Khadija. K. Al-Dulaimy **Microbiology /College Of Dentistry**
B.Sc., M.Sc., Med. Microbiol. **Al- Anbar University**

ANAEROBIC -SPORE FORMING-GRAM POSITIVE BACILLI

CLOSTRIDIA:

Clostridia comprise many species of Gram-positive, anaerobic spore-forming bacilli (but spores are not found in infected tissues); a few are aerotolerant. They are an important group of pathogens widely distributed in soil and in the gut of humans and animals. Clinically significant species of Clostridium include *C. perfringens*, which causes histotoxic (tissue destructive) infections (myonecrosis) and food poisoning; *C. difficile*, which causes pseudomembranous colitis associated with antibiotic use; *C. tetani*, which causes tetanus (lockjaw); and *C. botulinum*, which causes botulism (seen in table 13.1).

Clostridium spp.

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 *Clostridium tetani* (this shape is useful in laboratory identification of the organisms). In the various species, the spore is placed centrally, subterminally, or terminally). Some species are motile with peritrichous flagella (*e.g. C. tetani*), while others (*e.g. C.perfringens*) have a capsule.

Culture and identification

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

Physiology: Clostridia cannot use free oxygen as the terminal electron acceptor in energy production as do aerobic organisms. Instead, they use a variety of small organic molecules such as pyruvate as the final electron acceptors in the generation of energy. In the vegetative state, clostridia are also variably inhibited or damaged by O2 [Note: The reasons for this damage are not entirely

clear. One explanation is that these organisms produce oxygen radicals and peroxides and that some of them cannot detoxify these reactive molecules because they lack enzymes such as peroxidases, catalase, or superoxide dismutase.] Clostridia grow on enriched media in the presence of a reducing agent such as cysteine or thioglycollate (to maintain a low oxidation-reduction potential) or in an O2-free, gaseous atmosphere provided by an air-evacuated glove box, sealed jar, or other device. They lack cytochrome and cytochrome Oxidase and are unable to break down hydrogen peroxide because they lack Calalase and peroxidase. Therefore, H2O2 tend to accumulate to toxic concentrations in the presence of oxygen. Clostridia and other obligate anaerobes probably also lack superoxide dismutase and consequently permit the accumulation of the toxic free radical superoxide anion.

Epidemiology: Clostridia, part of the intestinal flora in humans and other mammals, and are found in soil, sewage, and aquatic settings, particularly those with high organic content. A number of clostridial species produce destructive and invasive infections when introduced into tissues; for example, by a break in the skin resulting from surgery or trauma. Their presence in infectious processes is opportunistic and often derives from the patient's normal flora. Endospore formation facilitates their persistent survival in the environment.
Spores are resistant to chemical disinfectants, and may withstand ultraviolet irradiation or boiling temperatures for some time, although not standard autoclaving conditions (121°C for fifteen minutes at increased pressure).

Clostridium perfringens

*C. **perfringens*** is a large, rod-shaped, nonmotile, gram-positive, encapsulated bacillus. It is ubiquitous in nature, with its vegetative form as part of the normal flora of the vagina and gastrointestinal (GI) tract. Its spores are found in soil. [Note: Spores are rarely seen in the body or following in vitro cultivation.] When introduced into tissue, however, *C. perfringens* can cause anaerobic cellulitis, and myonecrosis (gas gangrene). Some strains of *C. perfringens* also cause a common form of food poisoning.

- **Pathogenesis:** *C. perfringens* secretes a variety of exotoxins, enterotoxins, and hydrolytic enzymes that facilitate the disease process.
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- **Exotoxins*:*** *C. perfringens* elaborates at least twelve exotoxins, designated by Greek letters. The most important of these, and the one that seems to be required for virulence in tissue, is alpha toxin. Alpha toxin is a lecithinase (phospholipase C) that degrades lecithin in mammalian cell membranes, causing lysis of endothelial cells, as well as erythrocytes, leukocytes, and platelets. Other *C. perfringens* exotoxins have hemolytic or other cytotoxic

and necrotic effects, either locally or when dispersed in the bloodstream. *C. perfringens* strains are grouped, A through E on the basis of their spectrum of exotoxins. Type A strains, which produce both alpha toxin and enterotoxin, are responsible for most human clostridial infections.

