positive whiff test, must be ruled out before a diagnosis of bacterial vaginosis can be made. A pH of greater than 4.5 of the vaginal discharge supports the diagnosis of bacterial vaginosis.

Treatment and Prevention

The drug of choice is metronidazole. Treatment of sexual partners is not recommended. There is no vaccine.

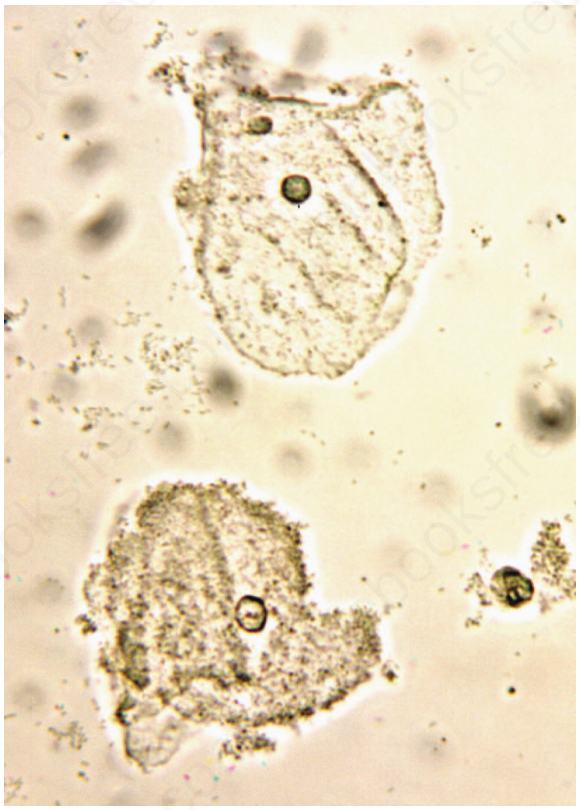

FIGURE 17–9 Clue cells in bacterial vaginosis. Note that the lower epithelial cell is a "clue cell" because its surface is covered with bacteria. The upper epithelial cell is *not* a "clue cell" because its surface has few bacteria. (Reproduced with permission from Usatine RP et al: *The Color Atlas of Family Medicine*, New York: McGraw-Hill, 2009. Courtesy of E.J. Mayeaux, Jr., MD.)

SELF-ASSESSMENT QUESTIONS

1. Which one of the following is a club-shaped, gram-positive rod that causes disease by producing an exotoxin that kills cells by inhibiting elongation factor-2, resulting in the inhibition of protein synthesis?
   (A) *Bacillus anthracis*
   (B) *Bacillus cereus*
   (C) *Clostridium perfringens*
   (D) *Corynebacterium diphtheriae*
   (E) *Listeria monocytogenes*
2. Which one of the following is a large gram-positive rod that causes necrosis of tissue by producing an exotoxin that degrades lecithin, resulting in the lysis of cell membranes?
   (A) *Bacillus anthracis*
   (B) *Bacillus cereus*
   (C) *Clostridium perfringens*
   (D) *Corynebacterium diphtheriae*
   (E) *Listeria monocytogenes*
3. Which one of the following sets of bacteria causes disease characterized by a pseudomembrane?
   (A) *Bacillus anthracis* and *Listeria monocytogenes*
   (B) *Bacillus cereus* and *Clostridium perfringens*
   (C) *Bacillus cereus* and *Clostridium tetani*
   (D) *Corynebacterium diphtheriae* and *Clostridium difficile*
   (E) *Corynebacterium diphtheriae* and *Listeria monocytogenes*
4. Disease caused by which one of the following sets of bacteria can be prevented by a toxoid vaccine?
   (A) *Bacillus anthracis* and *Clostridium botulinum*
   (B) *Bacillus anthracis* and *Clostridium perfringens*
   (C) *Bacillus cereus* and *Clostridium tetani*
   (D) *Corynebacterium diphtheriae* and *Clostridium tetani*
   (E) *Corynebacterium diphtheriae* and *Listeria monocytogenes*
5. Your patient in the pediatric intensive care unit is a 2-week-old boy with a high fever and the signs of meningitis. Gram stain of the spinal fluid reveals small gram-positive rods. Colonies on blood agar show a narrow zone of β-hemolysis. Which one of the following is the most likely cause of his neonatal meningitis?
   (A) *Bacillus anthracis*
   (B) *Bacillus cereus*
   (C) *Clostridium perfringens*
   (D) *Corynebacterium diphtheriae*
   (E) *Listeria monocytogenes*
6. Regarding the patient in Question 5, which one of the following is the best antibiotic to treat the infection?
   (A) Doxycycline
   (B) Gentamicin
   (C) Metronidazole
   (D) Trimethoprim-sulfamethoxazole
   (E) Vancomycin
7. Your patient is a 40-year-old woman with diplopia and other signs of cranial nerve weakness. History reveals she grows her own vegetables and likes to preserve them in jars that she prepares at home. She is fond of her preserved string beans, which is what she ate uncooked in a salad for dinner last night. Which one of the following is the most likely cause of this clinical picture?
   (A) *Bacillus anthracis*
   (B) *Clostridium botulinum*
   (C) *Clostridium perfringens*
   (D) *Clostridium tetani*
   (E) *Listeria monocytogenes*
8. Your patient is a 30-year-old man with a 2-cm lesion on his arm. It began as a painless papule that enlarged and, within a few days, ulcerated and formed a black crust (eschar). He works in an abattoir where his job is removing the hide from the cattle. A Gram stain of fluid from the lesion reveals large gram-positive rods. Which one of the following bacteria is likely to be the cause?
   (A) *Bacillus anthracis*
   (B) *Clostridium botulinum*
   (C) *Clostridium perfringens*
   (D) *Clostridium tetani*
   (E) *Listeria monocytogenes*
9. Your patient is a 30-year-old man who was brought to the emergency room following a motorcycle accident in which he sustained a compound fracture of his leg. He now has a high fever and a rapidly spreading cellulitis with crepitus in the area of the fracture. Large gram-positive rods are seen on the exudate. Necrotic tissue was debrided. Which one of the following is the best antibiotic to treat the infection?
   (A) Azithromycin
   (B) Ciprofloxacin
   (C) Gentamicin
   (D) Penicillin G
   (E) Vancomycin
10. Your patient is a 65-year-old woman who is several days post-op following removal of her carcinoma of the colon. She now spikes

a fever and has a cough, and chest X-ray shows pneumonia. While being treated with the appropriate antibiotics, she develops severe diarrhea. You suspect she may have pseudomembranous colitis. Which one of the following is the best antibiotic to treat the infection?

(A) Ceftriaxone
(B) Doxycycline
(C) Gentamicin
(D) Metronidazole
(E) Trimethoprim-sulfamethoxazole

ANSWERS

1. **(D)**
2. **(C)**
3. **(D)**
4. **(D)**
5. **(E)**
6. **(D)**
7. **(B)**
8. **(A)**
9. **(D)**
10. **(D)**

SUMMARIES OF ORGANISMS

Brief summaries of the organisms described in this chapter begin on page 656. 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.