

# Anaerobic Pathogenic Bacteria Associated with Fish and Fishery Products

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Based on the requirements of oxygen for growth and viability, bacteria are divided into Aerobes, Anaerobes, Facultative anaerobes and Microaerophilic (vide infra). Those bacteria which can grow only in the absence of oxygen are termed 'anaerobes.' Anaerobic bacteria which are pathogenic are found associated with fish, fishery products and fishery environments. Examples are *Clostridium botulinum*, *Clostridium perfringens* (also called *C. welchii*), *Campylobacter jejuni* and *Campylobacter coli*.

## *Clostridium botulinum*

*Clostridium botulinum* is an anaerobic, Gram positive, catalase negative, endosporeforming rod - shaped bacterium. When *C. botulinum* grow in food, they produce a toxin.

The toxin is elaborated into the food from the bacterial cell. This toxin is, therefore, called an 'exotoxin'. Chemically, the toxin is a protein. When we ingest the food containing the toxin, we get food poisoning. This food poisoning is called 'botulism'.

Botulism-toxin is a neurotoxin. It acts like the neuro active drug, 'curare'. Depending on the differences in the immunological response produced by the toxin when injected into test animals, the toxins produced by different strains of *Clostridium botulinum* are classified into 7 groups. The corresponding *C. botulinum* strains are designated as *C. botulinum* type A, B, C, D, E, F and G. Molecular weights of their toxins are in the range of 1,50,000 to 10,00,000 daltons (1 dalton = atomic weight of hydrogen).

Pharmacologically, ie, in their biological activity, all the seven toxins are similar and cause same type of food poisoning. Of these seven types, A, B, E and F cause botulism in man, B, C and D in animals and C and E in birds.

The source of botulism food poisoning is usually preserved foods such as meat and meat products, canned vegetables and fish, salted and smoked fish, surimi and imitation crab meat prepared from surimi meat. The spores of *C. botulinum* are resistant to heat and can withstand heat for several hours at 100°C and upto 20 min. at 120°C. This bacterium is usually present in soil, aquaculture farm sediments and sea bottom. If food is contaminated with the spores, they may survive heat treatment. Surviving spores can grow in favourable anaerobic conditions and produce toxin. But the toxin is heat labile. It will be destroyed at 80°C in 30-40 min. and at 100°C, in 10 min.

### ***Symptoms of botulism***

A number of symptoms may result from the ingestion of food containing botulism toxin. Symptoms begin usually within 12-36 hours after ingestion. Nausea and vomiting are the initial symptoms. In some cases, gastrointestinal disturbances follow. Thirst, difficulty of vision including diplopia (double vision), difficulty in swallowing, difficulty in speaking (aphonia) and difficulty in movement (ataxia) are the other symptoms. These may be followed by difficulty in breathing, leading to respiratory failure and death.

Mortality is upto 65%. Death depends on the amount of toxin ingested and absorbed. When large amounts of toxin are ingested, symptoms appear in a short while. Usually, death occurs within 48 hrs. in fatal cases.

### ***Treatment***

There is no satisfactory treatment for botulism. Once the disease is recognised as botulism, the only available remedy is to administer antitoxin injection. Also, other general symptomatic treatment for food poisoning is given. As the type of toxin may not be known, usually large doses of polyvalent antitoxin is given intramuscularly.

### ***Presence in fish***

Fish are obtained either by capture or by aquaculture. Fish caught from seas and oceans are relatively free from human pathogenic microorganisms. The major exceptions are *Clostridium botulinum* and some species of pathogenic *Vibrio*. Bottom feeding fish usually have the highest level of contamination with *C. botulinum*. Intestinal tracts and contents are the main reservoir. Incidence is markedly reduced by gutting the fish. Mainly, type E strains are commonly found associated with fish. Type A and B have also been occasionally present. *C. botulinum* type D is also reported to be present in marine environments and sediments from brackishwater culture farms in India.

### ***Control of C. botulinum***

Growth and toxin production of *C. botulinum* in food and food products are controlled by the temperature of storage, pH and acidity of the food, redox potential and composition of the surrounding atmosphere, water activity, salt content, presence of preservatives, etc.

#### ***Temperature***

There are two groups of *C. botulinum* based on their ability to degrade proteins, namely proteolytic and non-proteolytic. The proteolytic strains of *C. botulinum* can grow and produce toxin at 12.5°C at the lowest and 50°C at the highest, optimum being 35°C. The non-proteolytic strains grow and produce toxin, between 3.3°C and 45°C, the optimum being 30°C. Hence, refrigeration of food products above 3.3°C is not considered safe with respect to growth and toxin production by *C. botulinum*.

#### ***pH and acidity***

pH and acidity are important factors determining the processing parameters of heat processed foods. Optimum pH value for growth of *C. botulinum* is 6.8-7. Non proteolytic strains are capable of growing at a lower pH value of 5-5.2, while proteolytic strains can grow even at a pH value of 4.8. Generally, a pH value of 4.8 is accepted as the limiting value for *C. botulinum*.

