Most Clostridium species decompose proteins or form toxins and some do both. Their natural habitat is the soil or intestinal tract as saprophytes. The important pathogenic species are:

- **Clostridium botulinum**: Causes botulism
- **Clostridium tetani**: Causes tetanus
- **Clostridium perfringens**: Causes gas gangrene

**MORPHOLOGY**

- Large anaerobic gram positive motile rods.
- The spore is usually wider than the rods.
- Spores are placed centrally, terminally, or subterminally according to the genus.

**CULTURE**

An aerobic culture conditions are established by one of the following:

1. Agar plates or culture tubes are placed in air tight jar from which air is removed and replaced by N and CO$_2$.
2. Fluid media contain either:
   a. Fresh animal tissue (chopped meat)
   b. Reducing agent (Thioglycolate)

**COLONY FORMS**

- **Clostridium perfringens**: Large raised colonies with entire margins.
- **Clostridium tetani**: Smaller colonies with fine filaments.

Most Clostridia produce a zone of hemolysis on blood agar.

**Growth characteristics** of anaerobic microorganisms are:

1. Unable to utilize O$_2$ as the final oxygen acceptor.
2. Lack of cytochrome and cytochrome oxidase.
3. Unable to break down hydrogen peroxide H$_2$O$_2$ because they lack catalase of peroxidase so H$_2$O$_2$ will accumulate to toxic conc. in the presence of O$_2$.

**Clostridium botulinum**

It causes botulism, infant’s botulism, and rarely wound infection. It is found in soil and animal feces. The spores are subterminal highly resistant to heat. They resist boiling 3-5 hours. This resistance is diminished at acidic pH and salt. It produces toxin during life and autolysis of bacteria.
... Toxin ...

- Types of *Clostridium botulinum*: They are from A – H according to the type of toxin produced.
- Types A, B, and E are the most commonly associated with illness.
- Toxins of types A, B, and E have the following characteristics:
  1. They are among the most highly toxic substances known.
  2. They are neurotoxic proteins (MW = 150,000)
  3. Lethal dose for human is 1 – 2 mg
  4. They are destroyed by heating for 20 minutes at 100°C
  5. Toxin production is under the control of a viral gene (Bacteriophage yielded from toxigenic strain and it may infect non-toxigenic strain and convert it to toxigenic).

... Action of botulism toxin ...

It is a neurotoxic protein. All of its types (A, B, and E) are made of heavy and light chains linked by disulfide bonds. The heavy chain is thought to bind the toxin to the motor nerve end plate. The light chain blocks the calcium-mediated release of acetyl choline. The toxin acts by blocking the release of acetyl choline at synapses and neuromuscular junctions causing flaccid paralysis.

... Pathogenesis ...

Botulism is intoxication. It results from ingestion of food in which *Clostridium botulinum* spores germinate and produce toxins under anaerobic conditions. These foods are spiced, smoked, vacuum-packed, or canned alkaline foods (if eaten without smoking). The toxin acts by blocking the release of acetyl choline at synapses and neuromuscular junctions causing flaccid paralysis. Patients who recover don’t develop an antitoxin in the blood.

... Symptoms [ within 18 – 24 hours ] ...

- Visual disturbances
- Inability to swallow
- Speech difficulty
- Respiratory paralysis or cardiac arrest

... Infant botulism ...

It may result from honey feeding and cause signs of paralysis or sudden death.
© Lab diagnosis …

Toxin can be detected in the patient serum and left over food.

1. Mice are injected with the specimen and then neutralized by injections of antitoxin.
2. Culture of food remains of its growth test for toxin production.
3. Toxin is tested by hemoagglutination or radioimmunoassay (RIS).

© Treatment …

1. IV administration of antitoxin (trivalent antitoxin of types A, B, and E).
2. Adequate ventilation by mechanical respirator. This will reduce mortality form 65% to 25%
3. Infant botulism is recovered with supportive therapy alone.

© Control …

1. Boiling of home-canned food for 20 minutes to destroy the toxin.
2. Strict regulation of commercial canning
3. Avoiding swelled canned food or that with suspected appearance or odor.

❖ *Clostridium botulinum* is widely distributed in soil and contaminated fruits and vegetables. Inadequate precautions in processing and handling of a certain food will allow this organism to grow and produce one of the most powerful exotoxins known.

✓ *Clostridium tetani*

It causes tetanus, uterus, and tetanus neonatrum. It is distributed in soil and feces of animals. The spores are located at one end of the bacilli (drum-stick). It is differentiated into several types according to their specific flagellum antigens.

... Toxin ...

Vegetative cells of *Clostridium tetani* produce tetanospasmin that has the following properties:

1. It is a polypeptide in nature.
2. Its production is under the control of a plasmid gene.
3. The proteolytic enzymes split this toxin into two fragments of increased toxicity.
4. It contains $2 \times 10^7$ mouse lethal dose / mg
5. It acts upon CNS

... Mode of action ...

1. It inhibits the release of acetyl choline thus it interferes with neuromuscular transmission.
2. Inhibition of postsynaptic spinal neurons by blocking the release of an inhibiting mediator.

