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# Public Health Risks of Seafood Associated Bacterial Intoxication: An Overview

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**Abstract**

Foodborne diseases comprehend a wide spectrum of illnesses and are also a raising public health problem world wide. A disease caused by consuming contaminated foods and drinks. Innumerous microbes and toxic substances can contaminate foods. There are more than 250 known foodborne diseases till date. The majorities is infectious and are caused mainly by bacteria, some viruses, and parasites as well. Other foodborne diseases are also caused by chemicals, toxins, contaminating the food. Foodborne illness has been defined by the World Health Organization (WHO) as a disease of infectious or toxic nature caused by, or thought to be caused by, the consumption of food or water [1]. The WHO estimates that worldwide foodborne and waterborne diarrheal diseases taken together kill about 2.2 million people annually. The Centers for Disease Control and Prevention (CDC) estimates 48 million people suffer from foodborne illnesses annually, resulting in about 128,000 hospitalizations and 3,000 deaths.Too often, outbreaks of foodborne disease go unrecognized or unreported or are not investigated. Almost all foodborne microbes and toxins enter the body through the gastrointestinal tract andoften cause the primary symptoms.The frequently observed symptoms may include vomiting, nausea, abdominal cramps and diarrhea.

**Keywords:** Foodborne; Seafood; Bacteria

## Introduction

The main causes of foodborne illness are bacteria (66%), chemicals (26%), virus (4%) and parasites (4%). The two most common types of foodborne illness are intoxication and infection [2]. For the global estimates, 31foodborne hazards causing 32 diseases are included, being 11 diarrheal disease agents (1 virus, 7 bacteria, 3 protozoa), 7 invasive infectious disease agents (1 virus, 5 bacteria, 1 protozoan), 10 helminths and 3 chemicals (ref). Foodborne illness is a potential concern for many different types of foods, including seafood. The association between seafood exposure and illness is considered very much higher than other foods because specific symptoms are linked to certain types of the seafood and because of the early onset of symptoms. Toxins from the fish, shellfish are the most common cause of bacterial and chemical intoxications. Pathogens may be present at low levels when fish or shellfish are harvested, and others may be introduced during handling and processing or by unsanitary practices. This reinforces reporting of seafood borne illnesses.

Foodborne illnesses and intoxications are commonly categorized into two main categories:

1) Infections of the gastrointestinal tract by microbial pathogens

2) Intoxications resulting from consumption of preformed toxins or toxin precursors in foods [3].

During storage, indigenous spoilage bacteria will outgrow the indigenous pathogenic bacteria; normally the fish will spoil before becoming toxic due to the presence of huge amounts of pathogens. Pathogenic species of bacteria can be introduced into aquaculture ponds and coastal regions by human waste and animal manure and are usually found in fish and shellfish; especially crustaceans after the catch at fairly low levels.

### Seafood and public health

The connection between seafood and health is undeniable. Seafood contains mainly proteins and fat, a very low content of carbohydrates and fiber. It is an important part of a healthy diet and becoming the food of choice for the health-conscious. Doctors, nutritionists, and federal agencies recognize that seafood is indisputably a healthy part of human diet. Globally, seafood provides more protein than cattle, sheep, or poultry. It prominently includes fish, shellfish (mollusks and crustaceans). Nutritionists have known for decades that seafood is a low-fat source of high-quality protein and is the best dietary source of omega-3 fatty acids. Seafood is consumed all over the globe; it provides the world's prime source of high-quality protein: 14-16% of the animal protein consumed worldwide. Over one billion people rely on seafood as their primary source of animal protein [4]. The health benefits of eating seafood make it one of the best choices for children, active adults, and the elderly.

### Seafoodborne illness

Seafoodborne illness, or seafood poisoning, occurs mainly by human consumption of food harvested from the sea. This includes, but is not limited to, finfish and shellfish. Seafood and its products are responsible for a significant proportion of foodborne diseases worldwide. A number of bacterial illnesses may arise from the consumption of seafood that has either been contaminated at source or which becomes contaminated during the processing and packaging. Such illnesses may arise from infection with the bacteria




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**Table 1**: Ranking of food safety hazards.

| Ranking | Hazards | Relative Risk |
|---|---|---|
| **1** | Microbial content | 1,00,000 |
| **2** | Pollutant chemicals | 100 |
| **3** | Natural toxins | 100 |
| **4** | Pesticide residue | 1 |
| **5** | Food additive | 1 |

Source: Khora, 2015.

themselves or by the ingestion of toxins formed in the foodstuff prior to consumption. These toxins may occur naturally, may be chemical or biological contaminants, or may be metabolic products of infectious agents that are present in the food. Seafood is involved in an estimated 11% of foodborne outbreaks in the United States, 20% in Australia and over 70% in Japan, whose population has a huge tradition of eating raw seafood [5].

Foodborne illness often called food poisoning, it is caused by several pathogens or certain chemicals present in ingested food. Bacteria, viruses, and protozoa are mainly responsible for the illness.Some chemicals that can cause foodborne illness are natural components of food, while some of them may be accidentally added during production and processing either through improper handling or pollution. The main illnesses transmitted by seafood, fall into two categories: Seafoodborne infection and seafood poisoning or seafoodborne intoxication (Table 1) [6].

## Seafood as vehicle for bacteria

Seafood may be a vehicle for many bacterial pathogens. Pathogens may be present at low levels when finfish or shellfish are harvested, and others may be introduced during handling and processing and unsanitary practices. At least ten genera of bacterial pathogens have been implicated in seafoodborne diseases. Shellfish, especially the filter feeding bivalve mollusks (oysters, scallops, mussels, clams, and cockles) can accumulate pathogenic bacteria in the alimentary tract.

