

**THE GENUS**  
***CLOSTRIDIUM***

- This genus contains many species of grampositive, anaerobic and spore-forming rods.
- Some of them are pathogenic for humans and animals.

**The human pathogens in genus *Clostridium* may be categorized as follows:**

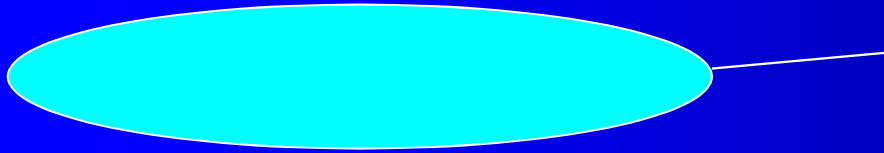
1. The gas gangrene group, the most important of which is *Clostridium perfringens*.
2. *Clostridium botulinum*, the cause of botulism.
3. *Clostridium difficile*, the cause of toxic enterocolitis.
4. *Clostridium tetani*, the cause of tetanus.

# The species *Clostridium tetani*

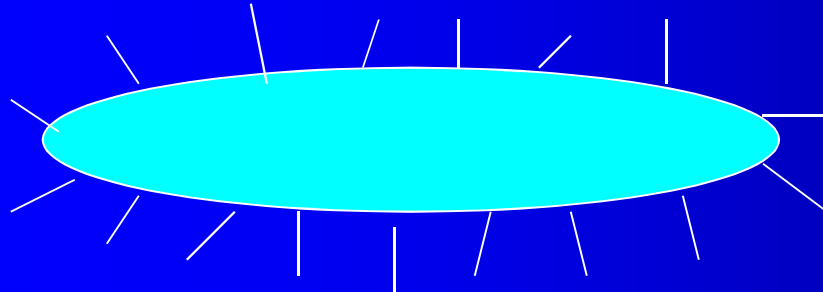
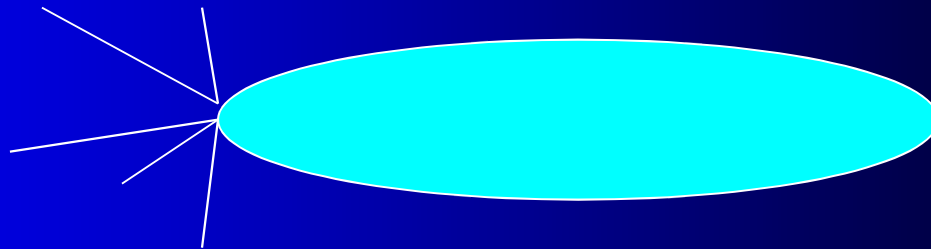
- Grampositive, straight and slender rods with rounded ends.
- Round terminal spores are formed after 2-4 days of incubation.
- *C. tetani* is flagellated and motile. It has numerous peritrichous flagella.
- Capsules are not formed.

# Bacterial flagella - three types of arrangement

Monotrichous – single polar flagellum



Lophotrichous – tuft of polar flagella



Peritrichous – flagella distributed over the entire bacterial cell

# The species

## *Clostridium tetani*

- *C. tetani* requires strict anaerobic conditions.
- Because of its motility, it spreads over the surface of anaerobic blood agar in a thin veil of growth.
- Some strains can grow in small spidery colonies.
- They weakly hemolyse on blood agar.
- They do not form acids from sugars.
- Aminoacids serve as main sources of energy.

# The species *Clostridium tetani*

- All *C. tetani* strains share a common somatic (O) antigen.
- The 10 types of *C. tetani* can be distinguished by specific flagellar (H) antigens. Antigenic types I and III most often cause tetanus in humans. However, this serotyping has not a significance for epidemiological practise.

# The species *Clostridium tetani*

- *C. tetani* spores remain viable in soil for many years.
- The spores are heat-stable.
- The spores of some strains are resistant to boiling in water for up to 3 hours. They are killed by autoclaving at 121 °C for 15 minutes. They may resist to 5% phenol for 10 hours or more.
- Vegetative cells of *C. tetani* are heat-labile.



# The species

## *Clostridium tetani*

- *C. tetani* produces an oxygen-labile hemolysin - tetanolysin. This toxin has only a negligible significance for the pathogenesis of tetanus. The most important product of *C. tetani* is neurotoxic exotoxin – tetanospasmin.
- The tetanospasmin production appears to be under control of plasmid gene.
- Vegetative cells produce tetanospasmin during the stationary phase and release it mainly when they lyse.

# The species *Clostridium tetani*

- Tetanospasmin is a heat-labile antigenic protein rapidly destroyed at 65 °C and by intestinal proteases.
- It is toxic to man and various animals when injected parenterally, but it is not toxic by the oral route.
- The LD<sub>50</sub> of the toxin for mice is 0.0001 μg, less than 1 μg is lethal to humans.

# The species *Clostridium tetani*

- *C. tetani* is not an invasive microorganism.
- The infection remains strictly localized in the area of devitalized tissue, into which the spores have been introduced.
- Germination of the spores and development of vegetative organisms that produce toxin are aided by:
  - necrotic tissue,
  - calcium salts,
  - associated pyogenic infections.

# The species *Clostridium tetani*

- Tetanospasmin acts by blocking the release of neurotransmitters (glycine and GABA "gamma-aminobutyric acid") on the level of the postsynaptic neuron junctions of the anterior horn cells of the spinal cord.
- Incubation: approximately 5 to 15 days.

