CLOSTRIDIUM SPECIES

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Scientific classification

Domain:	Bacteria
Phylum:	Firmicutes
Class:	Clostridia
Order:	Clostridiales
Family:	Clostridiaceae
Genus:	*Clostridium*

clostridia

The clostridia are large anaerobic, gram-positive, motile rods. Many 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 harmless saprophytes. Among the pathogens are the organisms causing botulism, tetanus, gas gangrene, and pseudomembranous colitis

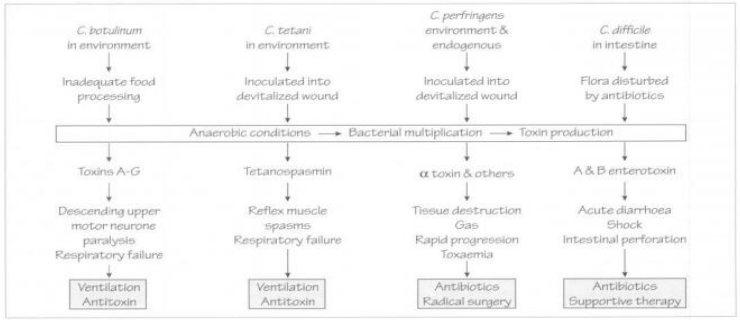
diseases :The remarkable ability of clostridia to cause is attributed to their

(1) ability to survive adverse environmental conditions through spore formation.

(2) rapid growth in a nutritionally enriched, oxygen-deprived environment.

(3) production of numerous histolytic toxins, and neurotoxins.and enterotoxins,

14 *Clostridium* species



Clostridium spp. are obligate, anaerobic, spore-forming, Gram-positive bacilli. More than 80 species are described, but only a few are human pathogens. All require anaerobic conditions to grow but do vary in their oxygen tolerance and their biochemical profile, e.g. their ability to ferment sugars or digest protein. Their normal habitat is in the soil, in aquatic sediments and in the intestinal tract of both humans and animals. They cause disease as a consequence of toxin production.

Tetanus

Clostridium tetani has both a peritrichous flagella and a large round terminal spore giving the organism a drumstick appearance. A strict anaerobe, it is non-saccharolytic and non-proteolytic. Colonies on blood agar are surrounded by a narrow band of haemolysis.

Epidemiology and pathogenesis

Infection occurs in wounds deep enough to produce anaerobic conditions. The organism produces tetanospasmin which prevents release of the inhibitory transmitter γ-aminobutyric acid (GABA) resulting in muscle spasms. Neonatal tetanus, which may occur if the umbilical stump is contaminated after delivery, is the cause of approximately 500 000 deaths each year.

Generalized tetanus is rare in developed countries (0.2 cases per million), usually occurring in older patients in whom immunity has declined. There is often a history of a trivial gardening injury.

Clinical features

Spastic paralysis and muscle spasms may develop at the site of the lesion; if time allows further toxin production, the condition may become generalized. Then perioral muscle spasm leads to the risus sardonicus ('the sardonic smile'), and spasm of the spinal muscles and legs to opisthotonus (when the head and heels are bent back towards each other). Spasms are painful and may be stimulated by light or sudden noise. There may be respiratory difficulties followed by secondary bacterial pneumonia. Diagnosis is based on history and clinical features: isolation of the organism is not diagnostic.

Treatment and prevention

Treatment aims to reduce symptoms by the use of muscle relaxants and to limit further toxin activity by the use of human tetanus immunoglobulin and toxin production by antibiotics, such as penicillin or metronidazole. Artificial ventilation should be instituted if required, and secondary pneumonia treated appropriately.

Infants are protected by passive immunity from their mothers, and develop active immunity when they receive

Morphology & Identification

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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 .