## Bacteria associated with seafood illness

Bacteria are the most abundant organism in the sea and are the causative agents of foodborne illness in 60% of cases requiring hospitalization [7]. They are diverse and participate in food webs. They can infect shellfish, finfish, turtles and mammals of the seas. Human can also be vulnerable if they eat infected seafood. The bacteria may cause a range of diseases in humans. A number of bacterial illnesses may arise from the consumption of seafood that has either been contaminated at source or which becomes contaminated during the processing.

Seafood includes finfish (salmon and tuna), marine mammals (seal and whale), and shellfish, crustaceans (shrimp, crab, and lobster) and mollusks (oysters, clams, and mussels) (Table 2).

## Seafoodborne bacterial intoxication

Seafood is one of the most perishable foods. Spoilage begins once the animal dies, normal defense mechanisms stop working, and a series of changes caused by bacteria, enzymes, and chemical actions start to cause spoilage.Seafood toxins cause a variety of illnesses of humans and animals in many areas of the world [3,8,9]. The risks that are associated with seafood consumption can be separated into two broad categories, those of foodborne illness and chronic illness.

A foodborne infection is when a person eats food containing harmful microorganisms, which then grow in the intestinal tract and cause illness. Some bacteria, cause foodborne illness via an infection. The foodborne bacteria that cause infection are: *Bacillus cereus, Clostridium botulinum*, *Clostridium perfringes*, and *Staphylococcus aureus*. These pathogens cause infection eitherby invading and multiplying in the lining of the intestines and/or other tissues or by invading and multiplying in the intestinal tract and releasing a toxin (bacteria only).

Hundreds of different foodborne diseases have been described. Most of these diseases are infections, caused by a variety of bacteria, viruses, and parasites that can be foodborne. It has also been reported that toxins are formed during intensive aquaculture of fish and shellfish [10]. In the USA, seafood ranked third on the list of products that caused foodborne disease between 1983 and 1992 [3,9]. A few bacteria associated with fecal contamination of seafood continue to pose a large-scale health threat through seafood consumption. The developmentofguidelines to minimize fecal contamination of shellfish and harvesting waters has greatly reduced the incidence of enteric bacteria in seafood [11]. Live finfish and shell fish, especially crustaceans may be contaminated with a number of pathogenic bacteria generally found in the aquatic environment such as *Cl. botulinum*, various *Vibrio spp*., Listeria monocytogenes and *Bacillus spp*. However, only the growth of these organisms can be regarded as a hazard. The severity of diseases related to these organisms may be high (botulism, cholera). The pathogenic strains mostly require temperatures > 5°C for growth and they are competing with the normal spoilage flora which proliferate comparatively more rapidly at low temperatures. Thus the products are likely to be spoiled before production of toxinsor development of high numbers of pathogens.

Seafoodborne intoxication is caused by ingesting food containing toxins formed by bacteria which resulted from the bacterial growth in the food item.Seafood includes mollusks (e.g., oysters, clams, and mussels), finfish (e.g., salmon and tuna), marine mammals (e.g., seal and whale), fish eggs (roe), and crustaceans (e.g., shrimp, crab, and lobster). Bacteria in food may cause illness in humans by infection or intoxication. Toxins are produced by harmful microorganisms, the result of a chemical contamination, or are naturally part of a plant or seafood. Some bacteria cause intoxication. Viruses and parasites do not cause foodborne intoxication. The foodborne bacteria that cause intoxication are: *Bacillus cereus*, *Clostridium botulinum*, Clostridium perfringens and *Staphylococcus aureus*. Seafood poisoning or intoxication occurs as a result of human consumption of food harvested from the sea. Foodborne intoxications occur when patients consume pre-formed toxins that are produced by certain types of bacteria when they grow and multiply in the food. *Clostridium botulinum* can produce a potent neurotoxin during growth under anaerobic conditions usually associated with vacuum packed, improperly canned, or fermented products. Bacteria such as *Staphylococcus aureus* can produce enterotoxins that cause foodborne illness.Preventing the growth of these bacterial pathogens is important to prevent infection or intoxication when seafood is eaten.

*Bacillus cereus*, Clostridium perfringens, and *Staphylococcus aureus*can form enterotoxins that cause acute gastrointestinal illness. The illness can typically be seen with *S. aureus* and *B. cereus* intoxication is the onset of nausea, vomiting, and mild diarrhea after

**Table 2**: Systematics of the bacteria responsible for seafood intoxication.

| Toxigenic bacteria | Phylum | Class | Order | Family | Genus | Species | Sporulation | Risky seafood |
|---|---|---|---|---|---|---|---|---|
| *B. cereus* (Frankland and Frankland, 1887) | *Firmicute* | *Bacilli* | *Bacillales* | *Bacillales* | *Bcillus* | *B. cereus* | Heat resistant endospores | Squid, prawn |
| *Cl. botulinum* (van Ermengem, 1896) | *Firmicute* | *Clostridia* | *Clostridial* | *Clostridiaceae* | *Clostridium* | *Cl. Botulinum* | Heat resistant endospores | Meat of blue crab, smoked fish |
| *Cl. perfringens* (Vellion and Zuber, 1898) | *Firmicute* | *Clostridia* | *Clostridial* | *Clostridiaceae* | *Clostridium* | *Cl. Perfringens* | Heat resistant endospores | Oyesters, fish fillets, canned and frozen prawn |
| *S. aureus* (Rosenbach, 1884) | *Coccus* | *Coccus* | *Bacillales* | *Staphylococcaceae* | | *Staphylococcus* | No spore | Oyesters, fish fillets and sefood contaminations from infected person |