- **Enterotoxin**: *C. perfringens* enterotoxin, a small, heat-labile protein, acts in the lower portion of the small intestine. The molecule binds to receptors on the epithelial cell surface and alters the cell membrane, disrupting ion transport (primarily in the ileum) and leading to loss of fluid and intracellular proteins. Interestingly, enterotoxin-producing strains are unusually heat resistant, the spores remaining viable for longer than an hour at 100°C, enhancing their threat as food-borne pathogens.
- **Degradative enzymes***: C. perfringens* is a metabolically vigorous organism that produces a variety of hydrolytic enzymes, including proteases, DNases, hyaluronidase, and collagenases, which liquefy tissue and promote the spread of infection.

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 either to exogenous or endogenous infection:

- **gas gangrene (myonecrosis)** resulting from infection of dirty ischaemic wounds (e.g. war injuries)
- **food poisoning** due to ingestion of food contaminated with enterotoxin producing strains.

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.

Culture and indentification

Grow 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.

Pathogenicity

Cause, gas gangrene and food poisoning.

Gas gangrene (myonecrosis). Wounds associated with traumatized tissue (especially muscle) may become infected with C. *perfringens* 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. Characterstic signs and symptoms include pain, oedema and creptation produced by gas in tissues.

Food poisoning. Some strains of *C. perfringens* produce an enterotoxin which induces food poisoning. This is due to the ingestion the 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-a-toxin administration.

Food poisoning. Symptomatic therapy only; no specific treatment.

The majority of infections resulting in necrosis of muscle are due to Clostridium species (gas gangrene) and group A streptococcus

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Increasing pain at the site of prior injury or surgery, together with signs of systemic toxicity and gas in the soft tissue, support the diagnosis of gas gangrene.

Clostridial infections usually involve a mixture of species; therefore, the use of broad-spectrum antibiotics is appropriate

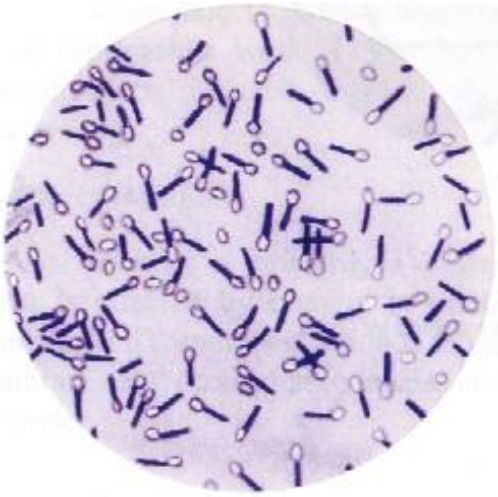
Clostridium tetani

Habitat and transmission

Clostridium 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 tow_ 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 toxin-mediated disease. The powerful, heat-labile neurotoxin (**tetanospasmin)** is produced at the wound site and released during cell lysis (Fig.13.4). 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. *Clostridium tetani* also produces an oxygen-labile haemolysin (tetanolysin); the clinical significance of this enzyme is not clear.