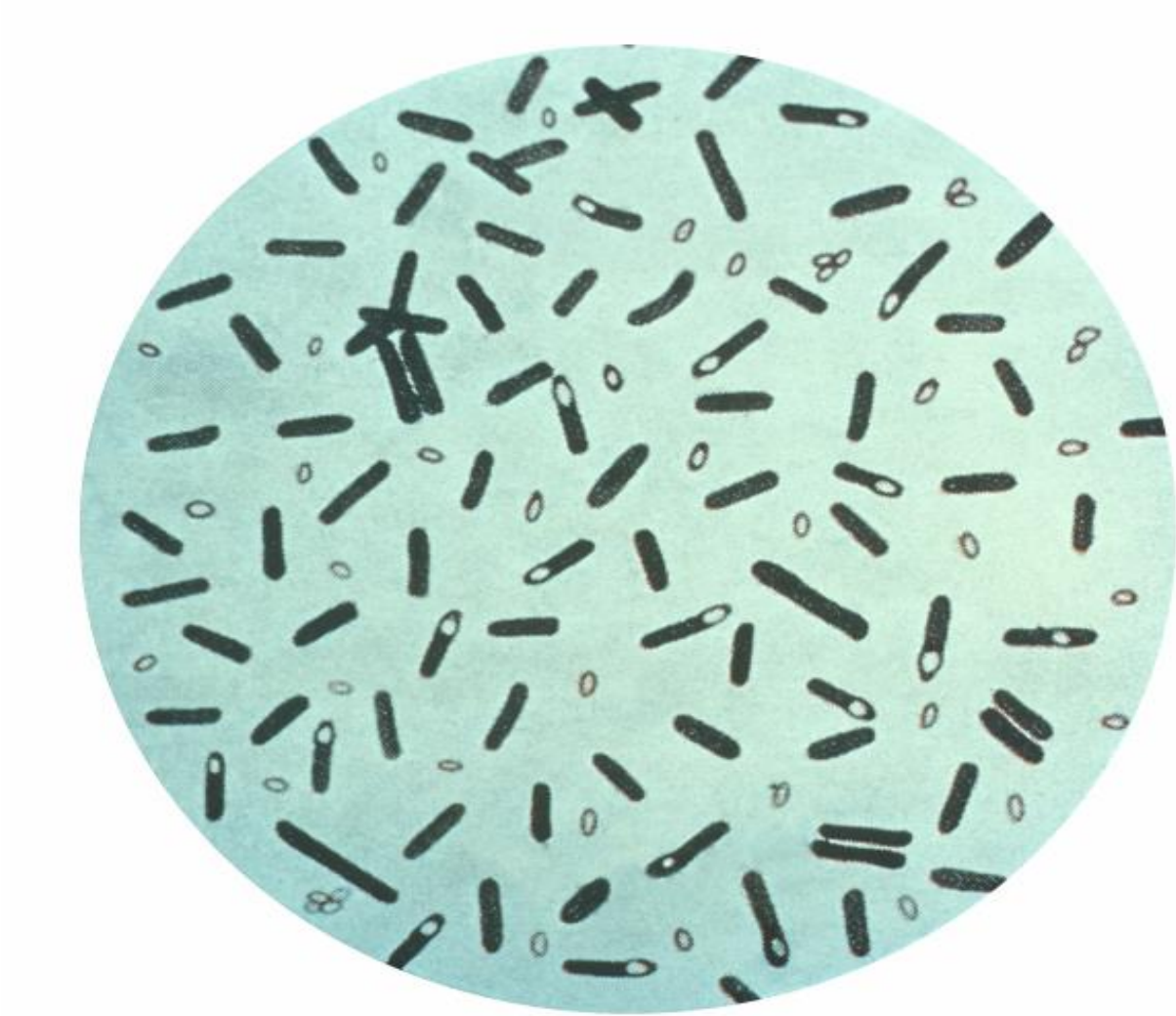
Culture

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

Clostridium botulinum

Typical Organisms

etiologic agents of botulism are a heterogeneous collection of large (0.6 to 1.4 × 3.0 to 20.2 μm), fastidious, sporeforming, anaerobic rods



Clostridium botulinum

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

Types are distinguished by the antigenic type of toxins they produce. Spores of the organism are highly resistant to heat, with standing 100 °C for several hours. Heat resistance is diminished at acid pH or high salt concentration.