**Table 3**: Pathogenesis of bacteria intoxications associated with seafood.

| Toxigenic bacterium | Name of the disease caused | Onset time | Predominant symptoms | Duration | Seafood vehicle | References |
|---|---|---|---|---|---|---|
| *Bacillus cereus* | *B. cereus* Food Poisoning | 10-16 hours | Abdominal cramps, watery diarrhea' nausea | 24-48 hours | Meat of blue crab, smoked fish | Granum et al., 1998 [84] |
| *Clostridium botulinum* | Botulism | 12-72 hours | Vomiting, diarrhea, blurred and double vision, weakness, paralysis | Variable | Oyesters, canned and frozen prawns | Gilbert, 1974[74] |
| *Clostridium perfringens* | Perfringens Poisoning | 6-24 hours | Intense abdominal cramps, watery diarrhea | Usually 24 hours | Squid, prawn | Hailegebreal, 2017 [85] |
| *Staphylococcus aureus* | Staphylococcal Poisoning | 1-6 hours | Sudden onset of severe nausea, vomiting, fever may be present | 24-48 hours | Oyesters, frozen shrimps and seafood contamination from infected person | Loir et al., 2003 [79] |

ingestion of contaminated food. Incubation periods are very short as preformed toxin is present in the ingested food. Intoxication with *Cl. perfringens* has a relatively long incubation period, because the toxin is not preformed but is produced in the gastrointestinal tract. The symptoms may include watery diarrhea and abdominal cramps after ingestion of contaminated food. Duration of symptoms is usually less than 24 h; therefore, many cases are likely going undiagnosed. Diagnosis is confirmed by isolation of the organism in samples of stool, or food. Additionally, *S. aureus* enterotoxin can be detected in food samples, which can be useful in situations in which the organism has been killed in food processing or preparation and therefore cannot be cultured (Table 3 and Table 4).

## *Bacillus cereus* intoxication

Characteristics of etiological agent: *Bacillus cereus species* are Gram positive, motile, facultative anaerobic, ubiquitous bacteria, characterized by their ability to form resistant spore coats. The sizes vary from 1.0–1.2μm by 3.0–5.0μm. There are about 48 known species in the genus *Bacillus* but only *B. anthracis* and *B. cereus* are associated with food poisoning in human. The food poisoning is a result of ingesting heat-stable enterotoxins. These are mesophilic bacteria that produce heat-resistant endosopores and grow between 10˚-50˚C and with an optimum temperature of 35˚C and 40˚C [12,13]. *Bacillus* cereus is commonly present in food production environments by virtue of its highly adhesive endospores, which spread to all kinds of foods. *B. cereus* toxicity ranges from the strains used as probiotics for humans [14] to highly toxic strains reported for food-related intoxications [15-17]. The bacteria cause two types of gastrointestinal disease, the diarrheal and the emetic syndromes, which are caused by very different types of toxins.

The emetic syndrome is caused by emetic toxin produced by the bacteria during the growth phase in the food. The diarrheal syndrome is caused by diarrheal toxins produced during growth of the bacteria in the small intestine [18].

**Public health effects:** Two distinct foodborne disease types, emetic and diarrheal, are associated with *B. cereus*. Both are generally mild and self-limiting. The symptoms of *B. cereus* diarrheal type food poisoning are similar to those of Clostridium perfringens food poisoning. The onset of watery diarrhea, abdominal cramps, and pain occurs 6-15 hours after consumption of contaminated food. Nausea may accompany diarrhea, but vomiting rarely occurs. Symptoms persist for 24 hours in most instances. The emetic type of food poisoning is characterized by nausea and vomiting within minutes to 6 hours after consumption of contaminated foods. Occasionally, abdominal cramps and diarrhea may also occur. Duration of symptoms is generally less than 24 hours. The symptoms of this type of food poisoning are similar to those caused by *Staphylococcus aureus* foodborne intoxication.

**Risky seafood:** *B. cereus* is commonly associated in seafoods like squid and prawn. The *bacillus* spores could sporulate because of the incorrect chilling after finishing the pasteurizing process (unpublished results).

Types of intoxications: *B. cereus* associated foodborne illness occurs as two distinct intoxication syndromes: emetic and diarrheal.

I. Emetic syndrome: Incubation: 0.5-6 hours [19,20].

Symptoms: Nausea, vomiting, malaise, occasionally followed by diarrhea.

II. Diarrheal syndrome: Incubation: 8-16 hours [21].

Symptoms: Abdominal pain, watery diarrhea, occasional nausea.

**Causative toxins:** The organism produces the following enterotoxins which are involved in a food borne intoxication: Two diarrheal enterotoxins: - hemolysin BL enterotoxin, non-hemolytic enterotoxin, and Emetic toxin. The emetic toxin causes emesis (vomiting) which is resistant to heat, pH and proteolysis but not antigenic [22]. The emetic toxin has been named cereulide, and

**Table 4**: Bacterial toxins and their actions causing intoxication.

| Pathogenic bacterium | Name of the toxin | Type of toxin | Syndromes | Intoxication dose | Mechanism of action | References |
|---|---|---|---|---|---|---|
| *B. cereus* | Emetic, diarrheal | Enterotoxin, Exotoxin | Emetic syndrome (vomiting), Diarrheal syndrome (watery diarrhea) | 15 mg i.v. | Symptoms arise by ingestion of a cyclic peptide toxin "cereulide" that is preformed in the food during the growth of *B.cereus* | Keith, 2015 [86] |
| *Cl. botulinum* | BONT A, BONT B, BONT C1, BONT D, BONT E, BONTF, BONT G | Neurotoxin | Infant botulism,Animal botulism | 1.2 ng i.p(for TypeA) | A, B, E :colonization on intestinal tract and produce toxin, C1, D, G: undetermined, E:ingestion of preformed toxin | |
| *Cl. perfringes* | α,β1,β2,ε,ta,tb,δ,λ,θ | Type A (α), Type B (α, β, ε), Type C (α, β),Type D (α, ε),Type E (α, I) | Food poisoning, Gas gangrene, Necrotizing enteritis | 3μg i.v.(for α) | Hydrolyses phopholipids in membranes of red and white blood cells, alteration of cell membrane permiability,protease activity | Granum, 1990 [84] |
| *S. aureus* | SEA, SEB, SEC, SED, SEE, TSST | Enterotoxin | TSST(Toxin shock syndrome), ET(Scaled skin syndrome), ENT(Food poisoning) | 40-60 ng i.v.(for enterotoxin A) | Organism grow and produce enterotoxin resulting diarrhea and vomiting | Addis and Sisay, 2015 [2] |

consists of a ring structure of three repeats of four amino and/or Oxy acids: [d-O-Leu-d-Ala-l-O-Val-l-Val]. This ring structure (dodecadepsipeptide) has a molecular mass of 1.2 kDa, and is chemically closely related to the potassium ionophore valinomycin [23].

