Clostridium Bacteria

Introduction:

The genus *Clostridium* includes all anaerobic, gram-positive bacilli and capable of forming endospores. Spores of clostridia are usually wider than the diameter of the rods in which they are formed, giving the bacillus a swollen appearance resembling a spindle. The name Clostridium is derived from the word „Kloster" (meaning a spindle).

Clinically significant species of *Clostridium* include

C. perfringens, which causes (gas gangrene) and food poisoning

C. difficile, which causes pseudomembranous colitis associated with antibiotic use

C. tetani, which causes tetanus

C. botulinum which causes botulism.

General features of Clostridium:

1.Morphology

The clostridia are 1) gram-positive typically large, 2) straight or slightly curved rods, 3-8 × 0.6-1 μm with slightly rounded ends. Pleomorphism forms are common. 3) Most species of clostridia are motile with peritrichous flagella except *Cl. perfringens* and *Cl. tetani* type VI which are non-motile. All clostridia are non-capsulated with the exception of *Cl. perfringens*.

2.Culture

Most species are obligate anaerobes. A few species grow in the presence of trace amounts of air and some actually grow slowly under normal atmospheric conditions. Clostridia grow on enriched media in the presence of reducing agent such as cysteine or thioglycollate (to maintain a low oxidation-reduction potential)

Liquid media like cooked meat broth (CMB) or thioglycollate media (containing reducing agent thioglycollate and 0.1% agar) are very useful for growing clostridia. A very useful medium is Robertson‟s cooked (RCM) meat broth. It contains unsaturated fatty acids which take up oxygen.

a-*Clostridium perfringens*: Gas gangrene

Morphology: It is a relatively large gram-positive bacillus (about 4-6 × 1 μm) with straight, parallel sides and rounded or truncated ends, occurring singly or in chains or small bundles. It is pleomorphic, capsulated and non-motile.Spores are typically oval, central or sub-terminal.

Cultural Characteristics

Clostridium perfringens is an anaerobe but can also grow under microaero¬philic conditions. It grows over a pH range of 5.5 to 8.0 and 50°C (optimum temperature range 37-45°C).

Robertson‟s cooked meat broth inoculated with mixtures of *Cl.perfringens* and other bacteria and incubated at 45°C for 4 to 6 hours serves as enrichment. Blood agar plates streaked and incubated at 37°C will have proportionally higher numbers of *Cl. perfringens*

It grows best on carbohydrate-containing media such as glucose blood agar. Colonies of most strains demonstrate a „target hemolysis, double zone of hemolysis‟ after overnight incubation on rabbit, sheep, ox, or human blood agar. It results from a narrow zone of complete hemolysis due to theta toxin and a much wider darker zone of incomplete hemolysis due to the α-toxin.

Pathogenesis:

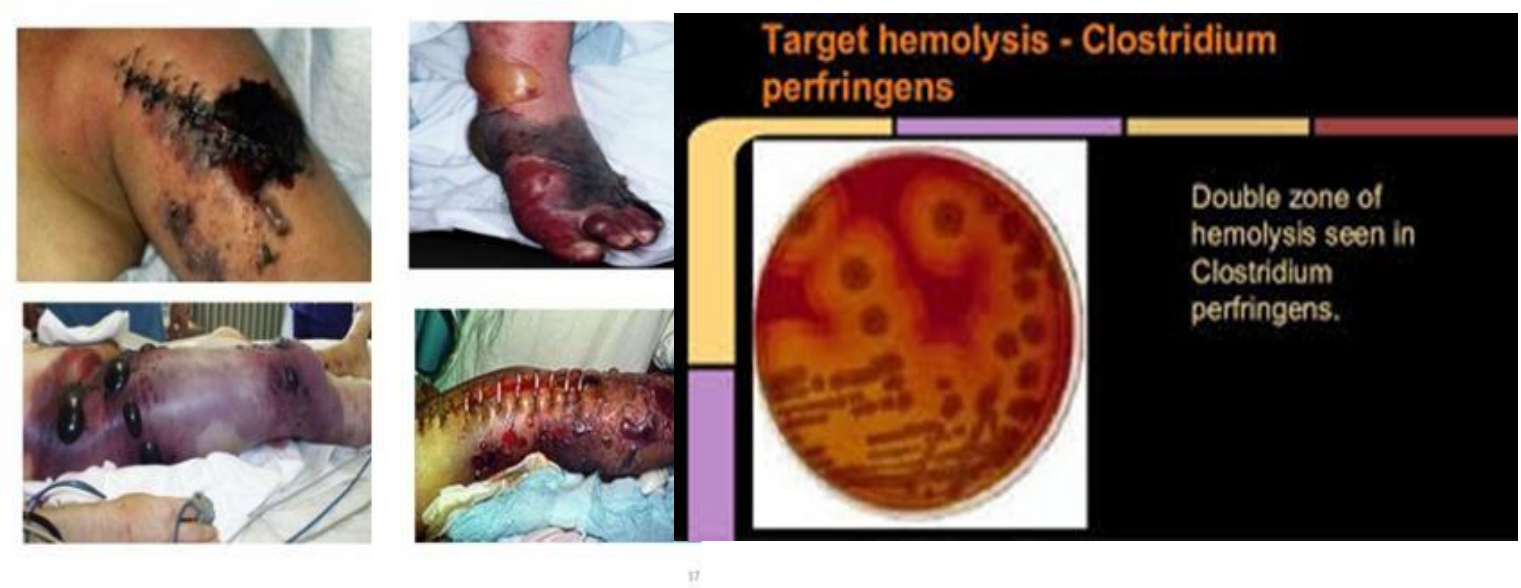
C. perfringens secretes a variety of exotoxins, enterotoxins, and hydrolytic enzymes that facilitate the disease process.

