Clostridium Species

The clostridia are large anaerobic, gram-positive, motile rods. Many species decompose proteins or form toxins, and some do both. Their natural habitat is the soil or the intestinal tract of animals and humans, where they live as saprophytes. Among the pathogens are the organisms causing **botulism, tetanus, gas gangrene,** and **pseudomembranous colitis.**

Morphology & Identification

Typical Organisms

Spores of clostridia are usually wider than the diameter of the rods in which they are formed. In the various species, the spore is placed centrally, subterminally, or terminally. Most species of clostridia are motile and possess peritrichous flagella.

Culture

Clostridia are anaerobes and grow under anaerobic conditions; a few species are aero-tolerant and will also grow in ambient air.in general, the clostridia grow well on the blood-enriched media used to grow anaerobes and on other media used to culture anaerobes .

Colony Forms

Some clostridia produce large raised colonies (eg, *C perfringens*); others produce smaller colonies (eg, *C tetani*). Some clostridia form colonies that spread on the agar surface. Many clostridia produce a zone of hemolysis on blood agar. *C perfringens* typically produces multiple zones of hemolysis around colonies.

Growth Characteristics

Clostridia can ferment a variety of sugars; many can digest proteins. Milk is turned acid by some and digested by others and undergoes "stormy fermentation" (ie, clot torn by gas) with a third group (eg, *C perfringens*). Various enzymes are produced by different species .

Antigenic Characteristics

Clostridia share some antigens but also possess specific soluble antigens that permit grouping by precipitin tests.

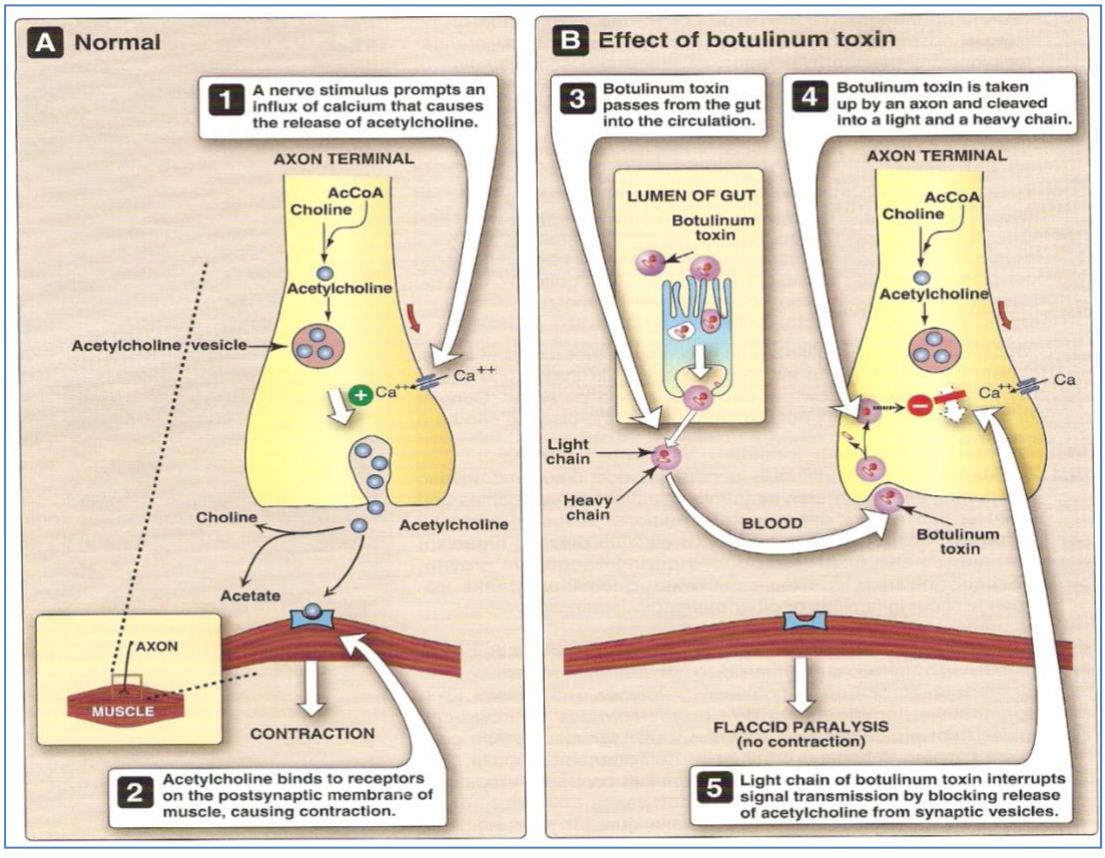
Clostridium Botulinum

Clostridium botulinum causes **botulism** is worldwide in distribution; it is found in soil and occasionally in animal feces.