Three types of enterotoxins associate with the diarrheal form of the disease. These are: the three component Enterotoxin Haemolysin BL (HBL), the three component Non-Haemolytic Enterotoxin (NHE) and the single component enterotoxin cytotoxin K. After consumption of food containing *B. cereus*, the enterotoxins are released into the small intestine during vegetative growth following spore germination, and by any surviving vegetative cells [24].

**Toxicity:** The lethal dose for enterotoxin (causing emetic syndrome) produced by *B. cereus*is 15 mg i.v. in mice [25]. The infective dose of *B. cereus* ranges from 104 to 1011 cells per gram of food [26].

**Incubation period**: The diarrheal form of *B. cereus* has an onset period of 8-16 h while the emetic form has an onset period of 1-6 hours. Recovery is usually complete in 24 hours [27,28].

**Signs and symptoms:** There are two types of illnesses associated with *B. cereus*. The most common is a diarrheal illness caused by a heat-labile toxin, accompanied by abdominal pain. An incubation period of 4-16 hours is followed by symptoms lasting 12-24 hours. The second type of disease state is an emetic illness caused by a heat-stable toxin, often associated with ingestion of rice that is not properly refrigerated after cooking. This illness is characterized by vomiting and nausea that usually occur within 1-5 hours upon ingestion of the contaminated food. This is sometimes referred as an intoxicant.

**Pathogenesis:** *B.cereus* causes self-limiting (24-48 hours) food-poisoning syndromes (a diarrheal type and an emetic type), opportunistic infections and is associated with clinical infections. The diarrheal form of *B. cereus* food poisoning is characterized by abdominal cramps, watery diarrhea, rectal tenesmus, and, occasionally, fever and vomiting. The emetic form of *B. cereus* food poisoning is characterized by nausea, vomiting, and malaise, occasionally with diarrhea [28]. *B. cereus* can cause wound infections, bacteremia, septicemia, meningitis, pneumonia, central nervous system infections, endocarditis, pericarditis, respiratory infections, and peripheral [27,29].

Infection in immunocompromised individuals can be life-threatening [30]. *B. Cereus* strains which harbor a plasmid bearing B. anthrax-like virulence factors can cause severe pneumonia in immunocompetent people [31]. **Modes of transmission:** The primary mode of transmission is via the ingestion of *B. cereus* contaminated food [32,33].

It forms spores and spreads easily [34]. In hospitals, *B. cereus* can be transmitted *via* contaminated linen [35,36].

**Morbidity and mortality:** Illness caused by *B. cereus* usually lasts less than 24 h. But in a few patients symptoms may last for a longer period [37,38].

**Laboratory diagnosis:** Confirmation of *B. cereus* as the etiologic agent in a foodborne outbreak requires either (1) Isolation of strains of the same serotype from the suspected food (≥ 105 *B. cereus* organisms per gram from food) (CDC, 1994) and feces or vomitus of the patient,

(2) Isolation of large numbers of a *B. cereus* serotype known to cause foodborne illness from the suspect food or from the feces or vomitus of the patient, or

(3) Isolation of *B. cereus* from suspect foods and determining their enterotoxigenicity by serological (diarrheal toxin) or biological (diarrheal and emetic) tests. The rapid onset time to symptoms in the emetic form of disease, coupled with some good evidence, is often sufficient to diagnose this type of food poisoning. MALDI-TOF MS has shown to be a competent tool for the rapid and accurate identification of *B. cereus* with reference to strain id: ATCC 14893 [33].

**Treatment:** Medication such as antibiotics may be prescribed as these are effective against bacterial infections. However, some strains of bacteria have developed a resistance to them, which cancels out their effectiveness. Vancomycin hydrochloride in combination with an aminoglycoside ensures more consistent antibiotic coverage of *Bacillus* species ocular infections. Other drugs that are highly active and likely to be bactericidal include imipenem, ciprofloxacin and gentamicin. Tetracycline, chloramphenicol, clindamycin and

erythromycin have activity against *Bacillus* species.

### *Clostridium botulinum* intoxications

**Characteristics of etiological agent:** *Clostridium botulinum* is Gram positive (Gram variable) anaerobic heat resistant spore bearing, non-capsulated bacilli that is widely distributed in soil, sediments of lakes, ponds and sea. They are highly pleomorphic and usually 5μM×1μm in size. Seven different strains of the bacteria (A-G) are categorized based on serologic specificity and toxins. Most reported human outbreaks are associated with fish and seafood products. Botulism in animals is caused mainly due to type C and D and rarely type A and B. All toxin-producing strains have placed under one of four groups; I, II, III and IV. The group I contains the proteolytics, group II the non proteolytic and group IV serological type *G. Cl. botulinum* type E is most common in seafood and its products is of major concern because it grows at very low temperatures 3˚–5˚C and produces little noticeable evidence of spoilage. Group III consists of type C and D [39].

Publichealth effects: Cl.botulinumproduces one of the most highly toxic substances. Seven toxin types (A, B, C, D, E, F, and G) are responsible for botulism symptoms botulinum toxin type A, a neurotoxin with a high fatality, is about 1,000 times more toxic than tetanus toxin. Types A, B, E, and F are mainly involved in botulism in humans, while types C and D are mainly involved in animals [40].

