Clostridium

C. perfringens: gas gangrene; food poisoning
C. tetani: tetanus
C. botulinum: botulism
C. difficile: pseudomembranous colitis

Physiology and Structure

Anaerobic, Large gram-positive rods, the spores are usually wider than the rods, and are located terminally or subterminally.Most clostridia are motile by peritrichous flagella.

Clostridium perfringens.

W. Welch and G. Nut-tall discovered the causative agent in 1892 . This organism occurs as a commensal in the intestine of man and animals. Outside of the host's body it survives for years in the form of spores. It is almost always found in the soil. The organism was isolated from 70-80 per cent of anaerobic infection cases during World War I, and from 91-100 per cent of cases during World War II.

Clostridium perfringens is large gram-positive bacilli,Spores are rarely observed Spore formation begin safter 3 to 3.5 hours of growth, the spores are enclosed by sporangia, Non-motile; capsulated and Hemolytic and metabolically active.

Many strains of *Cl. perfringens* lose their anaerobic properties on exposure to antibiotics, bacteriophage, and X-rays and may be cultivated under aerobic conditions.

The aerobic variants are non-toxic and non-pathogenic for laboratory animals.

*Clostridium perifrengens S*ubdivided into 5 types based on the four major lethal toxins they produce. Type A causes most of the human infections.

Antigenic structure and classification.

Six variants of *Cl. perfringens* are distinguished: A, B, C, D, E, and F. These variants are differentiated by their serological properties and specific toxins.

Toxins		**Bacterial Types**				
		A	**B**	**C**	**D**	**E**
	Lecithinase	+++	+++	+++	+++	+++
	Lethal, necrotizing	–	+++	+++	–	–
	Lethal	–	++	++	–	–
	Lethal, hemolytic	–	+	++	–	–

	Lethal, necrotizing	–	+++	–	+++	–
	Collagenase	+	+	+++	++	++
	Proteinase	–	+	–	++	+++
	Hyaluronidase	++	+	+	++	+
	Deoxyribonuclease	++	+	++	++	++

Four major lethal toxin:(alpha (), beta (), epsilon (), and iota () toxins)

Six minor toxin: (delta(), theta(), kappa(), lambda(), mu(), nu()toxins)

Variant **A** is commonly found as a commensal in the human intestine, but it produces anaerobic infections when it penetrates into the body by the parenteral route.

Variant **B** is responsible for dysentery in lambs and other animals.

Variant **C** causes hemorrhagic entero-toxaemia in sheep, goats, sucking pigs, and calves.

Variant **D** is the cause of infectious enterotoxaemia in man and animals, and

variant **E** causes enterotoxaemia in lambs and calves.

Variant **F** is responsible for human necrotic enteritis.

Pathogenicity and Immunity

Strains of *C. perfringens* are widely distributed in nature, and inhabit the intestine of humans and animals.

They (type A strains are most commonly isolated from human infections) cause a spectrum of diseases primarily by producing toxins and enzymes:

- **α-toxin** : (phospholipase C, lecithinase) is the most important toxin
  - Lyses of RBCs, platelets, leucocytes and endothelial cells
  - Increased vascular permeability with massive hemolysis and bleeding tissue destruction
  - Hepatic toxicity and myocardial dysfunction
- **β-toxin** is responsible for necrotic lesions in necrotizing enterocolitis
- Other necrotizing and hemolytic toxins

  - DNase
  - hyaluronidase

- **Enterotoxin** is heat labile toxin produced in colon → food poisoning produced primarily by type A strains

Clinical Diseases

Soft tissue infections

Portal of entry: trauma or intestinal tract.

Usually caused by mixed infection including toxigenic clostridia, proteolytic clostridia and various cocci and gram-negative organisms.

Three types of infections with increasing

severity: Cellulitis: gas formation in the

soft tissue.

Fasciitis or suppurative myositis: accumulation of gas in the muscle planes.

Myonecrosis or gas gangrene: a life-threatening disease.

Gas gangrene

Spores germinate → vegetative cells multiply, ferment carbohydrates and produce gas in the tissue. This results in distension of tissue and interference with blood supply → the bacteria produce necrotizing toxin and hyaluronidase, which → favor the spread of infection → tissue necrosis extends, resulting in increased bacterial growth, hemolytic anemia, then severe toxemia and death.

Incubation: 1-7 days after infection.

Symptoms: Crepitation in the subcutaneous tissue and muscle, foul smelling discharge, rapidly progressing necrosis, fever, hemolysis, toxemia, shock, renal failure, and death.

