• **BACILLUS SPECIES**
  The genus *Bacillus* includes large aerobic, gram-positive rods occurring in chains. Most members of this genus are saprophytic organisms prevalent in soil, water, and air and on vegetation, such as *Bacillus cereus* and *Bacillus subtilis*. Some are insect pathogens, such as *B. thuringiensis*. This organism is also capable of causing disease in humans. *B. cereus* can grow in foods and cause food poisoning by producing either an enterotoxin (diarrhea) or an emetic toxin (vomiting). Both *B. cereus* and *B. thuringiensis* may occasionally produce disease in immunocompromised humans (eg, meningitis, endocarditis, endophthalmitis, conjunctivitis, or acute gastroenteritis). *B. anthracis*, which causes anthrax, is the principal pathogen of the genus.

• **Morphology and identification**
  Large spore forming gram positive and are arranged in long chains; spores are located in the center of the nonmotile bacilli. Spore viable for up to 50 years Non hemolytic on sheep blood agar

• **growth Characteristics**
  The saprophytic bacilli use simple sources of nitrogen and carbon for energy and growth. The spores are resistant to environmental changes, withstand dry heat and certain chemical disinfectants for moderate periods, and persist for years in dry earth. Animal products contaminated with anthrax spores (eg, hides, bristles, hair, wool, bone) can be sterilized by autoclaving.

• **BACILLUS ANTHRACIS**
  Anthrax is primarily a disease of herbivores—goats, sheep, cattle, horses, and so on; other animals (eg, rats) are relatively resistant to the infection. Anthrax is endemic among agrarian societies in developing countries in Africa, the Middle East, and Central America. Humans become infected incidentally by contact with infected animals or their products. In animals, the portal of entry is the mouth and the gastrointestinal tract. Spores from contaminated soil find easy access when ingested with spiny or irritating vegetation

• **Clinical infection in humans**
  There are three types of anthrax

1- **Cutaneous anthrax**
  Cutaneous anthrax generally occurs on exposed surfaces of the arms or hands followed in frequency by the face and neck. A pruritic papule develops 1–7 days after entry of the organisms or spores through a scratch. Initially, it resembles an insect bite. The papule rapidly changes into a vesicle or small ring of vesicles that coalesces, and a necrotic ulcer develops. The lesions typically are 1–3 cm in diameter and have a characteristic central black eschar. Marked edema occurs. Lymphangitis and lymphadenopathy and systemic signs and symptoms of fever, malaise, and headache may occur. After 7–10 days, the eschar is fully developed. Eventually, it dries, loosens, and separates; healing is by granulation and
leaves a scar. It may take many weeks for the lesion to heal and the edema to subside. Antibiotic therapy does not appear to change the natural progression of the disease but prevents dissemination.

In as many as 20% of patients, cutaneous anthrax can lead to sepsis, the consequences of systemic infection—including meningitis—and death.

2- Inhalation anthrax (woolsorters’ disease).

The spores from the dust of wool, hair, or hides are inhaled; phagocytosed in the lungs; and transported by the lymphatic drainage to the mediastinal lymph nodes, where germination occurs. This is followed by toxin production and the development of hemorrhagic mediastinitis and sepsis, which are usually rapidly fatal. The incubation period in inhalation anthrax may be as long as 6 weeks. The early clinical manifestations are associated with marked hemorrhagic necrosis and edema of the mediastinum. Substernal pain may be prominent, and there is pronounced mediastinal widening visible on chest radiographs. Hemorrhagic pleural effusions follow involvement of the pleura; cough is secondary to the effects on the trachea. Sepsis occurs, and there may be hematogenous spread to the gastrointestinal tract, causing bowel ulceration, or to the meninges, causing hemorrhagic meningitis. The fatality rate in inhalation anthrax is high in the setting of known exposure; it is higher when the diagnosis is not initially suspected.

3- Gastrointestinal anthrax

This is rare in humans, and is extremely uncommon. It occurs by ingestion of raw meats abdominal pain, vomiting, and bloody diarrhea are clinical signs.

Virulence factors

- *B anthracis* isolates that do not produce a capsule are not virulent and do not induce anthrax in test animals.
- The poly-d-glutamic acid capsule is antiphagocytic.
- The capsule gene is present on a plasmid, pXO2.