*Clostridium botulinum* bacterium forms small spores that are resistant to drought and heat and capable of growing into new organisms. Under conditions with little oxygen (anaerobic), botulinum spores can germinate, resulting in the growth of bacteria and the production of the toxin.

Botulism is not transmitted from person to person. Botulism develops if a person ingests the toxin (or rarely, if the toxin is inhaled or injected) or if the organism grows in the intestines or wounds and toxin is released.

**Risky Seafood:** Many of the seafood-associated cases are caused by *Cl.botulinum* toxin type E as a result of eating fermented salmon heads, salmon eggs, and blubber and skin from marine animals (muktuk) [41]. *C. botulinum* type E spores are commonly found in fish and aquatic animals [38]. They can also be found inshellfish, fish intestines and gills and in mud from the sea. They grow and form toxin at a much lower temperature than the other types; they can grow at 5˚C in fish products [42-44].

Types of intoxications:

I. Food-borne botulism is usually spread by consuming food contaminated with the botulism toxin or spores. The genus Clostridium produces more protein toxins than other genera of bacteria [45].

II. Wound botulism occurs when the spores of Clostridum botulinum get into an open wound and are able to reproduce. It is a very rare condition. The toxin is produced at the site of infection and is absorbed later [37].

III. Infant botulism occurs when infants ingest Clostridum botulinum spores.The spores get deposited in the gut and later produce toxin.This is considered as toxicoinfection which usually occur in infants under 6 months. Honey and corn syrup are the food sources of infant botulism. Botulinum toxin can also be produced by *Cl. butyricum, Cl. barati*, and *Cl. argentiniense*, causing infant botulism [46].

IV. Adult intestinal colonization botulism is another form of botulism. It is similar to infant botulism, but occurs in older children and adults with bowel abnormalities such as colitis, intestinal bypass procedures.

**Causative toxins:** *Cl.botulinum* produces a very powerful endotoxin that is responsible for its pathogenicity.The toxin differs from other endotoxins in that it is not released during the life of the organism.It is produced intracellularly and appears in the medium after death, andanalysis of the cell.

The toxin has been isolated as a pure crystalline protein, which can be considered the most toxic substance known. It is a neurotoxin and acts slowly. It has a molecular weight of MW70, 000 and lethal dose for humans is probably 1-2μg [47]. The lethal dose is far below that is required to induce an antibody response.

**Toxicity:** The lethal doses (in mice) for *Cl.botulinum* neurotoxins Type A, B, E and F causing diseases in human are1.2 ng i.p. [48], 0.5 ng [49], 1.1 ng [50] and 2.5 ng i.v. [51], respectively.

**Incubation period:** For Foodborne botulism, it is typically 12-36 hours after toxin ingestion, but in rare cases as early as 6 hours or as late as 10 days after ingesting toxin.

**Signs and symptoms:** Botulism is a serious illness that causes paralysis or muscle weakness, including weakness in muscles needed for breathing. Signs and symptoms of foodborne botulism generally begin between 12 and 36 hours after ingestion of food. But, the start of symptoms can range from a few hours to several days, depending on the amount of toxin ingested. The toxin is produced at the site of infection.

The manifestations are nausea, vomiting and abdominal cramps, blurred or double vision, muscle weakness, drooping eyelids, difficulty swallowing, lethargy, poor feeding, pooled oral secretion and loss of head control. These symptoms appear when the toxin interrupts nerve impulses to the muscles, which paralyzes the muscles. If untreated, paralysis may lead to involve the hands, legs, and muscles of the respiratory tract.Death normally results from respiratory failure.

**Pathogenesis:** *Cl. botulinum* secretes one of the most powerful biological toxins. Its pathogenicity is due to the action of the toxin.The spores of *Cl.botulinum* are heat and acid resistant so they can easily lie dormant in most environments for long periods of time. If the spores are not killed, they will germinate in improperly cooked food and produce toxin.If the toxin is once ingested, it travels to the intestinal tract.It is able to pass through the high acidity of the stomach without any complications. The neurotoxin is not inactivated by gastric acid or proteolytic enzymes. The toxin goes to the peripheral nervous system through the blood and inhibits motor neurons. Once the neuron is compromised by the toxin the cell cannot secrete acetylcholine. As a result, the axon is impaired and the nerve is unable to function.The insertion of the neurotoxin is referred to as "intoxication" rather than an "infection" in the cell. In some cases, the toxin can spread to the central nervous system, but it is very rare.

Among the three types of botulism found (foodborne botulism,wound botulism and infant botulism), foodborne botulism is due to ingestion of pre-formed toxin. Human disease is usually caused by type A,B,E and very rarely F.Type C and D are usually associated with outbreaks in cattle and wild fowl.

Modes of transmission: Ingestion of toxin pre-formed in the food. This may occur when raw or improperly processed foods are stored in low oxygen conditions that allow growth of the organism. Most outbreaks occur due to fault in the preservation process of (e.g. Canning, fermentation, curing, smoking)

Examples of foods involved include vegetables (e.g. Fish and seafood products) (type E). Honey is a common vehicle of transmission of infant botulism.

**Morbidity and mortality:** Duration of botulism is long term and sometimes it may lead to death also [52].

**Laboratory diagnosis:** Diagnosis may be confirmed by the identification and isolation of the *bacillus* or the toxin in the food. Gram positive bacilli may be identified easily by the smear from the food. Typing is done by passive protection with type specific antitoxin. The toxin may sometime demonstrable from the patient's blood.Serological detection of a specific toxin remains the essential procedure for diagnosis in man and animals [53].

A retrospective diagnosis may be made by detection of antitoxin in patient's blood serum.

In recent years new techniques like MALDI-TOF MS have been developed for the identification of this species (*Clostridium botulinum* with reference to strain id: ATCC 19397) from seafood samples [33].