Can be also caused by other *Clostridium* species.

Food poisoning

Ingestion of food (sheep's milk cheese, milk, curds, sausages, cod, etc.) contaminated abundantly with *C. perfringens* results in toxinfections and intoxications. These conditions are characterized by a short incubation period (from 2 to 6 hours), vomiting, diarrhoea, headache, chills, heart failure, and cramps in the gastrocnemius muscle; the body temperature may either be normal, or elevated to 38 C.

Most outbreaks follow the ingestion of meat or gravy dishes that are heavily contaminated with vegetative cells of C *perfringens.* Interestingly, C *perfringens* type A strains produce a heat–labile enterotoxin only when the vegetative cells form spores in the small intestine, releasing the newly synthesized enterotoxin. Symptoms of acute abdominal pain and diarrhea begin 8 to 24 hours after ingestion of the contaminated food and usually subside within 24 hours. The toxin

appears to bind to specific receptors on the surface of intestinal epithelial cells in the ileum and jejunum.

The entire molecule then is inserted into the cell, membrane, but does not enter the cell. This induces a change in ion fluxes, affecting cellular metabolism and macromolecular synthesis. As the intracellular Ca^{2+} levels increase, cellular damage and altered membrane permeability occurs, resulting in the loss of cellular fluid and ions.

The enterotoxin causes marked hypersecretion in jejunum and ileum.

Necrotizing enteritis (pig-bel): a fatal disease (acute necrosis in jejunum attributed to β-toxin) in children in New Guinea caused by type C *C. perfringens* have been associated with the eating of pork or other high–protein foods.

Clotridium bacteremia usually occurs in patients with tumors

Resistance

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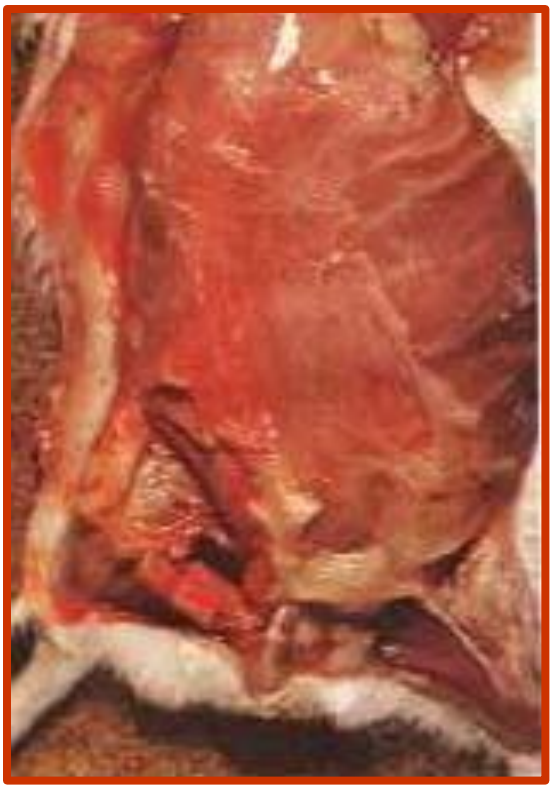
The spores withstand boiling for period of 8 to 90 minutes.

The vegetative forms are most susceptible to hydrogen peroxide, silver ammonia, and phenol in concentrations commonly employed for disinfection

Pathogenicity for animals.

Among laboratory animals, guinea pigs, rabbits, pigeons, and mice are most susceptible to infection. Postmortem examination of infected animals reveals oedema and tissue necrosis with gas accumulation at the site of penetration of the organism. Most frequently clostridia are found in the blood.



Clostridium tetani

- **Causes tetanus or lockjaw, a neuromuscular disease**

Morphology and Physiology-

- long thin gram-positive organism that stains gram negative in old cultures
- **round terminal spore** gives drumstick appearance
- motile by peritrichous flagella
- grow on blood agar or cooked meat medium with swarming

- beta-hemolysis exhibited by isolated colonies
- spores resist boiling for 20 minutes

Antigenic Structure-

flagella (H), somatic (0), and spore antigens. Single antigenic toxin characterizes all strains.

Pathogenesis and Immunity

Tetanospasmin is responsible for clinical manifestations of tetanus.

An A-B toxin, released when the bacteria lyse.

Subunit A is a zinc endopeptidase that acts on CNS: Inhibits release of an inhibitory mediator (e.g., GABA or glycine) which acts on postsynaptic spinal neurons (causing spastic paralysis).