Four forms of botulism have been identified: .1

(1) classic or foodborne botulism. .2

(2) infant botulism. .3

(3) wound botulism. .4

(4) inhalation botulism. .5

Clostridium botulinum

Foodborne botulism: initial presentation of blurred vision, dry mouth, constipation, and abdominal pain; progresses to bilateral descending weakness of the peripheral muscles, with flaccid paralysis

Infant botulism: initially nonspecific symptoms (e.g., constipation, weak cry, failure to thrive) that progress to flaccid paralysis and respiratory arrest

Wound botulism: clinical presentation same as with foodborne disease, although the incubation period is longer and fewer gastrointestinal symptoms are reported

Inhalation botulism: rapid onset of symptoms (flaccid paralysis, pulmonary failure) and high mortality from inhalation exposure to botulinum toxin

Toxins

During the growth and autolysis the toxin is liberated into the environment. Seven antigenic varieties of toxin (A–G) are known. Types A, B, and E (and occasionally 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. The toxin is a 150,000-MW protein that is 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. 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 1–2m g. 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 smoked, vacuum-packed, or canned offenders are spiced, alkaline foods that are eaten without cooking. In such foods, spores of C *botulinum* germinate; under anaerobic conditions, vegetative forms grow and produce toxin.

The toxin acts by blocking release of acetylcholine at synapses and neuromuscular junctions. Flaccid paralysis results. The electromyogram and edrophonium strength tests

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

- 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. Honey has been implicated as a possible vehicle for the spores

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

Treatment'Prevention, and Control

Patients with botulism require the following treatment measures:

(1) adequate ventilatory support

(2) elimination of the organism from the GI tract through the judicious use of gastric lavage and metronidazole or penicillin therapy,

(3) use of trivalent botulinum antitoxin versus toxins A, B, and E to inactivate unbound toxin circulating in the bloodstream. Ventilatory support is extremely important in reducing

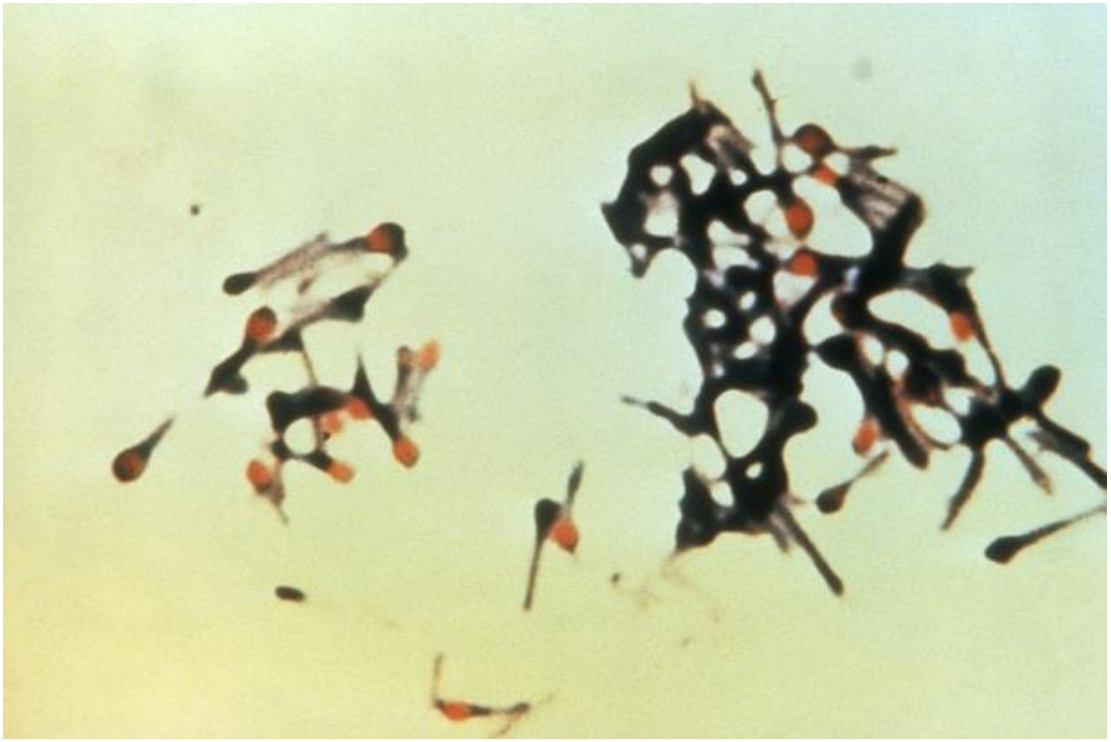
mortality. Protective levels of antibodies do not develop after disease, so patients remain susceptible to botulism. Disease is prevented by destroying the spores in food (virtually impossible for practical reasons), preventing spore germination (by maintaining the food in an acid pH or storage at 4° C or colder), or destroying the preformed toxin (all botulinum toxins are inactivated by heating at 60° C to 100° C for 10 minutes). Infant botulism has been associated with consumption of honey contaminated with spores, so children younger than 1 year should not eat honey