**Treatment:** Gas gangrene is treated with antibiotics and surgical debridement of gangrenous wounds. Tetanus and botulism require antibiotics, antitoxins and additional life-supporting measures. Antibiotic-associated diarrhea is treated by the withdrawal of antibiotics from patients and the use of metronidazole if necessary. *Cl. perfringens* food-poisoning is self limiting. Oral rehydration therapy is used in case of acute food poisoning syndromes, and antibiotics are seldom required.

### *Clostridium perfringens* intoxication

**Characteristics of etiological agent:** Clostridium perfringens is a plump, Gram positive, pleomorphic, capsulated nonmotile *bacillus* with straight parallel sides and rounded ends and spore forming. It is about 4-6μm×1μm in size usually found singly or in chains. Spores are central or subterminal.

Among all the species of Clostridium, Cl.perfringens (Cp) is the most widely distributed pathogen [54]. This species can produce up to 17 different types of toxins.Four of these, alpha, beta, epsilon, and iota, are responsible for the tissue lesions and the host's death [55-58] and are considered to be major toxins.

**Public health effects:** This organism was isolated from 2-3% of the seafood samples from SanFrancisco and Seattle retail markets in USA [59]. The way that *Cl. perfringens* enters the body determines the possible influence it will have about the person. A person can become infected with *Clostridium perfringens* from eating contaminated food. Once in the intestines, these bacteria produce toxins. It is the toxins that cause human illness. Epidemiological evidence suggests that *Cl.perfringens* plays an important role in the pathogenesis of both food-borne and non-food-borne human gastrointestinal (GI) illnesses.

**Causative toxins:** On the basis of type of toxin produced, *Cl.perfringens* strains are classified into five types A,B,C,D and E [60].

*Cl.perfringens* is one of the most prolific of toxin producing bacteria. Among these toxins, four major toxins (alpha, beta, epsilon, iota) are responsible for the pathogenicity.

I. Alpha toxin: The alpha toxin, found in type A strains of *Cl. Perfringens* causes gas gangrene and also hemolysis in infected species. For the mechanism of the alpha toxin of Cl. perfringens, requires zinc for activation, after which the toxin binds to the surface of the host cell, whereby a series of pathways lead to increased permeability in blood vessels [61].

II. Beta toxin: This lethal toxin is found in *Cl. perfringens* type B and type C strains. This toxin also results in necrosis by way of increased blood pressure, which is brought on by the presence of catecholiamine. Beta toxin is vulnerable to being degraded by proteolytic enzymes.The mechanism of action is still unknown, but it is clear that endogenous levels of trypsin in the intestinal tract of humans and mammals act as a defense against beta toxin infection [62].

III. Epsilon toxin: This toxin is produced by type B and type D strains of Cl. perfringens. It is isolated from animals, particularly sheep, goats, and cattle, but rarely from humans. Similar to the other toxins, epsilon toxin creates pores in tissues, which can result in leaked potassium ions and fluid leakage, which leads to greater complications, giving way to the symptoms associated with *Cl. Perfringens* infection [63].

IV. Iota toxin: The iota toxin is produced solely by type E strain of *Cl. perfringens* and is known as an AB toxin.In these types of toxins, one of the domains, A is usually the active portion, while the other domain, B, is the part of the toxin that binds to a receptorsite on the membrane of the host cell [64]. The iota toxin can cause tissue death in infected individuals.

**Toxicity:** The lethal doses (in mice) of Perfringens enterotoxin Alpha, Beta and Delta are 3μg i.v. [65]; <400ng [66] and 5μg i.v. [67], respectively.

Incubation period:Food Poisoning: 8-24 hours,Gas Gangrene: 1-4 days after the injury, but may also start within 10 hours.

**Signs and symptoms:** *Cl. perfringens* are found in soil, in the stool, and in the intestines of healthy people and of animals. Packages of uncooked meat or poultry, preserved fish, sea food frequently contains this species. Spores of Clostridium survive cooking. When the temperature drops back to less than about 140 degrees Fahrenheit, the spores germinate and begin to multiply.Symptoms are caused by a toxin produced by the multiplying bacteria.

The onset of Clostridium food poisoning is sudden, watery diarrhea accompanied by abdominal pain that may range from mild to severe. It does not spread directly from person to person, but someone with dirty hands can introduce Clostridium into food,

where it will germinate and multiply.

**Pathogenesis:** Cl.perfringens produces the following human diseases:

I. **Food poisoning:** Some strains of typeA can produce food poisoning. They are characterized by having heat resistant spores. Stomach pain followed by diarrhea may begin after having contaminated food.

II. **Gas gangrene:** Cl.perfringens type A is mainly responsible for causing gas gangrene. Gas gangrene is also known as Clostridial myonecrosis. The alpha toxin produced by *Cl.perfringens* destroys tissue and generates gas. The alpha-toxin enters into the plasma membrane of the cells and produces holes in the plasma membrane which disrupts the normal cell function and the tissue will begin to decompose from the inside out.All clostridial wound infection does not lead to gas gangrene rather to wound infection. But when the muscle tissues are invaded it may lead to producing gas gangrene.

III. **Necrotising enteritis:** *Cl.Perfringens* type C are mainly responsible for causing this severe and often fatal necrotizing enteritis.This condition is rare, but sporadic cases have been reported from different countries.This disease is said to be caused by the beta toxin characterized by the sudden onset of acute inflammation and necrosis of intestinal mucosa resulting in bloody diarrhea, abdominal cramps and shock [68].

IV. **Billiary tract infection:** Cl.perfringens has been reported to produces two very rare infection of the biliary tract-emphysyematous cholecystitis and postcholecystectomy septicaemia [69].

**Modes of transmission:** There are many direct and indirect methods by means of which *Cl.perfringens* can cause infection

**Food Poisoning:** Food-borne illness acquired by ingestion of large number of *Cl. perfringens* vegetative cells present in the food [70,71]. Food sources are usually cooked meat, vegetables, fish and other seafood which have been stored at ambient temperatures for a long time after cooking.

