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OTHER PATHOGENS CONNECTED WITH SEAFOOD

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We have already discussed about the incidence of pathogenic organisms such as Stephyulococcus aures, *Salmonella*, Vibrio cholerae and Vibrio parahaemolyticus in marine products. There are also some other pathogens involved in poisoning due to the consumption of seafood. They are Clostridium botulinum, *Clostridium* Welchil (Clostridium perfrigens), Bacillus cereus and Shigella.

Clostridium botulinum

Clostridium botulinum is a species of anaerobic, sporeforming, rod-shaped bacteria, producing a protein with characteristic neurotoxicity. Botulism, a severe food poisoning, results from ingestion of food containing botulinum toxin produced during the growth of this organism in food. Botulism is a rare type of food poisoning but the mortality rate is high. There have been 688 recorded outbreaks in the United States from 1899 to 1973. These outbreaks of botulism involved 1,784 cases and caused 978 deaths. In those outbreaks where the toxin type was determined, 158 due to Type A, 43 to Type B, 22 to Type E, and 1 to Type F. The foods implicated in two outbreaks contained both Type A and B toxins. The limited numbers of reports of C and D toxins as the causative agent of human botulism have not received general acceptance. However, all except Types F and G, which have not been as thoroughly studied, are important causes of animal botulism.

Antigenic types of C.*botulinum* are identified on the basis that their toxins are completelhy naturalized only by the homologous type of antitoxin and that cross neutralization by heterologous-type; antitoxins is absent or minimal. There are seven recognized types: A, B, C, D, E, F and G.

Aside from toxin type production, C.*botulinum* can be differentiated into 3 general groups on a basis of cultural, biochemical and physiological characters. Cultures producing Types C and D toxins are non-proteolytic on coagulated egg white or meat, and have a common metabolic pattern, which sets them apart from the others. All Type A and some B and F toxin-producing strains are proteolytic. All type E strains and the remaining B and F strains are non-proteolytic, with carbohydrate metabolic patterns differing from the C and D non-proteolytic group. Type G toxin producing strains have not been studied in sufficient detail for effective and satisfactory characterization.

C.botulinum is widely distributed in soils and in sediments of oceans and lakes. The finding of Type E in aquatic environments by many investigators correlates with the Type E cases of botulism being traceable to contaminated fish or other seafoods. Types A and B are most commonly encountered in foods subject to contamination with soil. In the United States, home canned vegetables are most commonly contaminated with A and B, but in Europe, meat products have also been important vehicles of foodborne illness.

Measures to prevent botulism include reduction of the microbial contamination level, acidification, reduction of moisture level, and whenever possible, destruction of all botulism spores in the food. Heat processing is the most common method of destruction. Propertly processed canned foods will not contain viable C.*botulinum*. Home canned food encounters more of botulism than commercially canned foods, which probably reflects the commercial canners' great awareness and better control of the required heat treatment.

A food may contain viable *C. botulinum* and still not be capable of causing botulism. If the organisms do not grow, no toxin is produced. Numerous foods satisfy the nutritional requirements of *C. botulinum* but not all of them provide the necessary anaerobic conditions. Both nutritional and anaerobic requirements are supplied by many canned foods and by various meat and fish products. Growth in otherwise suitable foods can be prevented if the product, naturally or by design, is acid (of low pH) has low water activity, has a high concentration of sodium chloride, has an inhibitory concentration of sodium nitrite, or has two or more of these conditions in combination. Refrigeration will not prevent growth and toxin formation by non-proteolytic strains unless the temperature is precisely controlled and kept below 3°C. The most common vehicles of botulism are those foods which are processed to prevent spoilage and are not usually refrigerated.

Optimum temperature for growth and toxin production of the proteolytic strains is close to 35°C; that of non-proteolytic strains is approximately 26°C. non-proteolytic types B, E and F are able to produce toxin at refrigeration temperatures 3-4 °C. Toxins of the non-proteolytics do not manifest maximum potential toxicity until they are activated with trypsin; toxins of the proteolytics generally occur in fully or close to fully activated form. These and other differences can be important in epidemiological and laboratory consideration of botulism outbreaks. Clinical diagnosis of botulism is most effectively confirmed in the laboratory by identifying botulinum toxin in the blood, faces or vomitus of the patients. Specimens must be collected before the administration of botulinum antitoxin to the patient. Identifying the causative food is most important to prevent additional cases of botulism.

Clostridium perfringens:

Food poisoning caused by *Clostridium perfringens* may occur when foods such as meat or poultry are cooked and held without adequate refrigeration before serving. The oxygen level can be sufficiently reduced during cooking to permit growth of the clostridia. The presence of small numbers of *C. perfringens* is not uncommon in raw meats, poultry, dehydrated soups and sauces, raw vegetables, spices etc. Since the spores of some strains are resistant to temperatures as high as 100°C for more than 1 hour, their mere presence in foods may be unavoidable. However, spores of this organism that survive cooking germinate and grow rapidly in cooked foods that is inadequately refrigerated. Therefore, the presence in foods of large numbers of *C. perfringens* is indicative of mishandling. In food poisoning outbreaks, demonstration of the presence of hundreds of thousands or more organisms of *C. perfringens* per gram of food supports a diagnosis of food poisoning caused by *C. perfringens* when it is substantiated by clinical and epidemiological evidence.

Illness occurs 8–15 after ingestion of the contaminated food. The symptoms, which are intense abdominal cramps, gas, and diarrhea (nausea and vomiting are rare), have been attributed to a protein enterotoxin produced during sporulation of the organism in the intestine. The enterotoxin can be demonstrated in sporulating cultures. A high correlation has been established between the ability of *C perfringens* strains to produce enterotoxin and their ability to cause food poisoning. However, difficulty has been experienced in obtaining consistent sporulation. etc., with some strains. Thus, a negative result with the guinea pig skin assay does not show conclusively that the strain is incapable of causing food poisoning.