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. . Types A and B have been associated with a variety of foods and type E predominantly with fish products. toxin is a 150,000-MW protein that is cleaved into 100,000-MW and 50,000-MW protein subunits 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. Proteolysis—by the light chain of botulinum toxin of the target SNARE proteins in the neurons inhibits the release of acetylcholine at the synapse, resulting in lack of muscle contraction and paralysis which called Flaccid paralysis . The SNARE proteins are synaptobrevin, SNAP 25, and syntaxin. The toxins of *C botulinum* types A and E cleave the 25,000-MW SNAP-25. Type B toxin cleaves synaptobrevin. *C botulinum* toxins are among the most toxic substances known . The lethal dose for a human is probably about 1–2 µg. The toxins are destroyed by heating for 20 minutes at 100 °C.



Pathogenesis

Although *C botulinum* types A and B have been implicated in cases of wound infection and botulism, most often the illness is not an infection. Rather, it is 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; under anaerobic conditions, vegetative forms grow and produce toxin.

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. *C botulinum* and botulinum toxin are found in feces but not in serum. It is assumed that *C botulinum* spores are in the babies' food, yielding toxin production in the gut.

Diagnostic Laboratory Tests

Toxin can often be demonstrated in serum from the patient, and toxin may be found in leftover food. Mice injected intraperitoneally die rapidly. The antigenic type of toxin is identified by neutralization with specific antitoxin in mice. *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, *C botulinum* and toxin can be demonstrated in bowel contents but not in serum. Toxin may be demonstrated by passive hemagglutination or radioimmunoassay.

Treatment

Potent antitoxins to three types of botulinum toxins have been prepared in horses. Since 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 ventilation must be maintained by mechanical respirator, if necessary. These measures have reduced the mortality rate from 65% to below 25%.