Toxins

- *Cl. tetani* produces two types of toxins:
  - **Tetanolysin**, which causes lysis of RBCs
  - **Tetanospasmin** is neurotoxin and essential pathogenic product
    - Tetanospasmin is toxic to humans and various animals when injected parenterally, but it is not toxic by the oral route

– Tetanospasmin which causes increasing excitability of spinal cord neurons and muscle spasm

Pathogenesis and Immunity

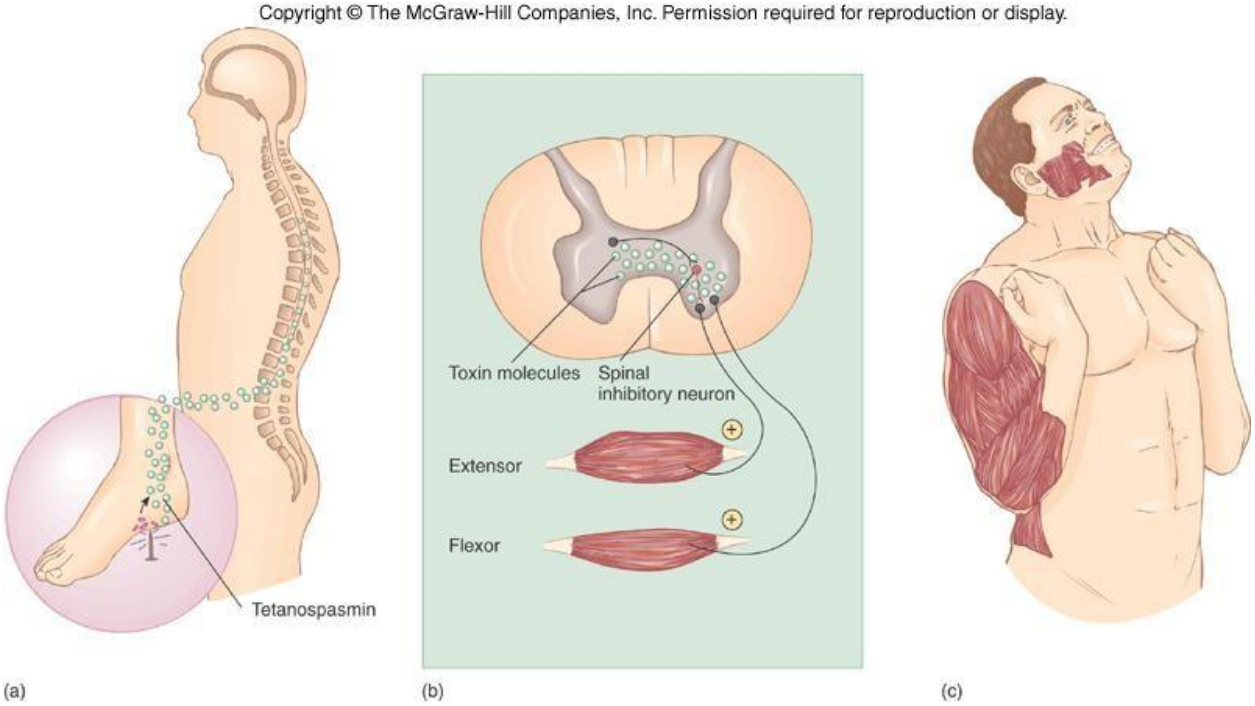
Contamination of devitalized tissue (wound, burn, injury, umbilical stump, surgical suture) with the spores germination of the spores release of tetanospasmin the toxin reaches CNS by retrograde axonal transport or via the bloodstream the toxin is fixed to gangliosides in spinal cord or brainstem and exerts its actions.

Germination of the spore and production of toxin are aided by conditions that lead to low oxidation-reduction potential:

Necrotic tissue;

calcium salts;

associated pyogenic infections.



Clinical Diseases

Generalized tetanus

Incubation period: 4-5 days.

Symptoms: convulsive tonic contraction of voluntary muscles. Spasms involve first the area of injury, then the muscles of the jaw (trismus or lockjaw; risus sardonicus). Other voluntary muscles become involved gradually, resulting in generalized tonic spasms (opisthotonos). Death usually results from interference with respiration. The mortality rate of generalized tetanus: ~50%. In more severe cases, the autonomic nervous systems are also involved.