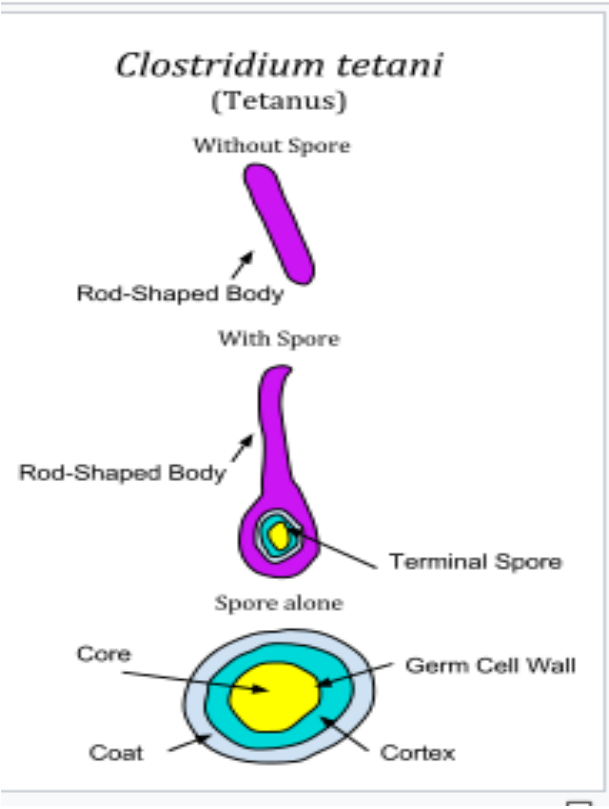
Epidemiology, Prevention, & Control

- Since spores of C *botulinum* are widely distributed in soil, they 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. 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 .

Clostridium tetani

C. tetani is a large (0.5 to 2 × 2 to 18 μm), motile, sporeforming rod. The organism produces round, terminal spores that give it the appearance of a drumstick. Unlike C. perfringens, C. tetani is difficult to grow because the organism is extremely sensitive to oxygen toxicity; when growth is detected on agar media, it typically appears as a film over the surface of the agar rather than discrete colonies. The bacteria are proteolytic but unable to ferment carbohydrates.




Clostridium tetani
(Tetanus)
Without Spore
Rod-Shaped Body
With Spore
Rod-Shaped Body
Terminal Spore
Spore alone
Core
Germ Cell Wall
Coat
Cortex

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.