Epidemiology, Prevention, & Control

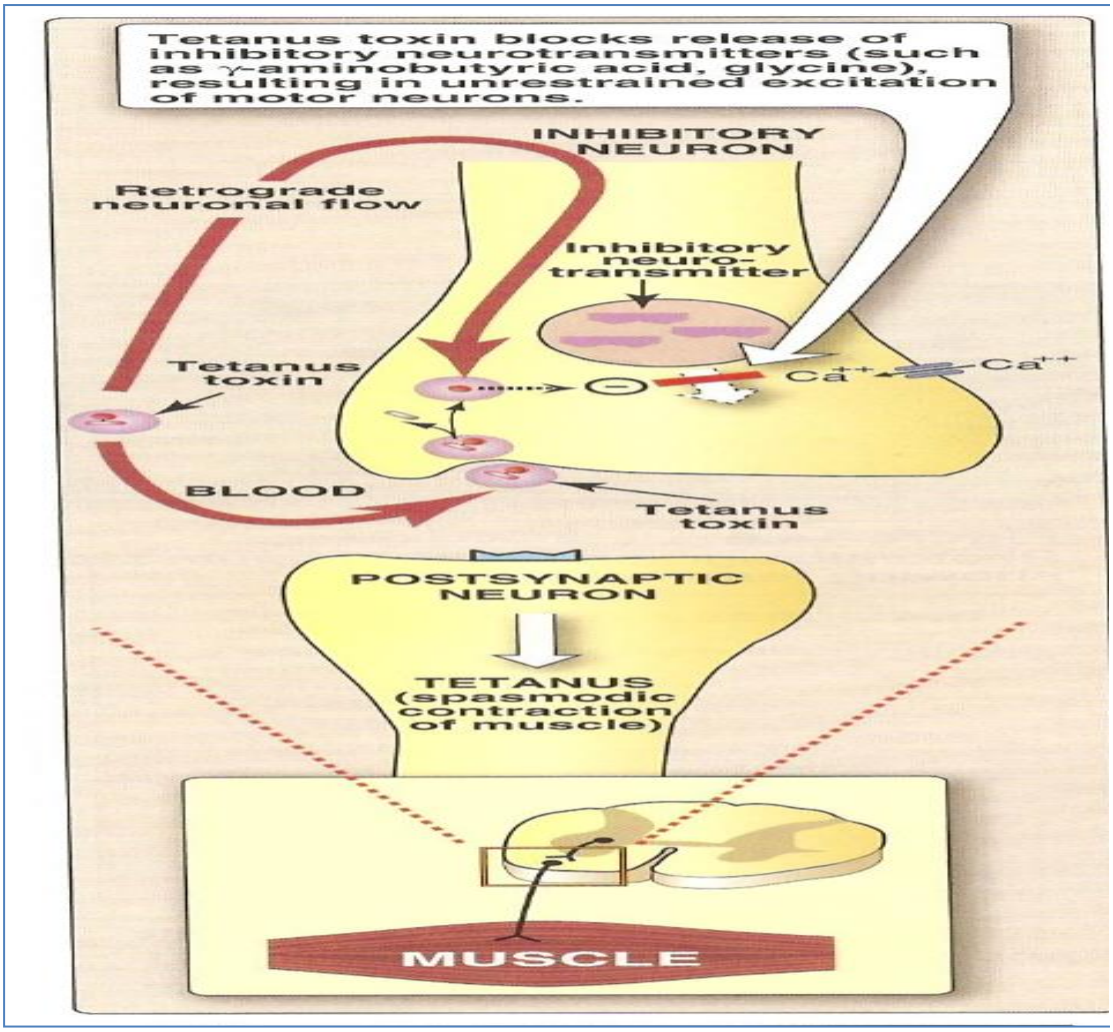
Since spores of *C botulinum* are widely distributed in soil, they often contaminate vegetables, fruits, and other materials. A large restaurant-based outbreak was associated with sautéed onions. When such foods are canned or otherwise preserved, they either must be sufficiently heated to ensure destruction 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. Toxoids are used for active immunization of cattle in South Africa.

Clostridium Tetani

Clostridium tetani, which causes **tetanus,** is worldwide in distribution in the soil and in the feces of horses and other animals. Several types of *C tetani* can be distinguished by specific flagellar antigens. All share a common O (somatic) antigen, which may be masked, and all produce the same antigenic type of neurotoxin, tetanospasmin.

Toxin

The vegetative cells of *C tetani* produce the toxin tetanospasmin , the toxin initially binds to receptors on the presynaptic membranes of motor neurons. It then migrates by the retrograde axonal transport system to the cell bodies of these neurons to the spinal cord and brain stem. The toxin diffuses to terminals of inhibitory cells, including both glycinergic interneurons and aminobutyric acid-secreting neurons from the brain stem. The toxin degrades synaptobrevin, a protein required for docking of neurotransmitter vesicles on the presynaptic membrane. Release of the inhibitory glycine and γ-aminobutyric acid is blocked, and the motor neurons are not inhibited. Hyperreflexia, muscle spasms, and spastic paralysis result. Extremely small amounts of toxin can be lethal for humans.



Pathogenesis

C tetani is not an invasive organism. The infection remains strictly localized in the area of devitalized tissue (wound, burn, injury, umbilical stump, surgical suture) into which the spores have been introduced. The volume of infected tissue is small, and the disease is almost entirely a toxemia. Germination of the spore and development of vegetative organisms that produce toxin are aided by (1) necrotic tissue, (2) calcium salts, and (3) associated pyogenic infections, all of which aid establishment of low oxidation-reduction potential.

The toxin released from vegetative cells reaches the central nervous system and rapidly becomes fixed to receptors in the spinal cord and brain stem and exerts the actions described above.

Clinical Findings

The incubation period may range from 4–5 days to as many weeks. The disease is characterized by tonic contraction of voluntary muscles. Muscular spasms often involve first the area of injury and infection and then the muscles of the jaw (trismus, lockjaw), which contract so that the mouth cannot be opened. Gradually, other voluntary muscles become involved, resulting in tonic spasms. Any external stimulus may precipitate a

tetanic generalized muscle spasm. The patient is fully conscious, and pain may be intense. Death usually results from interference with the mechanics of respiration. The mortality rate in generalized tetanus is very high.