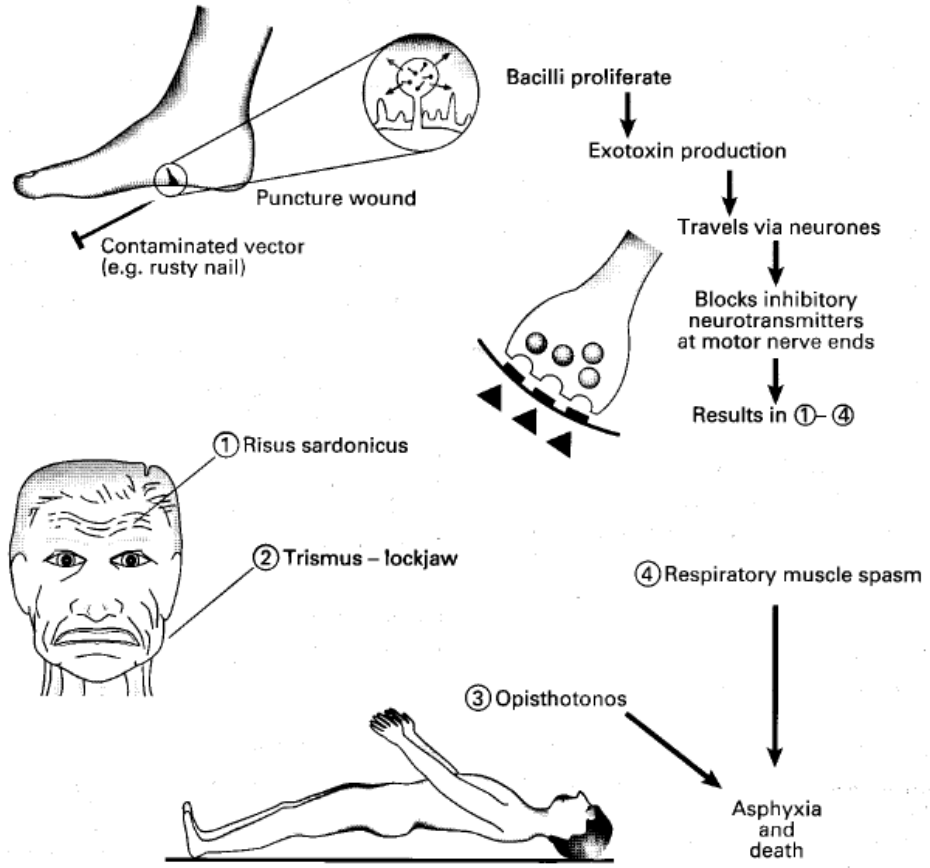


Fig. 13.4 Pathogenesis of tetanus and its sequelae.

In the oral cavity, the tetanus disease may be involved in the jaws, which become firmly fixed. In these cases the Patient cannot talk and has difficulty in opening his mouth, hence the term 'lockjaw' . The tight masseter muscles causing the trismus can be felt on palpating the cheek .Dysphagia, or difficulty in swallowing, a clinical aspect that is caused by spasm of the muscles of deglutition. Also, Opisthotons, in which the body is stiffened, and a slight rise in temperature, difficulties of breathing leading to death from asphyxia.

Treatment and prevention

1-Active immunization with toxoid.
2-Proper care of wounds contaminated with soil, etc.
3-Prophylactic use of antitoxin.
4-Administration of penicillin.

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*

Botulism

C. botulinum causes botulism, which occurs in several clinical forms. Botulism is caused by the action of a neurotoxin that is one of the most potent poisons known. It causes a flaccid paralysis. Contact with the organism itself is not required; hence, the disease can be a pure intoxication.Botulism is a life-threatening neuroparalytic disease caused by antigenically distinct, heat-labile, protein toxins of *C.botulinum*. Although seven toxin types (A,B,C,D,E,F,G) are produced by different strains of *C.botulinum*, most cases of botulism in humans are caused by types A,B,E, and F. Of these, type F is the least commen.

- **Epidemiology:** C. botulinum is found worldwide in soil and aquatic sediments, and the spores frequently contaminate vegetables and meat or fish. Under appropriate conditions, including a strictly anaerobic environment at neutral or alkaline pH, the organism germinates and toxin is produced during vegetative growth. Because the toxin is often elaborated in food, outbreaks frequently occur in families or other eating groups.
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- **Pathogenesis:** There are several types of botulinum toxin, designated A through G, but human disease is almost always caused by types A, B, or E. The botulinum serotypes and tetanus toxin constitute a homologous set of proteins whose neurotoxicity arises from proteolytic cleavage of specific synaptic vesicle peptides, causing subsequent failure of neurotransmission. In contrast to tetanus toxin, which causes constant contraction (spasms), botulinum toxins affect peripheral cholinergic synapses by blocking the neuromuscular junction and inhibiting release of the neurotransmitter, acetylcholine, preventing contraction and causing flaccid paralysis .

Clinical significance:

Classic botulism is a food poisoning in which a patient first begins to experience difficulties in focusing vision, swallowing, and other cranial nerve functions, 12 to 36 hours after ingesting toxin-containing food but not necessarily viable organisms. There is no fever or sign of sepsis. A progressive paralysis of striated muscle groups develops, and mortality is about fifteen

percent; the patient usually succumbing to respiratory paralysis. Recovery is protracted, lasting several weeks.

