

Lecture 12 :Anaerobic bacteria

Clostridium perfringens is a Gram-positive, rod-shaped, non motile anaerobic, spore-forming pathogenic bacterium of the genus *Clostridium perfringens* is the third most common cause of food poisoning. Infections due to *C. perfringens* show evidence of tissue necrosis, bacteremia, and gas gangrene.

Classification

Phylum:	Firmicutes
Class:	Clostridia
Order:	Clostridiales
Family:	Clostridiaceae
Genus:	<i>Clostridium</i>

Biochemical test :

Catalase: Negative, Spot indole: Positive, Lecithinase: Positive, Lipase: Negative, Litmus Milk: Stormy Fermentation,

) *Cl. perfringens* characterized by:

- It ferments many carbohydrates with acid & gas
- Nagler reaction is positive

) Food poisoning

C. perfringens bacteria are the third most common cause of foodborne illness, with poorly prepared meat and poultry, or food properly prepared but left to stand too long, the main culprits in harboring the bacterium. The clostridium perfringens enterotoxin mediating the disease is heat-labile (inactivated at 74 °C). It can be detected in contaminated food (if not heated properly), and feces. Incubation time is between six and 24 (commonly 10–12) hours after ingestion of contaminated food.

Since *C. perfringens* forms spores that can withstand cooking temperatures, if cooked food is let stand for long enough, germination can ensue and infective bacterial colonies develop. Symptoms typically include abdominal cramping, diarrhea, vomiting, and fever. The whole course usually resolves within 24 hours, but can last up to two weeks in older or infirm hosts.

Diagnosis:

C. perfringens can be identified by Nagler's reaction where the suspect organism is cultured on an egg yolk media plate. One side of the plate contains anti-alpha-toxin, while the other side does not. A streak of suspect organism is placed through both sides. An area of turbidity will form around the side that does not have the anti-alpha-toxin, indicating uninhibited lecithinase activity. In addition, laboratories can diagnose the bacteria by determining the number of bacteria in the feces. Within the 48 hours from when the disease began, if the individual has more than 10^6 spores of the bacteria per gram of stool, then the illness is diagnosed as *C. perfringens* food poisoning.

This test is done to detect the lecithinase activity

- The plate is incubated anaerobically at 37 C for 24 h
- Colonies of *Cl. perfringens* are surrounded by zones of turbidity due to lecithinase activity and the effect is specifically inhibited iff *Cl. perfringens* antiserum containing α . antitoxin is present on the medium



Nagler's test

) The toxins of *Cl. perfringens*

- α toxin (phospholipase C, lecithinase) is the most important toxin
 -) Lyses of RBCs, platelets, leucocytes and endothelial cells
- β -toxin is responsible for necrotic lesions in necrotizing enterocolitis
- Enterotoxin is heat labile toxin produced in colon → food poisoning

Prevention

The growth of *C. perfringens* spores can be prevented by most importantly cooking food, especially beef, and poultry, thoroughly to the recommended temperatures. Leftover food should be refrigerated to a temperature below (4°C) within two hours of preparation.

Treatment: Penicillin G and amoxicillin

Clostridium tetani : is a rod-shaped, anaerobic species of pathogenic bacteria, of the genus *Clostridium*. Like other *Clostridium* genus species, it is Gram-positive, and its appearance

on a gram stain resembles tennis rackets or drumsticks. *C. tetani* is found as spores in soil or in the gastrointestinal tract of animals. *C. tetani* produces a potent biological toxin, tetanospasmin, and is the causative agent of tetanus, a disease characterized by painful muscular spasms that can lead to respiratory failure.

C. tetani is a rod-shaped, obligate anaerobe which stains Gram positive in fresh cultures; motile established cultures may stain Gram negative. During vegetative growth, the organism cannot survive in the presence of oxygen, is heat-sensitive and exhibits flagellar motility. As the bacterium matures, it develops a terminal spore, which gives the organism its characteristic appearance. *C. tetani* spores are extremely hardy as they are resistant to heat and most antiseptics. The spores are distributed widely in manure-treated soils and can also be found on human skin. The incubation period is typically 3-21 days. The period of illness is 14 days. *C. tetani* is not contagious.



Vaccination

Tetanus can be prevented through the highly effective tetanus vaccine. The vaccine can be formulated as simple or adsorbed tetanus vaccine, combined tetanus and killed polio vaccine, or the older (diphtheria, tetanus, pertussis) (DPT) vaccine

Toxicity

C. tetani usually enters a host through a wound to the skin, then it replicates and spread in blood stream then moves up nerves . High risk individuals are people exposed to soil or animal feces. The spores are widely distributed in soil and in the intestines and feces of horses, sheep, dogs, cats, rats, and chickens. Once an infection is established, *C. tetani* produces two exotoxins, tetanolysin and tetanospasmin, incubation 1-2 days.

Toxin action

-) *Cl. tetani* produces two types of toxins:
 -) Tetanolysin, which causes lysis of RBCs
 -) Tetanospasmin is neurotoxin and essential pathogenic product

Treatment: metronidazole and Penicillin G