Localized tetanus (confined to the musculature of primary site of infection)

Cephalic tetanus (site of infection: head)

Neonatal tetanus (infection of the umbilical wound): mortality > 90%, and developmental defects are present in survivors

Disease	Clinical Manifestations
Generalized	Involvement of bulbar and paraspinal muscles (trismus or lockjaw, risus sardonicus, difficulty swallowing, irritability, opisthotonos); involvement of autonomic nervous system (sweating, hyperthermia, cardiac arrhythmias, fluctuations in blood pressure)
Cephalic	Primary infection in head, particularly ear; isolated or combined involvement of cranial nerves, particularly seventh cranial nerve; very poor prognosis
Localized	Involvement of muscles in area of primary injury; infection may precede generalized disease; favorable prognosis
Neonatal	Generalized disease in neonates; infection typically originates from umbilical stump; very poor prognosis in infants whose mothers are nonimmune

Clostridium novyi.

The organism was discovered by F. Novy in 1894. It ranks second among the causative agents of anaerobic infections. Soil examination reveals the presence of the organism in 64per cent of the cases.

Morphology

Clostridium novyi (oedematiens) a Gram-positive, endospore- forming, obligate anaerobic bacteria of the class clostridia. It is ubiquitous, being found in the soil and faeces

Toxin production.

Cl. novyi A produces alpha-, gamma-, delta-, and epsilon-toxins;

Cl. novyi B produces alpha-, beta-, zeta-, and eta-toxins.

Cl. novyi C is marked by low toxigenicity.

In cultures *Cl. novyi* liberates active haemolysin which possesses the properties of lecithinase.

Antigenic structure and classification.

Cl. novyi is differentiated into four variants A, B, C and D.

Variant A is responsible for anaerobic infections in man,

and type B causes infectious hepatitis, known as the black disease of sheep.

Variant C produces bacillary osteomyelitis in buffaloes
variant D is responsible for haemoglobinuria in calves.

Resistance.

Spores survive in nature for a period of 20-25 years with-out losing their virulence. Direct sunlight kills them in 24 hours, boiling destroys them in 10-15 minutes. Spores withstand exposure to a 3 percent formalin solution for 10 minutes.

Pathogenicity for animals. *Cl. novyi* causes necrotic hepatitis (black disease) in sheep. In association with non-pathogenic clostridia it produces bradsot (acute hemorrhagic inflammation of the mucous membranes of the true stomach and duodenum, attended with formation of gases in the alimentary canal and necrotic lesions in the liver) and haemoglobinuria in calves.

A subcutaneous injection of the culture into rabbits, white mice, guinea pigs, and pigeons results in a jelly-like oedema usually without the formation of gas bubbles. Postmortem examination displays slight changes in the muscles; the oedematous tissues are pallid or slightly hyperaemic.

Human diseases

The type and severity of the disease caused depends on penetration of the tissues. The epithelium of the alimentary tract, in general, provides an effective barrier to penetration. However, spores may escape from the gut and lodge in any part of the body and result in spontaneous infection should local anaerobic conditions occur.

Clostridium septicum.

The clostridia are pleomorphous The organisms are motile, peritrichous, and produce no capsules in the animal body. The spores are central or subterminal. The clostridia are Gram-positive but Gram-negative organisms occur in old cultures.

Toxin production.

Cl. septicum produces a lethal exotoxin, necrotic toxin, haemotoxin, hyaluronidase, deoxyribonuclease, and collagenase. The organism haemolyses human, horse, sheep, rabbit, and guinea pig erythrocytes

Antigenic structure and classification. On the basis of the agglutination reaction, serovars of *Cl. septicum* can be distinguished, which produce identical toxins, the differential properties being associated with the structure of the H-antigen *Cl. septicum* possesses antigens common to Cl. *chauvoei* which is responsible for anaerobic infections in animals.

Resistance is similar to that of *Cl novyi.*

Pathogenicity for animals.

Among domestic animals horses, sheep, pigs, and cattle may contract the disease. Infected guinea pigs die in18-48 hours. Postmortem examination reveals crepitant haemorrhagic oedema and congested internal organs. The affected muscles have a moist appearance and are light brown in colour. Long curved filaments which consist of clostridia are found in impression smears of microscopical sections of the liver