1.**Exotoxins**: *C. perfringens* secretes at least 12 exotoxins. The most important of these required for virulence in tissue, is alpha toxin. **Alpha toxin is a lecithinase** that degrades lecithin in mammalian cell membranes, causing lysis of endothelial cells, as well as RBC, WBC and platelets.

2.**Enterotoxin**: a small protein, acts in the lower portion of the small intestine, and causes loss of fluid and intracellular proteins.

3.Degradative enzymes: it is produces of hydrolytic enzymes, including proteases, DNases, hyaluronidase, and collagenases, which liquefy tissue and promote the spread of infection.

Myonecrosis (gas gangrene): Clostridial spores are introduced into tissue, for example, by contamination with infected soil, or by endogenous transfer from the intestinal tract. Severe and open wounds are a prime predisposing condition. Alpha toxin and other exotoxins are secreted and extensive cell killing follow (ensues). Production of enzymes that break down ground substance facilitates the spread of infection.



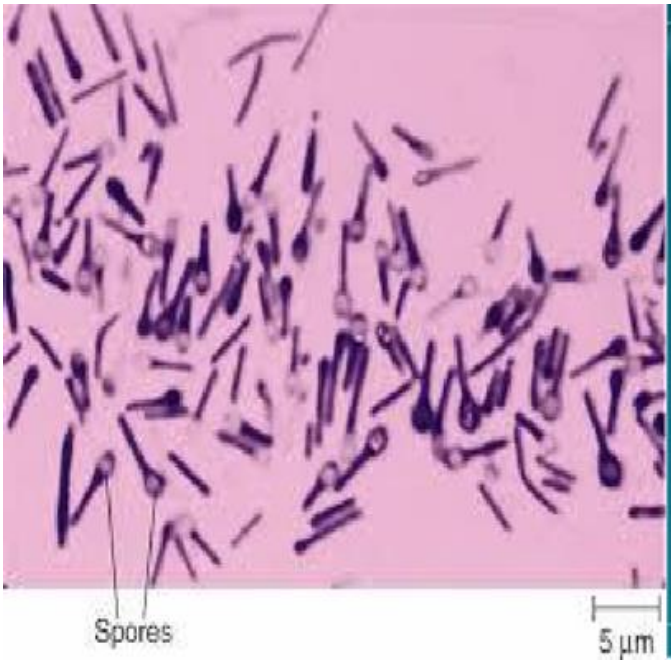
Food poisoning: *C. perfringens* is a common cause of food poisoning. The symptoms; nausea, abdominal cramps, diarrhea occurs 8 to 18 hr. after eating contaminated food.

Treatment

Treatment must begin immediately. High doses of antibiotics, typically penicillin and clindamycin, are given, and all dead and infected tissue is removed surgically. About one of five people with gas gangrene in a limb requires amputation.

b- *Clostridium tetani*: Tetanus disease

Morphology: It is a gram-positive, slender bacillus, 2 to 5 × 0.4-1 mm with rounded ends. The spores are spherical, terminal and twice the diameter of vegetative cells giving them typical drumstick appearance. It tends to be pleomorphic and sometimes filamentous. It is non-capsulated and motile by peritrichous flagella.



Cultural Characteristics

Cl. tetani is an obligate anaerobe. The optimal temperature for growth is 37°C, and the optimal pH is 7.4. It can grow well in cooked meat broth (CMB), thioglycollate broth, nutrient agar and blood agar. In cooked meat broth (CMB), growth occurs as turbidity and there is also some gas formation. The meat is not digested but becomes black on prolonged incubation. On blood agar the bacilli produce a swarming (thin spreading film) growth. On horse blood agar, the colonies of *Cl. tetani* are surrounded by a zone of α-hemolysis, which subsequently develops into β-hemolysis, due to the production of an oxygen-labile hemolysin known as tetanolysin.