**Gas Gangrene/ Anaerobic Cellulitis:** Infection can occur through contamination of wounds (fractures, bullet wounds) with dirt or any foreign material contaminated with *Cl. perfringens*.

**Morbidity and mortality:** Most people who suffer from Clostridium perfringens intoxication are uncomfortable, but death is not common. People usually recover in 24 hours or less. It is unknown how deadly a release of purified toxin would be, but any effects will be related to the strain of bacteria used and the amount taken into the body.

**Laboratory diagnosis:** *Cl. perfringens* can be diagnosed by Nagler's reaction in which the suspect organism is cultured on an egg yolk medium 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 lecithinaseactivity [72].

The *Cl. perfringens* can be identified by MALDI-TOF MS by taking some reference strains of the same species [33].

**Treatment:** Laboratories diagnose *Cl. perfringens* food poisoning by detecting a type of bacterial toxin in feces or by tests to determine the number of bacteria in the feces. Oral rehydration or, in severe cases, intravenous fluids and electrolyte replacement can be used to prevent or treat dehydration. Antibiotics are not recommended.

### *Staphylococcus aureus* intoxication

**Characteristics of etiological agent:** *Staphylococcus aureus*isGram positive, non-motile, facultative anaerobic, spherical non-sporing cocci, approximately 1µm in diameter, arranged in grape-like clusters. This cluster formation is due to cell division occurring in three plains with daughter cells lying in close proximity. *S. aureus* is one of the main causes of hospital and community-acquired infections, which can result in serious consequences [73]. Nosocomial *S. aureus* infections affect the bloodstream, skin, soft tissues and lower respiratory tracts.It can be a cause of central venous catheter-associated bacteremia. *S. aureus* is often responsible for toxin-mediated diseases, such as Toxic Shock Syndrome (TSS), scalded skin syndrome and Staphylococcal Foodborne Diseases (SFD) like Staphyloenterotoxocosis or Staphyloenterotoxemia caused by enterotoxins.

**Public health effects:** *S. aureus* have long been recognized as one of the most important bacteria that cause disease in humans. *Staphylococcus aureus* is an important pathogen due to a combination of toxin-mediated virulence, invasiveness, and antibiotic resistance. This bacterium is a significant cause of nosocomial infections, as well as community-acquired diseases. The spectrum of staphylococcal infections ranges from pimples and furuncles to toxic shock syndrome and sepsis. On the other hand, some infections, such as staphylococcal food poisoning, rely on one single type of virulence factor: the Staphylococcal enterotoxins. The symptoms of staphylococcal food poisoning are abdominal cramps, nausea, vomiting, sometimes followed by diarrhea (never diarrhea alone). The onset of symptoms is rapid (from 30 min to 8 hours) and usually spontaneous remission is observed after 24 hours.

**Risky seafood:** Oysters, Frozen marine shrimps are found to begood sources of *S. aureus* [74].

Causative toxins: Many *S. aureus* virulence factors can be described as toxins. Toxins are usually defined as poisonous substances. *Staphylococcus aureus* produces a wide variety of exo-proteins that contribute to its ability to colonize and cause disease in mammalian hosts.

This organism produces 5 serologically different enterotoxins that are involved in foodborne intoxication. They are:

1. *Staphylococcal* enterotoxinA(SEA)
2. *Staphylococcal* enterotoxinB(SEB)
3. *Staphylococcal* enterotoxinC(SEC)
4. *Staphylococcal* enterotoxinD(SED)
5. *Staphylococcal* enterotoxinE(SEE)

Nearly all strains secrete a group of enzymes and cytotoxins which includes four hemolysins (alpha, beta, gamma, and delta), nucleases, proteases, lipases, hyaluronidase and collagenase.

I. **Membrane damaging toxins:** These toxins cause pore formation in the membrane, leading to the efflux of vital molecules and metabolites, and therefore are cytolytic.

II. **Toxins that interfere with the receptor:** *S. aureus* produces a variety of cytolytic toxins. Most are infamous for lysing red and/or white blood cells. Those that lyse red blood cells are called hemolysins. Alpha-toxin is probably the best-known toxin of *S. aureus* independently, alpha-toxin also causes apoptosis in human monocytes, T and B cells [75].

III. **Secreted enzymes:** These toxins degrade host molecules or affect important host defense mechanisms.

Enterotoxins are secreted toxins of ~ 20 to 30 kD that interfere with intestinal function and typically cause emesis and diarrhea. The most famous *S. aureus* superantigen, the 22-kD Toxic Shock Syndrome toxin (TSST), causes toxic shock syndrome (TSS) by stimulating release of IL-1, IL-2, TNF-α, and other cytokines (Todd, 1988).

*S. aureus* beta-toxin is a sphingomyelinase of type C that degrades the sphingomyelin present on the surface of a variety of host cells, leading to cell lysis.

**Toxicity:** The lethal doses (in mice) of *S. aureus*enterotoxin A and Alpha toxin are 40-60 ng i.v. [76,77] and 110 ng i.v. [78], respectively.

**Incubation period:** Onset of symptoms after consuming contaminated food is usually 30 minutes to 8 hours [79]. Colonies of *S. aureus* can be carried for an undetermined amount of time; some individuals may carry it chronically, and some may carry it intermittently [80].

**Signs and symptoms:** *S.aureus* may cause disease due to the production of toxins. Boils, impetigo, food poisoning, cellulitis, and toxic shock syndrome are all examples of diseases that can be caused by this species.

Symptoms and signs of a localized staph infection include a collection of pus, such as a boil, furuncle, or abscess. The area is typically tender or painful and may be reddened and swollen. Staphylococcal food poisoning is a gastrointestinal illness. It is caused by eating foods contaminated with toxins produced by *Staphylococcus aureus*. Food workers who carry *Staphylococcus* and then handle food without washing their hands contaminate foods by direct contact.