Pathogenesis in human

As of 2006, between 1000 and 3000 cases of clostridial myonecrosis were reported in the United States each year, typically accompanied by another pre-existing medical condition. C. septicum is one of several bacteria responsible for myonecrosis, otherwise known as gas gangrene. Infection by *C. septicum* was once thought to be extremely rare, however anaerobic laboratory techniques allowed for the discovery of the true potential of this infectious microbe. Infections are typically seen in settings of immunodeficiency, trauma, surgery, malignancy, skin infections/burns, and septic abortions. Sites prone to infection are those with poor vascular supply, although because of pH, electrolyte and omsotic differences, the colon may promote the growth of *C. septicum* better than most other anatomical regions. One of the more aggressive progenitors of gas gangrene, *C. septicum* infection progresses very

rapidly, with a mortality rate of approximately 79% in adults, typically occurring within 48 hours of infection. The greatest survival rates are typically seen in patients without pre-existing medical conditions, and with infection localized to the extremities. Gas gangrene proceeds via disruption of blood flow to the infected site, resulting in diminished levels of oxygen and nutrients ultimately causing premature cell death and tissue necrosis. Four toxins have been isolated from *C. septicum*: the lethal alpha toxin, DNase beta-toxin, hyaluronidase gamma toxin, and the thiol-activated/septicolysin delta toxin. Alpha toxin causes intravascular hemolysis and tissue necrosis and is well known as the main virulent factor in *C. septicum*. Symptoms of infection include pain, described as a heaviness or pressure that is disproportionate to physical findings, tachycardia, and hypotension. Tissue necrosis then causes edema and ischemia resulting in metabolic acidosis, fever, and renal failure.[3] The carbon dioxide and hydrogen produced during cellular respiration move through tissue planes, causing their separation, producing features characteristic of palpable emphysema. This also results in a magenta-bronze skin discoloration and bulla filled with a foul-smelling serosanguinous fluid.

***C. botulinum* — agent of botulism,** a rare, but severe (lethal) neuroparalytic disease

Morphology and Physiology

- heterogeneous group of fastidious, strictly anaerobic bacilli
- **motile by peritrichous flagella**
- heat-resistant **spores (ovoid, subterminal)**
- proteolytic and non-proteolytic

Antigenic Structure

- species divided into **four groups (I-IV)** based on type of toxin produced and proteolytic activity
- **seven antigenically distinct botulinum toxins (types A to G)**
- somatic antigens - heat stable and heat labile; spore antigens - more specific

Pathogenicity Determinants

- **lethal foodbome intoxication with toxin types A,B,E,or F**; shorter incubation period, poor prognosis
- phage-mediated, systemic-acting A-B neurotoxin (botulinum toxin = botulin) released at cell lysis

Mode of Action - one of most extremely potent neurotoxins known
(1 ng of purified toxin contains about 200,000 minimal lethal doses (MLDs) for a 20g mouse)

- **A-B toxin ingested, binds specific receptors on peripheral cholinergic nerve endings**

  (neuromuscular junctions) where it blocks release of presynaptic acetylcholine (excitatory neurotransmitter) blocking muscle stimulation & resulting in flaccid paralysis

- **Early**: nausea, vomiting, weakness, lassitude (lack of energy), dizziness, constipation
- **Later**: double vision, difficulty in swallowing and

speaking

- Final: death due to respiratory paralysis

Botulinum Food Poisoning

- Botulism – intoxication associated with inadequate food preservation
- *Clostridium botulinum* – spore-forming anaerobe; commonly inhabits soil and water

Pathogenesis

- Spores are present on food when gathered and processed.
- If reliable temperature and pressure are not achieved air will be evacuated but spores will remain.
- Anaerobic conditions favor spore germination and vegetative growth.
- Potent toxin, botulin, is released.
- Toxin is carried to neuromuscular junctions and blocks the release of acetylcholine, necessary for muscle contraction to occur.
- Double or blurred vision, difficulty swallowing, neuromuscular symptoms

Infant and Wound Botulism

- Infant botulism – caused by ingested spores that germinate and release toxin; flaccid paralysis

- Wound botulism – spores enter wound and cause food poisoning symptoms

***Clostridium difficile* infection (CDI)**

is a symptomatic infection due to the spore-forming bacterium, *Clostridium difficile*. Symptoms include watery diarrhea, fever, nausea, and abdominal pain. It makes up about 20% of cases of antibiotic-associated diarrhea. Complications may include pseudomembranous colitis, toxic megacolon, perforation of the colon, and sepsis.

clostridium difficile infection is spread by bacterial spores found within feces. Surfaces may become contaminated with the spores with further spread occurring via the hands of healthcare workers. Risk factors for infection include antibiotic or proton pump inhibitors use, hospitalization, other health problems, and older age. Diagnosis is by stool culture or testing for the bacteria's DNA or toxins. If a person tests positive but has no symptoms it is known as *C. difficile* colonization rather than an infection.