Diagnosis

The diagnosis rests on the clinical picture and a history of injury, although only 50% of patients with tetanus have an injury for which they seek medical attention. The primary differential diagnosis of tetanus is strychnine poisoning. Anaerobic culture of tissues from contaminated wounds may yield *C tetani*, but neither preventive nor therapeutic use of antitoxin should ever be withheld pending such demonstration. Proof of isolation of *C tetani* must rest on production of toxin and its neutralization by specific antitoxin.

Prevention & Treatment

The results of treatment of tetanus are not satisfactory. Therefore, prevention is all-important. Prevention of tetanus depends upon (1) active immunization with toxoids; (2) proper care of wounds contaminated with soil, etc; (3) prophylactic use of antitoxin; and (4) administration of penicillin. The intramuscular administration of 250–500 units of human antitoxin (tetanus immune globulin) gives adequate systemic protection (0.01 unit or more per milliliter of serum) for 2–4 weeks. It neutralizes the toxin that has not been fixed to nervous tissue. Active immunization with tetanus toxoid should accompany antitoxin prophylaxis.

Patients who develop symptoms of tetanus should receive muscle relaxants, sedation, and assisted ventilation. Sometimes they are given very large doses of antitoxin (3000–10,000 units of tetanus immune globulin) intravenously to neutralize toxin that has not yet been bound to nervous tissue. Surgical debridement is vitally important because it removes the necrotic tissue that is essential for proliferation of the organisms. Hyperbaric oxygen has no proved effect.

Penicillin strongly inhibits the growth of *C tetani* and stops further toxin production. Antibiotics may also control associated pyogenic infection. When a previously immunized individual sustains a potentially dangerous wound, an additional dose of toxoid should be injected to restimulate antitoxin production.

Control

Tetanus is a totally preventable disease. Universal active immunization with tetanus toxoid should be mandatory. Three injections comprise the initial course of immunization, followed by another dose about 1 year later. Initial immunization should be carried out in all children during the first year of life. A "booster" injection of toxoid is given upon entry into school. Thereafter, "boosters" can be spaced 10 years apart to maintain serum levels of more than 0.01 unit antitoxin per milliliter. In young children, tetanus toxoid is often combined with diphtheria toxoid and pertussis vaccine.

Clostridia that Produce Invasive Infections

Many different toxin-producing clostridia (*Clostridium perfringens* and related clostridia) can produce invasive infection (including **myonecrosis** and **gas gangrene**) if introduced into damaged tissue. About 30 species of clostridia may produce such an effect, but the most common in invasive disease is *Clostridium perfringens* (90%).

Toxins

The invasive clostridia produce a large variety of toxins and enzymes that result in a spreading infection. Many of these toxins have lethal, necrotizing, and hemolytic properties. The alpha toxin of *C perfringens* type A is a lecithinase, and its lethal action is proportionate to the rate at which it splits lecithin (an important constituent of cell membranes) to phosphorylcholine and diglyceride. The theta toxin has similar hemolytic and necrotizing effects but is not a lecithinase. DNase and hyaluronidase, and collagenase that digests collagen of subcutaneous tissue and muscle, are also produced.

Some strains of *C perfringens* produce a powerful enterotoxin, especially when grown in meat dishes. When more than 10^8 vegetative cells are ingest and sporulate in the gut, enterotoxin is formed. The enterotoxin is a protein (MW 35,000) that may be a nonessential component of the spore coat; it is distinct from other clostridial toxins. It induces intense diarrhea in 6–18 hours. The action of *C perfringens* enterotoxin involves marked hyper-secretion in the jejunum and ileum, with loss of fluids and electrolytes in diarrhea. Much less frequent symptoms include nausea, vomiting, and fever. This illness is similar to that produced by *B cereus* and tends to be self-limited.

Pathogenesis

In invasive clostridial infections, spores reach tissue either by contamination of traumatized areas (soil, feces) or from the intestinal tract. The spores germinate at low oxidation-reduction potential; vegetative cells multiply, ferment carbohydrates present in tissue, and produce gas. The distention of tissue and interference with blood supply, together with the secretion of necrotizing toxin and hyaluronidase, favor the spread of infection. Tissue necrosis extends, providing an opportunity for increased bacterial growth, hemolytic anemia, and, ultimately, severe toxemia and death.