# Symptoms of tetanus

- Sudden difficulties with mastication due to rigidity of masticatory muscles.
- Elevated temperature.
- The patient cannot open his mouth, this effect is named as trismus.
- Risus sardonicus is another sign in which trismus is combined with facial spasm.
- In severe cases, spasms of the back muscles produce the opisthotonus.
- The patients are fully conscious, and pain may be very intensive.

# Symptoms of tetanus

- In a later stage of the disease, high temperature is usually present.
- Tachycardia.
- Generalized tonic spasms are more and more frequent, prolonged and intensive.
- Breathlessness and cyanosis are expressed when the respiratory muscles are affected by spasms.
- Laryngospasmus can be also present.
- In fatal cases death results from exhaustion and respiratory or circulatory failures.
- Tetanus of newborns follows infections of the umbilical stump.
- Others.

- Localized tetanus is another form of *C. tetani* disease. It remains confined to the muscles at the site of primary wound and infection. This form has a good prognosis.
- Another variant of localized tetanus is so called cephalic tetanus. The incubation of this variant is very short and its prognosis is considerably poor.

## There are two basic types of tetanus:

- The most often *C. tetani* infection in humans is of a descendent type which spreads through lymphatic and blood routes to nerve fibres. It begins with spastic symptoms on the face. This type has a shorter incubation and worse prognosis.
- The less frequent form is an ascendent type of tetanus. Spasm begin in the environment of a wound. This type has a longer incubation and better prognosis.



# The tetanus

- The mortality caused by the generalized disease represents more than 50%.
- Mortality is the highest in the neonates, elders and in the patients with cardiac diseases.
- Tetanus of newborns has the highest mortality, even more than 90%.

# The tetanus - treatment

- Surgical wound treatment is vitally important because it removes the necrotic tissue that is essential for proliferation of the *C. tetani* strains.
- Tetanus antitoxin of human origin.
- Penicillin (or other antibiotics) strongly inhibits the growth of *C. tetani* and stops further toxin production. Antibiotics may also control associated pyogenic infection.

# The tetanus - treatment

Examples of antibiotic therapy:

- penicillin G 4x5-10 mil. IU, 10 days
- gentamicin 3x80 mg (or 1x240 mg) + clindamycin 4x900 mg, 10 days
- others (like metronidazol)

Prevence: Application of toxoid

# The tetanus

## Epidemiology:

- Sources: exogenous, endogenous.
- Transmission:
  - Direct contact of wound with sources.
  - Perforation of the intestinal wall after injuries, operations or during pathological processes.

# *Clostridium botulinum*

- *C. botulinum* is a strictly anaerobic grampositive bacillus. It is motile with peritrichous flagella. Its spores are oval and subterminal. It is a widely distributed saprophyte occurring in soil, vegetables, fruits, and others.
- The widespread occurrence of *C. botulinum* in nature, its ability to produce a potent neurotoxin in food, and the resistance of its spores to inactivation combine to make it a formidable pathogen of man and range of animals and birds.

# *Clostridium botulinum*

- Botulinal toxins are among the most poisonous natural substances known.
- Seven main types of *C. botulinum* designated A-G produce antigenically distinct toxins with pharmacologically identical actions.

# *Clostridium botulinum*

- Botulism is a severe, often fatal, form of food poisoning characterized by pronounced neurotoxic effects.
- The preformed toxin in the food is absorbed from the intestinal tract. Although it is protein, it is not inactivated by the intestinal proteolytic enzymes.
- The toxin primarily affects the cholinergic system and seems to block release of acetylcholine, chiefly at points in the peripheral nervous system.

# Clinical presentation

- Descending symmetrical paralysis beginning with cranial nerve involvement, induced by botulinum toxin. Onset begins with blurry vision, followed by ocular muscle paralysis, difficulty speaking and inability to swallow. Respiratory paralysis may occur in severe cases. Mental status is unaffected.
- Usual incubation period is 10 – 12 hours. Incubation is shortest for type E strain (hours), longest for type A strains (up to 10 days), and is inversely proportional to the quantity of toxin consumed (food botulism).
- Wound botulism (types A or B) may follow *C. botulinum* entry into IV drug abuser injection site, surgical or traumatic wounds.
- Infant (less than 1 year) botulism (most commonly type A or B) is acquired from *C. botulinum* containing honey.



# Therapy of botulism

- Application of antitoxin
  - antitoxin neutralizes only free toxin, and does not reverse toxin-induced paralysis
  - botulism is toxic-mediated infection and antibiotic therapy (wound botulism) is adjunctive
- Prognosis:
  - good if treated early, before respiratory paralysis

# Clostridia that produce invasive infections

- Many different toxin-producing clostridia can produce invasive infections (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%).

# *Clostridium difficile*

- This organism has a direct relationship with pseudomembranous colitis, usually is association with broad-spectrum antibiotic therapy.
- Pseudomembranous colitis is diagnosed by endoscopic observation of pseudomembranes or microabscesses in patients who have diarrhea and have been given antibiotics. The diarrhea may be watery or bloody, and the patient frequently has associated abdominal cramps, leukocytosis, and fever.

# ***Clostridium difficile* - pseudomembranous colitis**

- Administration of antibiotics results in proliferation of drug-resistant *C. difficile*, that produce two toxins (toxin A, toxin B). Both toxins are found in the stools of patients with pseudomembranous colitis.
- **Treatment:**
  - metronidazol 3 x 500 mg p.os or i.v.**
  - vancomycin 4x125mg p.os**
  - fidaxomicin 2x100mg, 10 days p.os**