***A note on* Clostridium botulinum**

The agent of botulism, a form of food-poisoning, has powerful toxins that can be used in bioterrorism and warfare. 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

Table 13.1 Common *Clostridium* species associated with human disease

Clostridium spp.	Disease
C. perfringens	Gas gangrene, food-poisoning, bacteraemia, soft-tissue infections
C. tetani	Tetanus
C. botulinum	Botulism (foodborne, infant, wound)
C. difficile	Pseudomembranous colitis, antibiotic-associated diarrhoea
Other species (e.g. *C. septicum, C. ramosum, C. novyi, C. bifermentans*)	Bacteraemia, gas gangrene, soft-tissue infections

AEROBIC -SPORE FORMING-GRAM POSITIVE BACILLI

BACILLUS SPEACIS

The genus Bacillus comprises the Gram (+) rods that grow aerobically on nutrient agar and form resistant endospores, the vegetative-bacilli are large and straight, most are motile with lateral flagella. They are common environmental organisms and are frequently isolated in laboratories as contaminants of media or specimens. Most member of this genus are saprophytic organism prevalent in soil, water and air such as *B. cereus* and *B. subtilis, B. anthracis* is the most

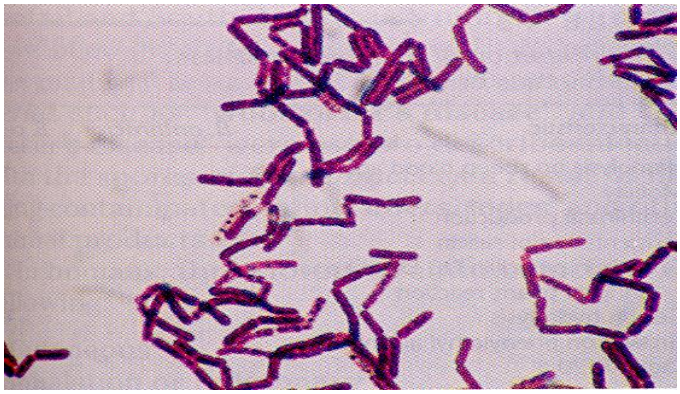
important pathogen of the group. Anthracis is an example of a zoonotic disease and the *B. anthracis* the causative organism play an important role in the history of medical microbiology because of the work of Robert Koch who first showed that a causative organism could be isolated from the blood of infected animals, artificially grown in pure culture and then used to reproduce the disease in animals.

BACILLUS ANTHRACIS

Anthrax, the classical disease caused by *B. anthracis*, is primarily a disease of herbivorous animals; humans become infected by having contact with infected animals and animal products. The causative agent, *B. anthracis*, is maintained in the soil as resistant endospores and, in this form, may remain infective for years.

DESCRIPTION:

B. anthracis is a non- motile straight, the spore is oval, refractile, central in position, the organism is G (+) aerobe and facultative anaerobe with a temp, range for growth of 12-45^{0}C (optimum, 35^{0}C), it grows on all ordinary media as typical colonies with a wavy margin and small projection, they so- called medusa head appearance.



SPORS:

Spores are never found in the tissues but appear when the organism is grown on artificial media, they stain only with special spore- staining procedures, the spores are resistant to lethal effects of heat, drying chemical disinfectants and UV radiation. However, autoclaving at 121^{0}C (15 1b/in or 1 bar) destroys them in 15 min.

Epidemiology:

Anthrax is an enzootic disease of worldwide occurrence. [Note: An enzootic disease is endemic to a population of animals (that is, its occurrence changes little over time). This is as compared to an epizootic disease, which attacks a large number of animals at the same time (similar to a human epidemic).] Anthrax affects principally domestic herbivores"sheep, goats, and horses"and is transmitted to humans by contact with infected animal products or contaminated dust. Infection is usually initiated by the subcutaneous inoculation of spores through incidental skin abrasions. Less frequently, the inhalation of spore-laden dust causes a pulmonary form of anthrax. [Note: Sometimes an occupational hazard, this form of pneumonia is known as woolsorter's disease.] *B. anthracis* spores may remain viable for many years in contaminated pastures, or in bones, wool, hair, hides, or other animal materials. These spores, like those of clostridia, are highly resistant to physical and chemical agents. In the United States, a veterinary vaccine in widespread use makes domestic animal sources of the disease quite rare. Contaminated agricultural imports may account for the few cases seen, and lead occasionally to the quarantine of goods from endemic areas.

virulence factors of *B. anthracis*

B. anthracis produces toxins that are composed of three distinct proteins: protective antigen (PA), edema factor (EF), and lethal factor (LF). Virulent strains also are encapsulated.