Anthrax toxins are made up of three proteins, protective antigen (PA), edema factor (EF), and lethal factor (LF). PA binds to specific cell receptors, and after proteolytic activation, it forms a membrane channel that mediates entry of EF and LF into the cell. EF is an adenylate cyclase; with PA, it forms a toxin known as edema toxin. LF plus PA form lethal toxin, which is a major virulence factor and cause of death in infected animals and humans. When injected into laboratory animals (eg, rats), the lethal toxin can quickly kill the animals. The anthrax toxin genes are encoded on another plasmid, pXO1.

Pathology

In susceptible animals and humans, the organisms proliferate at the site of entry. The capsules remain intact, and the organisms are surrounded by a large amount of proteinaceous fluid containing few leukocytes from which they rapidly disseminate and reach the bloodstream. In resistant animals, the organisms proliferate for a few hours, by which time there is massive accumulation of leukocytes. The capsules gradually disintegrate and disappear. The organisms remain localized.

Clinical Findings
In humans, approximately 95% of cases are cutaneous anthrax, and 5% are inhalation. Gastrointestinal anthrax is very rare

**Diagnostic Laboratory Tests**

Specimens to be examined are fluid or pus from a local lesion, blood, pleural fluid, and cerebrospinal fluid in inhalational anthrax associated with sepsis and stool or other intestinal contents in the case of gastrointestinal anthrax. Stained smears from the local lesion or of blood from dead animals often show chains of large gram-positive rods. Anthrax can be identified in dried smears by immunofluorescence staining techniques.

- When grown on blood agar plates, the organisms produce nonhemolytic gray to white, tenacious colonies with a rough texture and a ground-glass appearance. Comma-shaped outgrowths (Medusa head, “curled hair”) may project from the colony. Demonstration of capsule requires growth on bicarbonate-containing medium in 5–7% carbon dioxide.
- Detection of the capsule by fluorescent antibody
- Identification of toxin genes by polymerase chain reaction (PCR).
- A rapid enzyme-linked immunoassay (ELISA) that measures total antibody to PA has been approved by the U.S. Food and Drug Administration (FDA), but the test result is not positive early in disease.

**Resistance and Immunity**

Immunization to prevent anthrax is based on the classic experiments of Louis Pasteur. In 1881, he proved that cultures grown in broth at 42–52°C for several months lost much of their virulence and could be injected live into sheep and cattle without causing disease; subsequently, such animals proved to be immune. Active immunity to anthrax can be induced in susceptible animals by vaccination with live attenuated bacilli, with spore suspensions, or with PAs from culture filtrates. Animals that graze in known anthrax districts should be immunized for anthrax annually. Other immunotherapies available or in development include anthrax immunoglobulin and human monoclonal antibodies with high affinity for PA.

**Treatment**

- Many antibiotics are effective against anthrax in humans, but treatment must be started early. Ciprofloxacin is recommended for treatment; penicillin G, along with gentamicin or streptomycin, has previously been used to treat anthrax.
- In the setting of potential exposure to *B anthracis* as an agent of biologic warfare, prophylaxis with ciprofloxacin or doxycycline should be continued for 4 weeks while three doses of vaccine are being given or for 8 weeks if no vaccine is administered.
- Some other gram-positive bacilli, such as *B cereus*, are resistant to penicillin by virtue of β-lactamase production.
- Doxycycline, erythromycin, and ciprofloxacin may be effective alternatives to penicillin.

**Epidemiology, Prevention, and Control**


• Soil is contaminated with anthrax spores from the carcasses of dead animals. These spores remain viable for decades. Perhaps spores can germinate in soil at a pH of 6.5 at proper temperature.
• Grazing animals infected through injured mucous membranes serve to perpetuate the chain of infection. Contact with infected animals or with their hides, hair, and bristles is the source of infection in humans. Control measures include
• (1) disposal of animal carcasses by burning or by deep burial in lime pits, (2) decontamination (usually by autoclaving) of animal products, (3) protective clothing and gloves for handling potentially infected materials, and (4) active immunization of domestic animals with live attenuated vaccines. Persons with high occupational risk should be immunized.