A loss in the viability of *C. perfringens* cells will occur if foods are frozen or held under prolonged refrigeration unless special precautions are taken to maintain the cells in viable condition. Such losses may cause difficulty in establishing *C. perfringens* as the specific

cause of a food poisoning outbreak. If the suspected food is held at low temperature for more than a day or two without the special precautions described below, the (-toxin indicator method may be useful for estimating the maximum *C. perfringens* population reached in the suspected food. Production of (-toxin by *C. perfringens* is proportional to the number of metabolizing cells except with a few weekly toxigenic strains. (-toxin can be extracted and quantitated to obtain a reliable estimate of the maximum population levels of this proganism in the food. This method is particularly valuable for determining the extent of growth of *C. perfringens* in food samples which have been subjected to freezing or prolonged refrigeration and for which low plate count values may be expected.

Bacillus cereus:

Food poisoning caused by Bacillus cereus may occur when foods are prepared and held for several hours without adequate refrigeration before serving. B. cereus is an aerobic sporeformer that is common in soil, on vegetables, and in many raw and processed foods. Consumption of foods which contain large numbers of B. cereus (106 or more/g) may result in food poisoning. Foods which have been incriminated in past outbreaks include cooked meat and vegetables, boiled or fried-rice, vanilla sauce, custards, soups, and raw vegetable sprouts.

Two types of illness have been attributed to the consumption of foods contaminated with *B. cereus*. The first and best known type is characterized by abdominal pain and diarrhoea; it has an incubation period of 4–16 hr and symptoms which last for 12-24 hours. The second type is characterized by an acute attack of nausea and vomiting which occurs within 1-5 hr after a meal; diarrhea is not a common feature in this type of illness.

Shigella

Shigella is a bacterium belonging to the family Enterobacteriaceae and is often associated with intestinal diseases such as bacillary dysentery. Dysentery is a clinical condition of multiple aetiology, characterised by the passage of loose motion mixed with blood and mucus. This organism was named after Shiga, who isolated if for the first time in 1896.

Morphology and cultural characteristics

Shigellae are short Gram-negative rods; about 0.5 x 1.3m in size. They are non-motile, non-sporing, non-capsulated and are aerobes and faculative anaerobes, growing within a temperature range of 10- 40°C. The optimum growth temperature is 37°C and pH 7.4.

Natural habit and epidemiology

Man is the only known natural host for Shigella. The transmission of the organism from one individual to another is by the following means:

- Direct-hand to mouth-through contaminated fingers.
- Contaminated food, water, ice etc.
- Contaminated contact surfaces.
- Flies etc.
- Food handlers who are carriers of this organism.

Classification

Shgellae are classified into four species or subgroups based on a combination of biochemical and serological characteristics. They Are:

Shigella dysenteriae (Sub-group A)
Shigella flexneri (Sub-group B)
Shigella boydii (Sub-group C)
Shigella sonnei (Sub-group D)
Of these *dysenteriae* is the cause of the most severe type of bacillary dysentery and is most frequently associted with complications.

Resistance

Shigellae are not highly resistant. They are killed at 60°C in 10 minutes. 1 % phenol solution destroys the organism in 30 minutes. In water and ice they remain viable for 1-6 months. Boiling or chlorination of water destroys the organism. In faeces, they die within a few hours due to the acidity produced by the growth of coliforms.

Control measures

As the infection due to this organism is execlusively from human sources, the following will prevent or control the contamination and transmission of this organism.

- Good personal hygiene
- Sanitary food handling practices
- Temperature control
- Restriction of known carriers from food preparation duties
- Use of properly chlorinated water and ice

Shigellae in marine products

As this organism is transmitted due to improper personal hygiene and through media such as water, ice, flies, food contact surfaces etc., the marine products, which require more human handling, are also susceptible to contamination with this organism. Shigellosis is very much less compared to Salmonellosos. Although food-borne, there are a few cases of shigellosis on consumption of marine products. During the period 1964-68, 25 outbreaks of shigellosis were reported in USA. In two of these, fish salads were considered to be the vehicle and the source of contamination was from the worker who prepared the salad. The Centre of Disease Control in USA have reported two incidences of shigellosis on consumption of fishery products in 1980 and in one of these cases, a food handler has been shown as the source of infection. During 1984, there was an incidence of shigellosis in Netherlands due to the consumption of cooked frozen shrimp resulting in the death of 14 persons. But so far there is no reported incidence of shigellosis in India due to the consumption of marine products. The Central Institute of Fisheries Technology, Cochin collected and analysed some marine products during 1984-85 to see whether our seafood are contaminated with shigellae. The samples were drawn from different fish processing factories at Cochin. The samples were analysed for shigella as per the method suggested by the International Commission on Microbiological Specifications for Foods (ICMSF, 1978). The details of the samples collected and results obtained are given in Table 1. It is seen from the table that all the samples tested were free from this pathogen. The results further indicate that Shigella is not, so far, a serious problem with regard to our marine products.

TABLE 1 Details of seafood samples tested for *Shigella*

Type of sample	Number of samples tested	Result
Frozen shrimp PD	78	*Shigella* absent
Frozen shrimp HL	06	"
Frozen raw lobster tails	17	"
Frozen boiled clam meat	11	"
Frozen crab meat	03	"
Frozen cuttle fish	02	"
Raw shrimp PD	11	"
Total	118	*Shigella* absent