Staphylococcal toxins are fast acting, sometimes causing illness in as little as 30 minutes after eating contaminated foods, but symptoms usually develop within three to six hours. Patients typically experience several of the following: nausea, retching, vomiting, stomach cramps, and diarrhea within 24 to 48 hours. The illness cannot be passed to other people and it typically lasts for one day, but sometimes it can last up to three days.

**Pathogenesis:** *S.aureus* expresses many cell surface-associated and extracellular proteins that are potential virulence factors. Staphylococcal diseases may be classified as cutaneous and deep infections, acute toxemia including food poisoning, exfoliative diseases and 'Toxic Shock Syndrome'.

The commonest deep infection is acute osteomyelitis, the majority of which is caused by *Staphylococcus* spp.Staphylococcal food poisoning results when food contaminated with enterotoxin produced by *Staphylococcus aureus*. The types of food generally responsible are meat, fish, milk and milk products.After consumption of food, the organisms grow and produce enterotoxin resulting diarrhea and vomiting within 6 hours.

Stripping of the superficial layers of the skin from the underlying tissues by the exfoliative toxin is the cause for exfoliative syndrome.

The Toxic Shock Syndrome is a multisystem dysfunction (Todd, 1988) caused by *S.aureus* which exhibits symptoms like vomiting, fever, diarrhea, and hypotension.TSST-1 is responsible for most cases of Toxic Shock Syndrome.

**Modes of transmission:** Staphylococcal food poisoning occurs when food is consumed that contains Staphylococcal enterotoxin produced by *S. aureus*. Food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands are regarded as the main source of food contamination via direct contact or through respiratory secretions [81].

**Morbidity and mortality:** Staphylococcal food poisoning is a very common disease whose actual incidence is probably underestimated for many reasons, which includemisdiagnosis, unreported minor outbreaks, improper sample collection and improper laboratory examination [82].

**Laboratory diagnosis:** Direct microscopy with Gram stained smear is useful in case of pus. The organism is readily cultured from nasopharynx or skin, or by culture of suspicious lesions. *Staphylococci* has a characteristic glistening, opaque, yellow to white appearance on blood agar. Patterns of α or β hemolysis may also be visible. Further identification of *staphylococcal* isolates is available using commercial test kits. *S. aureus* isolates may also be identified by phage typing or by 16S ribosomal DNA typing. Serological tests may sometimes be done in the diagnosis of deep infections.Determination of causative agent if present in food sources is done with relevant epidemiological markers- eg: Biotyping, Serotyping, PCR, Phage typing etc.PCR-based techniques are commonly used for typing, as they are easy, fast, and cost-effective.

MALDI-TOF MS can also be used for the accurate detection of the bacteria present in the seafood by taking some references from the same species [33].

**Treatment:** As *S.aureus* is resistant to many of the drugs; appropriate antibiotic should be chosen on the basis of the antibiotic sensitivity tests. Benzyl penicillin is the most effective antibiotic, if the strain is found to be sensitive [83].

## Discussion and Conclusion

Bacterial seafood intoxication refers to the ingestion of toxins (poisons) contained within the seafood, including exotoxins produced by bacteria. All are Gram positive, one is aerobic, cocci, cluster shaped non spore-forming (*Staphylococcus aureus*), the rest three are bacilli but among them one is aerobic and spore-forming (*Bacillus* cereus) whereas, the other two are anaerobic and spore-forming (*Clostridium botulinum* and *Clostridium perfringens*) are predominant seafood intoxicant bacteria.

Out of these, *Cl.botulinum* is indigenous to the aquatic environment. *Cl.perfringens* and *Bacillus cereus* are indigenous to the general environment, whereas, *S. aureus* are from the animal/human reservoir.But all have been associated with seafood safety risks, through actual intoxication. The intoxication dose often varies from species to species as well as considerably for a number of pathogens based on the health of the consumers.

All these bacteria of particular strains produce enterotoxins which cause seafood intoxications.B.cereus produces two types of toxins: diarrheal (hemolysin BL enterotoxin,non-hemolytic enterotoxin) and emetic toxin. *S.aureus* producesfive toxins (SEA, SEB, SEC, SED and SEE). *Cl.perfringes* Produces Enterotoxin (CPE) in the gastrointestinal tract by enterotoxigenic strains of *Cl.perfringens*. *Cl.botulinum* produces potent botulinum toxins (A, B, E, F and G which causes human botulism but toxin C and D cause disease in animals).

Seafood intoxications caused by non-invasive bacteria that secrete toxins while adhering to the intestinal wall are enterotoxigenic *E.coli*, *Vibrio cholera* and *Camphylobacter jejuni*.The other seafood intoxications that follow an intracellular invasion of the intestinal epithelial cells, caused by Shigella and *Salmonella spp*.

Except *S. aureus*, the rest is heat-resistant. Spore forming bacteria may remain long duration in seafood. Botulinumtoxin is neurotoxic and are of the most lethal poisons. Mortality is high up to 60-100% of the affected persons, hence used as Bioweapon. Botulinum toxin type A, B and E are most commonly associated with human intoxications. There are no Botulism outbreaks associated with Shellfish (Mollusks and Crustaceans). There are some resembles of illness caused by *Cl. perfringens* and *B. cereus*. Further, symptomatic similarities to *S. aureus* intoxication (*B. cereus* emetic type) or *Cl.perfringens* food poisoning (*B. cereus* diarrheal type). Toxin-mediated gastroenteritis is generally self-limited, but cause acute illness.

To prevent outbreaks of intoxicated illnesses, it is imperative to keep seafood refrigerated and to ensure proper cooling of hot seafood to refrigerator temperature. Control strategies include monitoring harvest waters, identification and implementation of process controls as well as consumer education.

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