• **BACILLUS CEREUS**

Food poisoning caused by *B. cereus* has two distinct forms, the emetic type, which is associated with fried rice, and the diarrheal type, which is associated with meat dishes and sauces. *B. cereus* produces toxins that cause disease that is more an intoxication than a foodborne infection. The emetic form is manifested by nausea, vomiting, abdominal cramps, and occasionally diarrhea and is self-limiting, with recovery occurring within 24 hours. It begins 1–5 hours after ingestion of rice and occasionally pasta dishes. *B. cereus* is a soil organism that commonly contaminates rice. When large amounts of rice are cooked and allowed to cool slowly, the *B. cereus* spores germinate, and the vegetative cells produce the toxin during log-phase growth or during sporulation. The diarrheal form has an incubation period of 1–24 hours and is manifested by profuse diarrhea with abdominal pain and cramps; fever and vomiting are uncommon. *B. cereus* is an important cause of eye infections, such as severe keratitis, endophthalmitis, and panophthalmitis. Typically, the organisms are introduced into the eye by foreign bodies associated with trauma. *B. cereus* has also been associated with localized infections and with systemic infections, including endocarditis, meningitis, osteomyelitis, and pneumonia; the presence of a medical device or intravenous drug use predisposes to these infections. *B. cereus* is resistant to a variety of antimicrobial agents, including penicillins and cephalosporins. Serious non-foodborne infections should be treated with vancomycin or clindamycin with or without an aminoglycoside. Other *Bacillus* species are rarely associated with human disease.

*Bacillus subtilis* cause laboratory contamination
**Clostridium**

**Habitat**
Soil, water, decaying animal and plant matter, and human and animal intestines.

**Characteristics**
Gram-positive rods, but older cultures may stain irregularly. All species form characteristic endospores, which create a bulge in the bacterial body, for instance, the drumstick-shaped *C. tetani* (this shape is useful in laboratory identification of the organisms). Some species are motile with peritrichous flagella (e.g. *C. tetani*), while others (e.g. *C. welchii*) have a capsule.

**Culture and identification**
Grow anaerobically on blood agar or Robertson’s cooked meat medium (liquid culture). Although *C. tetani* and *Clostridium novyi* are strict anaerobes, *Clostridium histolyticum* and *C. welchii* can grow in the presence of limited amounts of oxygen (aerotolerant). The saccharolytic, proteolytic and toxigenic potentials of the organisms are useful in identification.

**Clostridium welchii**

**Habitat and transmission**
Spores are found in the soil, and vegetative cells are normal flora of the colon and vagina. This bacterium causes two discrete diseases, due to either exogenous or endogenous infection:

**Characteristics**
A short, fat bacillus. Spores are not usually found as they are formed under nutritionally deficient conditions. More tolerant of oxygen than other clostridia.

**Toxins**
A variety of toxins (at least 12), including collagenase, proteinase and hyaluronidase, are formed, the most notable of which is the α-toxin, which lyses the phospholipids of eukaryotic cell membranes (i.e. a phospholipase). *C. welchii* is divided into five types (A–E) on the basis of toxins formed; type A is the human pathogen.

**Culture and identification**
Grows well on blood agar under anaerobic conditions, producing β-haemolytic colonies; some are non-haemolytic. The saccharolytic characteristic is used for identification purposes as it ferments litmus milk, producing acids and gases responsible for the so-called ‘stormy-clot’ reaction. Nagler’s reaction The neutralization of the α-toxin of the organism growing on agar plates by a specific antitoxin is useful in identification. In this test, the organism is streaked on an agar plate containing egg yolk (which contains high concentrations of phospholipase), half of the plate having been spread with antitoxin; an opaque reaction develops, surrounding the growth of *C. welchii* in the untreated half of the plate, while in the other half, no such reaction occurs as the toxin is neutralized by the antitoxin.

**Pathogenicity**
Causes gas gangrene and food poisoning. Gas gangrene (myonecrosis) Wounds associated with traumatized tissue (especially muscle) may become infected with C. welchii and other clostridia, with severe, life-threatening spreading infection. Activity of the bacillus in injured tissue results in toxin and enzyme production, allowing the organism to establish and multiply in the wound. Characteristic signs and symptoms include pain, oedema and crepitation produced by gas in tissues.