**Pathogenesis**

*Clostridium tetani* is not an invasive organism. The infection remains strictly localized in the area of dead tissue (into which the spores have been introduced). Germination of spores to vegetative organisms that produce toxin is aided by:

1. Necrotic tissue
2. Calcium salts
3. Associated pyogenic infections

All aid in the establishment of low oxidation – reduction potent.

- Tetanospasmin released from vegetative cells will reach the CNS via the blood and result in generalized muscular spasm.

**Clinical findings of tetanus**

Duration is 4 to 5 days – many weeks. There is muscular contraction of the voluntary muscles (1st area of infection) then the muscles of the jaw (Lock-Jaw disease). Later, other voluntary muscles are involved causing generalized spasm resulting in respiratory paralysis and cardiac failure which lead to death (50%).

- Uterus tetanus: Follows septic abortion.
- Tetanus neonatrum: Follows contamination of the umbilical cord of newborns when it is cut by contaminated food.

**Prevention**

1. Active immunization with toxoid (detoxified toxin) to stimulate Ab.
2. Proper care of wound (Remove the necrotic tissue).
3. Prophylactic use of antitoxin.
4. Administration of penicillin (to inhibit Clostridium and pyogenic bacteria).
   - Treatment with antitoxin in tetanus neonatrum is life saving.

**Control**

Active immunization of children with tetanus toxoid 3 injections:

- In the 1st year
- Booster injection at entry to school
- Boosters are spaced 7-10 years
Usually in young children: In immunization, tetanus toxoid is combined with diphtheria toxoid and Pertussis vaccine (DTP).

**Laboratory diagnosis**

Diagnosis rests on clinical pictures.
1. Anaerobic culture of necrotic tissue.
2. Growth is tested for toxin production.
3. Neutralization of the toxin produced with specific antitoxin.

**Clostridium perfringens**

It produces invasive infection. It is responsible for 90% of myonecrosis and gas gangrene cases infecting contaminated wounds (e.g. compounds fracture and post partum uterus). There are other 30 species of Clostridium which cause the rest 10% of infection.

**Morphology**

They are found in the soil and the intestine of man and animals. They are anaerobic large G+ve rods. They produce subterminal non-bulging spores (rarely produce spores in laboratory media). They produce capsule in the patient’s tissue.

*Clostridium perfringens* also causes profuse diarrhea (food poisoning).

**Toxin**

There are 5 types of *Clostridium perfringens* (A, B, C, D, and E). They are differentiated on the basis of production of 4 major toxins (α, B, E, and lota).

α toxin is responsible for severe toxemia in gas gangrene and has the following properties:
1. It is lethal for lab animals.
2. It is Ca⁺², Mg⁺² – dependent lecithinase.
3. Causes lysis of RBCs.
4. Produced by all types of *Clostridium perfringens*.
5. It splits lecithin of cytoplasmid membrane → Phosphorylendin + Diglyceride
6. It has necrotizing and hemolytic effect.

**Enzymes**

*Clostridium perfringens* produce enzymes that digest subcutaneous tissue and muscles.

a. DNAase
b. Hyaluronidase
c. Collagenase
**Enterotoxin** causes profuse diarrhea (food poisoning).

***Pathogenesis***

1. **Gas gangrene:**
   The spores reach traumatized tissue from soil or intestine of patients. The spores will germinate to vegetative cells. Vegetative cells will multiply and ferment carbohydrates of tissue producing CO$_2$ gas. Distention of tissue and interference with blood supply together with secretions of necrotizing toxins and enzymes $\rightarrow$ spread of infection and necrosis of tissue.
   The necrosis extends $\rightarrow$ bacterial growth, hemolytic anaemia, and severe toxemia and death.  
   In gas gangrene, a mixed infection is the rule (Clostridia + G+ve cocci + G-ve bacilli).

2. **Uterine gas gangrene:** May follow instrumental abortion because Clostridium perfringens is present in the genital tract of 5% of women.

3. **Clostridial bacteremia** is frequent in patients with neoplasms.

4. **Food poisoning** due to enterotoxin.

***Clinical findings***

The infection spread from a contaminated wound in 1-3 days to produce:

- a. Crepitation in the subcutaneous tissue.
- b. Foul smelling discharge.
- c. Necrosis.
- d. Fever.
- e. Hemolysis
- f. Toxemia
- g. Shock
- h. Death

***Treatment***

1. Immediate surgical debridement of all dead tissue.
2. Administration of antibiotics (ampicillin).
3. Polyvalent antitoxin could be used.
4. Hyperbaric oxygen detoxifies the patient rapidly.
... Laboratory diagnosis ...

A. Specimen: Tissue form wounds, pus, and deep swabs.
B. Gram stain.
C. Culture on:
   1. Chopped meat and glucose media.
   2. Thioglycolate media.
   3. Blood agar media
   ➔ Incubated anaerobicly
D. Action on milk.
E. Biochemical shirt (Sugar from)
F. Lecithin’s activity
G. Test for toxin production

*Clostridium perfringens* food poisoning

Usually it follows the ingestion of large no. of *Clostridium perfringens* that have grown in warmed meat dishes. The toxin is formed when Clostridium sporulate in gut with the onset of diarrhea. There is no vomiting or fever in 6 – 18 hours. It lasts only 1 – 2 days. It is self limited.

**Toxin** is heat labile enterotoxin that has a mechanism of action which resembles that of *E. coli*. 