PATHOGENESIS:

Anthracis is a disease of sheep, cattle, horses and many other animals. The infection is usually acquired by the entry of spores through injured skin or mucous membrane, rarely by inhalation of spores into the lung. In animals the portal of entry is the mouth and the gastrointestinal tract. The spores germinate in the tissue at the site of entry and growth of the vegetative organisms results information of a gelatinous edema and congestion. Bacilli spread via lymphatics to the blood stream and they multiply freely in the blood and tissues shortly before and after the death of the animal.

Clinical infection:

in human anthrax present two forms :

- External or Cutaneous (malignant pustule and anthrax edema).
- Internal(pulmonary anthrax or wool- sorters disease, Gastrointestinal anthrax, and Oropharyngeal anthrax).

Malignant pustule: Apapule first develops within 12- 36 hours after entry of the organisms or spores through a scratch. This papule rapidly changes into a vesicale, then a pustule and finally a necrotic ulcer from which the infection may disseminate giving rise to septicemia. The absence of pain in cutanous lesions serves to distinguish anthrax from such painful lesions as Staphylocoecal.

pulmonary anthrax: The spore of anthrax in dust or wool fibers may result in respiratory anthrax (wool- sorters disease). This condition carries a high mortality due to the intense inflammation, hemorrhage and septicemia which result from the multiplication of organisms in bronchi and spread to the lungs, lymphatics and blood stream.

Oropharyngeal anthrax and **gastrointestinal anthrax**

Are acquired by ingestion of inadequately cooked foods containing either spores or vegetative bacilli. Both of these forms of infection may became systemic and, like the pulmonary form, have a high mortality rate if untreated. The incubation period of gastrointestinal anthrax is 2 to 7 days. Symptoms begin with fever and nausea with bloody vomiting. Subsequently, abdominal pain and bloody diarrhea appear. Oropharyngeal involvement is suggested by cervical and oral pain and edema. Eschars may be noted on the posterior pharynx, tonsillar areas, or the hard palate.Progression of the infection results in dysphagia, sever sore throat, and cervical lymphadenopathy . Death results from overwhelming sepsis.

Oral aspect: There are occasional oral aspects to anthrax. An infection of the palate has been reported and this was caused by a toothbrush with imported animal bristles.

Bacillus species have been isolated from periodontal pocket, in per apical involvement of teeth and from the root canal of infected but pulp less teeth. The bacterimia due to bacillus species may occur on dental extraction.

Treatment:

B. anthracis is sensitive to a variety of antibiotics. Cutaneous anthrax responds to doxcycline, ciprofloxacin, or erythromycin. Penicillin is not recommended because of inducible -lactamase in B. anthracis. Multidrug therapy (for example, ciprofloxacin plus rifampin plus vancomycin) is recommended for inhalation anthrax.

Prevention and Control.

Control measures include:
1-Disposal of animal carcasses by burning or by deep burial in lime pites.
2-Decontamination (usually by autoclaving) of animal products.
3-protective clothing and gloves for handling potentially infected materials.
4-Active immunization of domestic animals with live attenuated vaccines.

B. anthracis is a potential bioterrorism agent because it can be easily grown in large quantities, is resistant to destruction, and can be formulated into an aerosol for wide dissemination. Physicians must be prepared to recognized anthrax even though it is rarely seen in the United States.

Aggressive therapy is indicated for inhalation anthrax both because of the severity of the disease and the fact that the disease is often not diagnosed until late in the course of the illness.

Bacillus cereus

Like other Bacillus species. ***Bacillus cereus*** is commonly found in soil and may also be isolated from straw and rice. *B. cereus* produces several potential virulence factors(hemolysin II, and hemolysin III) in addition to the toxin associated with gastrointestinal infections, and these factors are thought to play a role in nongastrointestinal infections.

Opportunistic *Bacillus* Species infections

Serious Bacillus species infections have been associated with operative procedures, immunosuppression, traumatic wounds, burns, hemodialysis, and parenteral drug abuse. Isolates recoverd from nongastrointestinal infections produce a variety of putative virulence factors, including hemolysins, necrotizing exotoxins, and phospholipases. Analyses of large series of cases involving significant Bacillus infections indicate their involvement in the clinical syndromes as below.
1-Bacteremia and endocarditis.
2- Ocular infections.
3- Musculoskeletal infections.
4- Nosocomial infections.

Reference:

1- Lippincott's Illustrated Reviews: Microbiology, 2nd Edition