Food poisoning Some strains of C. welchii produce an enterotoxin that induces food poisoning. This is due to the ingestion of large numbers of vegetative cells from contaminated food, which then sporulate in the gut and release enterotoxin. The disease is characterized by watery diarrhoea with little vomiting.

**Treatment and prevention**

Gas gangrene Rapid intervention with:
1. extensive debridement of the wound
2. antibiotics (penicillin or metronidazole)
3. anti-α-toxin administration.

Food poisoning Symptomatic therapy only; no specific treatment.

**Clostridium tetani**

**Habitat and transmission**

C. tetani is present in the intestinal tract of herbivores, and spores are widespread in soil. Germination of spores is promoted by poor blood supply and necrotic tissue and debris in wounds.

**Characteristics**

Long, thin bacilli with terminal spores giving the characteristic ‘drumstick’ appearance. Produces an extremely potent neurotoxin, tetanospasmin, by vegetative cells at the wound site. Another less powerful toxin, tetanolysin, is haemolytic in nature.

**Culture and identification**

Grows on blood agar, anaerobically, as a fine spreading colony. Identification in vitro is by a toxin neutralization test on blood agar, or in vivo by inoculation of culture filtrate into mice. The ‘two-mouse model’ is used: one animal is protected with antitoxin and the other is unprotected; the latter dies with typical tetanic spasms.

**Pathogenicity**

The agent of tetanus (lockjaw), which is a typical toxinmediated disease. The powerful, heat-labile neurotoxin (tetanospasmin) is produced at the wound site and released during cell lysis. It is retrogradely carried via the peripheral nerves (intra-axonally) to the central nervous system where it blocks inhibitory mediators at spinal synapses. This causes sustained muscle spasm and the characteristic signs of spasm of jaw muscles (lockjaw, trismus) and facial muscles (risus sardonicus), and arching of the body (opisthotonos). Toxin genes are plasmid-coded. C. tetani also produces an oxygen-labile haemolysin (tetanolysin); the clinical significance of this enzyme is not clear.

**Treatment and prevention**

Antitoxin (hyperimmune human α-globulin) administered with or without toxoid, depending on the immunization history of the patient. Prevention is by tetanus toxoid (a component of
the diphtheria–tetanus–pertussis (DTP) vaccine) with boosters every 10 years. Proper wound debridement and administration of penicillin (to inhibit clostridial growth and secondary infection) are other important management measures.

**Clostridium difficile**

Found in the faeces of 3–6% adults and almost all healthy infants, *C. difficile* is the agent of antibiotic-associated colitis, which may lead to sometimes lethal pseudomembranous colitis. It multiplies in the gut under the selective pressure of antibiotics. Although clindamycin was earlier singled out as the main cause of colitis, it is now known that common drugs such as ampicillin may occasionally precipitate the disease. Treatment is to withhold the offending antibiotic and administer oral vancomycin or metronidazole. As much as 25% of the common antibiotic-associated diarrhoea is considered to be due to *C. difficile*.

**Clostridium botulinum**

*C botulinum*, which causes botulism, is worldwide in distribution; it is found in soil and occasionally in animal feces. The agent of botulism, a form of food poisoning, has powerful toxins. In contrast, minute doses of botulinum toxin, injected periodically, are popular in beauty therapy as facial muscle relaxants to minimize wrinkles for a youthful appearance, so-called botox treatment utilized in aesthetic dentistry in some parts of the world. Types of *C botulinum* are distinguished by the antigenic type of toxin they produce. Spores of the organism are highly resistant to heat, withstanding 100°C for several hours. Heat resistance is diminished at acid pH or high salt concentration.