Clostridium tetani

Generalized tetanus: generalized musculature spasms and involvement of the autonomic nervous system in severe disease (e.g., cardiac arrhythmias, fluctuations in blood pressure, profound sweating, dehydration)

Localized tetanus: musculature spasms restricted to localized area of primary infection

Neonatal tetanus: neonatal infection primarily involving the umbilical stump; very high mortality

Toxins

- The vegetative cells of C *tetani* produce the toxin tetanospasmin (MW 150,000) that is cleaved by a bacterial protease into two peptides (MW 50,000 and 100,000) linked by a disulfide bond. 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

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 with held 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 in an effort to neutralize toxin that has not yet been bound to nervous tissue. However, the efficacy of antitoxin for treatment is doubtful except in neonatal tetanus, where it may be lifesaving.

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

Control

- Tetanus is a totally preventable disease. Universal active immunization with tetanus toxoid should be mandatory. Tetanus toxoid is produced by detoxifying the toxin with formalin and then concentrating it. Aluminum-salt-adsorbed toxoids are employed. 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(*Cl.perfringens*)

C. perfringens is a large (0.6 to 2.4 × 1.3 to 19.0 μm), rectangular, gram-positive rod , with spores rarely observed either in vivo or after in vitro cultivation, an important characteristic that differentiates this species from most other clostridia. Colonies of C. perfringens are also distinctive, with their rapid, spreading growth on laboratory media and β-hemolysis on blood-containing media.

C. perfringens

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%). An enterotoxin of C *perfringens* is a common cause of food poisoning

Clinical Diseases

Clostridium perfringens

Soft-Tissue Infections

Cellulitis: localized edema and erythema with gas formation in the soft tissue; generally nonpainful

Suppurative myositis: accumulation of pus (suppuration) in the muscle planes, without muscle necrosis or systemic symptoms

Myonecrosis: painful, rapid destruction of muscle tissue; systemic spread with high mortality

Gastroenteritis

Food poisoning: rapid onset of abdominal cramps and watery diarrhea with no fever, nausea, or vomiting; short duration and self-limited

Necrotizing enteritis: acute, necrotizing destruction of jejunum, with abdominal pain, vomiting, bloody diarrhea, and peritonitis

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, a collagenase that digests collagen of subcutaneous tissue and muscle, are also produced.

Toxins

- Some strains of C *perfringens* produce a powerful enterotoxin, especially when grown in meat dishes. When more than 10^8 vegetative cells are ingested 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 hypersecretion 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 uterine infections followed instrumental abortions. *Clostridium sordellii* has many of the properties of C *perfringens*. C *sordellii* has been reported to cause a toxic shock syndrome after medical abortion . Endometrial infection with C *sordellii* is implicated. Clostridial bacteremia is a frequent occurrence in patients with neoplasms. In New Guinea, C *perfringens* type C produces a necrotizing enteritis (pigbel) that can be highly fatal in children.

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. At times, the infection results only in anaerobic fasciitis or cellulitis.

C *perfringens* food poisoning usually follows the ingestion of large numbers of clostridia that have grown in warmed meat dishes. The toxin forms when the organisms sporulate in the gut, fever—.with the onset of diarrhea—usually without vomiting or in 6–18 hours. The illness lasts only 1–2 days

Diagnostic Laboratory Tests

Specimens consist of material from wounds, pus, and 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.

Treatment&Prevention & Control

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, Cl. novyi, Cl. 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

Clostridium difficile

C. difficile is a large (0.5 to 1.9 by 3.0 to 17 μm) anaerobic rod that freely forms spores in vivo and in culture. The organism grows rapidly in culture although the vegetative cells die rapidly when exposed to oxygen. C. *difficile* produces a variety of volatile fatty acids that produce a characteristic "barnyard" smell in culture.

Clinical Diseases

Clostridium difficile

Antibiotic-associated diarrhea: acute diarrhea generally developing 5 to 10 days after initiation of antibiotic treatment (particularly clindamycin, penicillins, cephalosporins, fluoroquinolones); may be brief and self-limited or more protracted

Pseudomembranous colitis: most severe form of *C. difficile* disease, with profuse diarrhea, abdominal cramping, and fever; whitish plaques (pseudomembranes) over intact colonic tissue seen on colonoscopy

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.