### ***Water activity and salt content***

Water activity is very critical in controlling the growth of microorganisms. Water activity can be regulated by the addition of NaCl to the food or curing medium. Usually *C. botulinum* strains do not grow below an  $a_w$  of 0.94. Type E-strains cannot grow below  $a_w$  of 0.97.

Salt tolerance varies with the type of strain. Usually proteolytic strains can tolerate a maximum of 10% NaCl concentration. Non-proteolytic strains do not survive in 5% NaCl in curing medium. *C. botulinum* type E cannot tolerate more than 4% NaCl.

Smoking of cured or salted fish is traditionally practised in certain countries. Much of the inhibitory effect of smoking is due to drying and consequent reduction in  $a_w$ . But certain ingredients of the smoke, like formaldehyde, have an inhibitory effect, particularly on Type E-strains in smoked fish.

Preservatives like sorbic acid and potassium sorbate are used in foods to inhibit spoilage microflora, particularly yeasts and molds. Undissociated sorbic acid has been found to inhibit the growth of *C. botulinum* strains in foods.

### ***Clostridium perfringens (C. welchii)***

*Clostridium perfringens* (also referred to as *C. welchii*) is a Gram-positive, rod shaped bacterium, which forms endospores. It is a common microorganism, frequently found in faeces of man and animals. It is also found in raw meat, poultry and other foods, including dry products. The body surface and alimentary canal of several species of fish harbour this organism. *C. perfringens* can also gain access during handling and processing of fish.

Eating of food contaminated with large numbers of live cells of this bacterium can cause food poisoning. The live cells of *C. perfringens* reaching the intestine through food, produce an enterotoxin in the intestine, which is responsible for the food poisoning.

Symptoms of food poisoning occur within 8 to 22 hrs. after consuming the contaminated food. Incubation period is usually 8-22 hrs. The common symptoms are abdominal pain, diarrhoea, nausea and rarely vomiting. These may last for 12-48 hrs.

### ***Control***

The spores of *C. perfringens* can survive heat and dehydration. Spores which survive cooking, germinate and multiply in food during warm storage of food. Cooked meat, poultry, fish, pies and gravies are excellent media for growth of the bacterium.

Generally, *C. perfringens* has a growth temperature range of 15 to 50°C. But there are reports of its growth at temperatures as low as 6°C. However, keeping food well refrigerated below 10°C would prevent food poisoning due to *C. perfringens*.

### ***Campylobacter***

*Campylobacter* is a microaerophilic organism, even though it grows anaerobically in presence of certain compounds like nitrate and fumarate. For growth, it requires very low levels of oxygen (3 to 15%) and carbon dioxide (3 to 5%).

*Campylobacter* is a Gram-negative, slender, spiral, rod shaped bacterium. It is polar flagellated, either at one and or on both ends of the cell. It has a characteristic “corkscrew-like” motility. Of the many species of *Campylobacter*, *Campylobacter jejuni* and *C. coli* are the only enteropathogenic species frequently involved in food poisoning, causing enteritis in man.

These enteropathogenic species viz. *Campylobacter jejuni* and *C. coli* are of zoonotic origin. They have been isolated from food, animals and birds. They have also been isolated from environmental sources like water. Milk, poultry, red meat, shellfishes and water have been responsible for large and sporadic outbreaks of *Campylobacter* enteritis.

In an outbreak in the USA, raw clams were found to be the vehicle of infection. *Campylobacter* has also been isolated from oysters. Sewage pollution of the growing beds of clams and oysters might have contributed to the accumulation of *Campylobacter* in those animals.

The symptoms of *Campylobacter* enteritis tend to be abrupt. The incubation period varies between 2 to 11 days. Before the onset of the intestinal symptoms, influenza-like prodromal symptoms such as headache, shivering and dizziness may occur for a few hours to 2-3 days. These are followed by abdominal pain, nausea and vomiting. In certain cases, rise in body temperature upto 40°C and delirium may occur. Diarrhoea may occur in certain cases, but the severity is less than that of Salmonellosis. Severe abdominal pain is a major feature of *Campylobacter* enteritis. The infection may be of limited duration, lasting less than one week. Symptoms of *C. jejuni* and *C. coli* enteritis are essentially the same, but severity is less in the case of *C. coli* enteritis. Chemotherapy is indicated in severe cases.

### ***Control***

*Campylobacter* is easily destroyed at temperature of pasteurisation and cooking. They are destroyed within 8 minutes at 50°C and 2 min. at 55°C. They can readily survive at refrigerated temperatures. Freezing does not destroy *Campylobacter* completely. They are destroyed by drying. But *Campylobacter* can survive greatly in vacuum or controlled atmosphere packed foods, than in foods packed in air.