In gas gangrene (clostridial myonecrosis), a mixed infection is the rule. In addition to the toxigenic clostridia, proteolytic clostridia and various cocci and gram-negative organisms are also usually present. *C perfringens* occurs in the genital tract of 5% of women. Clostridial bacteremia is a frequent occurrence in patients with neoplasms. Also, *C perfringens* type C produces a necrotizing enteritis (pigbel) that can be highly fatal in children. Immunization with type C toxoid appears to have preventive value.

Clinical Findings

From a contaminated wound (eg, a compound fracture, postpartum uterus), the infection spreads in 1–3 days to produce crepitation in the subcutaneous tissue and muscle, foul-smelling discharge, rapidly progressing necrosis, fever, hemolysis, toxemia, shock, and death. Treatment is with early surgery (amputation) and antibiotic administration. Until the advent of specific therapy, early amputation was the only treatment.

Diagnostic Laboratory Tests

Specimens consist of material from wounds, pus, tissue. The presence of large gram-positive rods in Gram-stained smears suggests gas gangrene clostridia; spores are not regularly present.

Material is inoculated into chopped meat-glucose medium and thioglycolate medium and onto blood agar plates incubated anaerobically. The growth from one of the media is transferred into milk. A clot torn by gas in 24 hours is suggestive of *C perfringens*. Once pure cultures have been obtained by selecting colonies from anaerobically incubated blood plates, they are identified by biochemical reactions (various sugars in thioglycolate, action on milk), hemolysis, and colony form. Lecithinase activity is evaluated by the precipitate formed around colonies on egg yolk media. Final identification rests on toxin production and neutralization by specific antitoxin. *C perfringens* rarely produces spores when cultured on agar in the laboratory.

Treatment

The most important aspect of treatment is prompt and extensive surgical debridement of the involved area and excision of all devitalized tissue, in which the organisms are prone to grow. Administration of antimicrobial drugs, particularly penicillin, is begun at the same time. Hyperbaric oxygen may be of help in the medical management of clostridial tissue infections. It is said to "detoxify" patients rapidly.

Antitoxins are available against the toxins of *C perfringens, Clostridium novyi, Clostridium histolyticum,* and *Clostridium septicum,* usually in the form of concentrated immune globulins. Polyvalent antitoxin (containing antibodies to several toxins) has been used. Although such antitoxin is sometimes administered to individuals with contaminated wounds containing much devitalized tissue, there is no evidence for its efficacy. Food poisoning due to *C perfringens* enterotoxin usually requires only symptomatic care.

Prevention & Control

Early and adequate cleansing of contaminated wounds and surgical debridement, together with the administration of antimicrobial drugs directed against clostridia (eg, penicillin), are the best available preventive measures. Antitoxins should not be relied on. Although toxoids for active immunization have been prepared, they have not come into practical use.

Clostridium Difficile & Diarrheal Disease

Pseudomembranous Colitis

Pseudomembranous colitis is diagnosed by detection of one or both *C difficile* toxins in stool and by endoscopic observation of pseudomembranes or microabscesses in patients who have diarrhea and have been given antibiotics. Plaques and microabscesses may be localized to one area of the bowel. The diarrhea may be watery or bloody, and the patient frequently has associated abdominal cramps, leukocytosis, and fever. Although many antibiotics have been associated with pseudomembranous colitis, the most common are ampicillin and clindamycin. The disease is treated by discontinuing administration of the offending antibiotic and orally giving either metronidazole or vancomycin.

Administration of antibiotics results in proliferation of drug-resistant *C difficile* that produces two toxins. Toxin A, a potent enterotoxin that also has some cytotoxic activity, binds to the brush border membranes of the gut at receptor sites. Toxin B is a potent cytotoxin. Both toxins are found in the stools of patients with pseudomembranous colitis. Not all strains of *C difficile* produce the toxins, and the *tox* genes apparently are not carried on plasmids or phage.

Antibiotic-Associated Diarrhea

The administration of antibiotics frequently leads to a mild to moderate form of diarrhea, termed antibiotic-associated diarrhea. This disease is generally less severe than the classic form of pseudomembranous colitis. As many as 25% of cases of antibiotic-associated diarrhea may be associated with *C difficile.*