Pathogenesis:

Tetanus toxin, called **tetanospasmin**, is an extremely potent toxin .Tetanus has an incubation period varying from four days to several weeks. A shorter period is usually associated with more severe disease and wounds closer to the brain. Tetanus presents as a spastic paralysis, in which muscle spasms often first involve the site of infection. In the early stages of the disease, the mouth (jaw) muscles are affected, so that the mouth cannot open (trismus). Gradually, other voluntary muscles become involved, and any external stimulus precipitates a painful spasm, and sometimes convulsions. Death, which occurs in fifteen to sixty percent of cases, is usually the result of paralysis of chest muscles leading to respiratory failure

Treatment

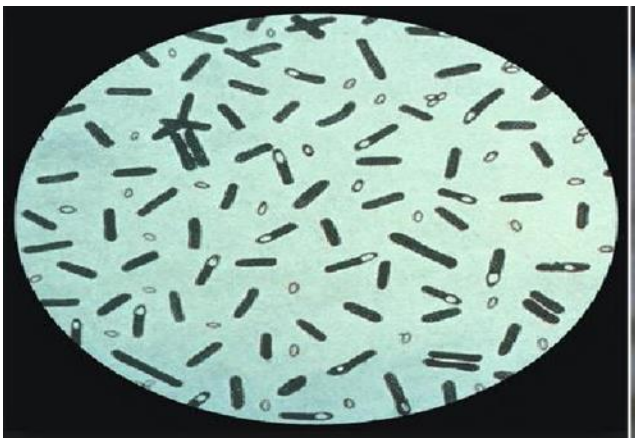
These agents are used to eradicate clostridial organisms in the wound, which may produce tetanus toxin. They are administered to patients with clinical tetanus; however, their efficacy is questioned. Penicillin G was long considered the drug of choice, but metronidazole is now considered the antibiotic of choice

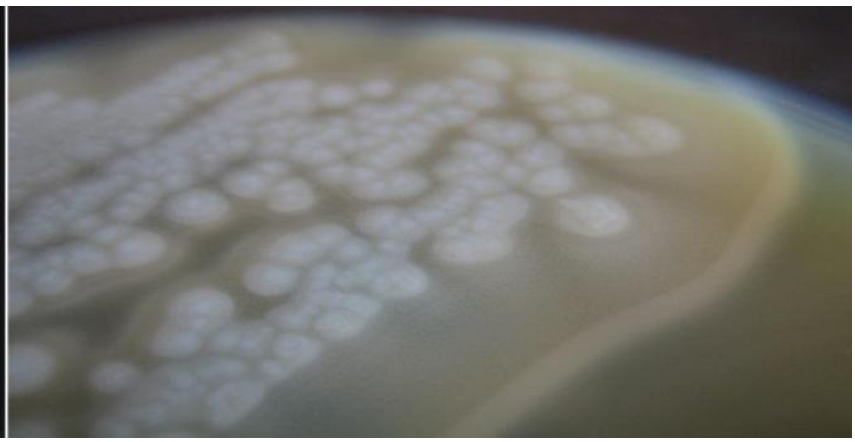
c- Clostridium botulinum

C. botulinum (from the Latin botulus, "sausage") causes botulism. Botulism is a severe, often fatal, form of food poisoning characterized by pronounced neurotoxic effects. The disease has been caused by a wide range of foods, usually preserved hams, sausages, home-preserved meats and vegetables, canned products such as fish, liver contact .

Morphology: *C. botulinum* is a strictly anaerobic gram-positive bacillus (about 5 x 1 mm). It is non-capsulated, motile with peritrichous flagella and produces spores which are oval, sub-terminal and bulging.

Cultural Characteristics: It is a strict anaerobe. Optimum temperature is 35°C but some strains may grow even at 1 to 5°C. Good growth occurs on ordinary media. Surface colonies are large, irregular, and semi-transparent, with fimbriate border. On horse blood agar, all strains except those of type G are beta-hemolytic. On egg- yolk agar (EYA) all types except G produce opalescence and a pearly effect (as in above photo)





Pathogenesis:

There are several types of botulinum toxin. Botulism is caused by the action of a neurotoxin that is one of the most potent poisons known. It causes a **flaccid paralysis**.

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, botulinum toxins affect peripheral cholinergic synapses by blocking the neuromuscular junction and inhibiting release of the neurotransmitter, acetylcholine, preventing contraction and causing flaccid paralysis.

Treatment

In addition to that described, guanethidine and 4-aminopyridine have been used for the treatment of botulinum paralysis but have not been shown to be effective. The use of local antibiotics such as penicillin G or metronidazole may be helpful in eradicating *Clostridium botulinum* in wound botulism.

Clostridium botulinum	*Clostridium tetani*
causes flaccid paralysis.	**causes spastic paralysis**
affected the neurons of PNS	affected the neurons of CNS
botulinum exotoxin called **botulinumtoxin** , designated A through G	Tetanus exotoxin, called **tetanospasmin** (A&B)
The botulinum toxin constitute a set of proteins whose neurotoxicity arises from proteolytic cleavage of specific synaptic vesicle peptides, causing subsequent failure of neurotransmission. botulinum toxins affect peripheral cholinergic synapses by blocking the neuromuscular junction and inhibiting release of the neurotransmitter, acetylcholine, preventing contraction and causing flaccid paralysis.	The A fragment blocks neurotransmitter release at inhibitory synapses, and thus causes severe, prolonged muscle spasms. The A fragment has been shown to be a protease; it cleaves a small synaptic vesicle protein (synaptobrevin) and prevent the flow of inhibitory neurotransmitters. tetanus toxin, which causes contraction (spasms)