**Toxin**

During the growth of *C botulinum* and during autolysis of the bacteria, toxin is liberated into the environment. Seven antigenic varieties of toxin (A–G) are known. Types A, B, E, and F are the principal causes of human illness. Types A and B have been associated with a variety of foods and type E predominantly with fish products. Type C produces limberneck in birds; type D causes botulism in mammals. Type G is not associated with disease. The toxin is a 150,000-MW (molecular weight) protein that is cleaved into 100,000MW and 50,000-MW proteins linked by a disulfide bond. Botulinum toxin is absorbed from the gut and binds to receptors of presynaptic membranes of motor neurons of the peripheral nervous system and cranial nerves. *C botulinum* toxins are among the most toxic substances known: The lethal dose for a human is probably about 1–2 μg/kg. The toxins are destroyed by heating for 20 minutes at 100°C. Rare strains of *C butyricum* and *C barati* have also been shown to produce botulinum neurotoxin and cause botulism in humans. Strains that produce toxins A, B, or F are associated with infant botulism.

**Pathogenesis**

Most cases of botulism represent an intoxication resulting from the ingestion of food in which *C botulinum* has grown and produced toxin. The most common offenders are spiced, smoked, vacuum packed, or canned alkaline foods that are eaten without cooking. In such foods, spores of *C botulinum* germinate; that is, under anaerobic conditions, vegetative forms grow and produce toxin. In infant botulism, honey is the most frequent vehicle of infection. The pathogenesis differs from the way that adults acquire infection. The infant
ingests the spores of \textit{C botulinum} (or \textit{C butyricum} or \textit{C baratii}), and the spores germinate within the intestinal tract. The vegetative cells produce toxin as they multiply; the neurotoxin then gets absorbed into the bloodstream. The toxin acts by blocking release of acetylcholine at synapses and neuromuscular junctions. The result is flaccid paralysis. The electromyogram and edrophonium strength test results are typical.

\textbf{Clinical Findings}

Symptoms begin 18–24 hours after ingestion of the toxic food, with visual disturbances (incoordination of eye muscles, double vision), inability to swallow, and speech difficulty; signs of bulbar paralysis are progressive, and death occurs from respiratory paralysis or cardiac arrest. Gastrointestinal symptoms are not regularly prominent. There is no fever. The patient remains fully conscious until shortly before death. The mortality rate is high. Patients who recover do not develop antitoxin in the blood. In the United States, infant botulism is as common as or more common than the classic form of paralytic botulism associated with the ingestion of toxin-contaminated food. The infants in the first months of life develop poor feeding, weakness, and signs of paralysis (floppy baby). Infant botulism may be one of the causes of sudden infant death syndrome. \textit{C botulinum} and botulinum toxin are found in feces but not in serum.

\textbf{Diagnostic Laboratory Tests}

Toxin can often be demonstrated in serum, gastric secretions, or stool from the patient, and toxin may be found in leftover food. \textit{C botulinum} may be grown from food remains and tested for toxin production, but this is rarely done and is of questionable significance. In infant botulism, \textit{C botulinum} and toxin can be demonstrated in bowel contents but not in serum. Other methods used to detect toxin include ELISAs and PCR, but the latter may detect organisms that carry the gene but do not express toxin.

\textbf{Treatment}

Potent antitoxins to three types of botulinum toxins have been prepared in horses. Because the type responsible for an individual case is usually not known, trivalent (A, B, E) antitoxin must be promptly administered intravenously with customary precautions. Adequate respiration must be maintained by mechanical ventilation if necessary. These measures have reduced the mortality rate from 65\% to below 25\%. Although most infants with botulism recover with supportive care alone, antitoxin therapy is recommended.

\textbf{Epidemiology, Prevention, and Control}

Because spores of \textit{C botulinum} are widely distributed in soil, they often contaminate vegetables, fruits, and other materials. A large restaurant-based outbreak was associated with sautéed onions. When such foods are canned or otherwise preserved, they either must be sufficiently heated to ensure destruction of spores or must be boiled for 20 minutes before consumption. Strict regulation of commercial canning has largely overcome the danger of widespread outbreaks, but commercially prepared foods have caused deaths. A chief risk factor for botulism lies in home-canned foods, particularly string beans, corn, peppers, olives, peas, and smoked fish or vacuum-packed fresh fish in plastic bags. Toxic foods may be spoiled and rancid, and cans may “swell,” or the appearance may be innocuous. The risk from home-canned foods can be reduced if the food is boiled for more than 20 minutes before consumption.