Chapter 7

Orientation to Hazards: Biological I

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Fish and fishery products have gained importance in recent years because of availability of macro and micronutrients. These foods are considered safer in comparison to other meat products. Safety of fish and fishery products is a concern for human health. Various pathogenic bacteria due to unhygienic handling practices may get into the contacts of foods. The failure to prevent raw fishes and shellfish to come in contact with cooked or ready-to eat foods (cross contamination), and lack of proper temperature control are significant factors resulting pathogens to grow and become causative agents for foodborne illness. Bacterial and viral Pathogens are the primary food safety concern with regard to seafood. To prevent the outbreak of foodborne illnesses, it is crucial for food professionals to understand all aspects of biological contaminants from how they grow and reproduce to how they contaminate food and infect humans.

Biological contaminants of food are harmful and hazardous substances of biological origin in the food that can cause foodborne illness when they are consumed. Biological contaminants commonly found in seafood include bacterial pathogens, viral pathogens and parasites. Pathogen contamination and growth is often an important factor in food-borne illness. Pathogenic bacteria can cause illness in human, either by infection or intoxication. Food borne infections are caused by swallowing live pathogens that grow within the body, usually in the intestinal tract. Intoxication is a condition caused by swallowing preformed toxins i.e. toxins produced by microorganisms in the food before it is eaten. Most of the pathogenic bacteria are not present in fish caught from off-shore waters, but contamination occurs during handling of the raw material. Uncontrolled time and temperature conditions are favourable for organisms to grow and multiply at a faster rate. Food can become contaminated at any point during production, distribution and preparation. Everyone along the production chain, from producer to consumer, has a role to play to ensure the food we eat does not cause diseases.

Bacterial Pathogens:

1. *Bacillus cereus*
2. *Campylobacter jejuni and C. coli*
3. *Clostridium botulinum*
4. *Clostridium perfringens*
5. *Pathogenic Escherichia coli*
6. *Listeria monocytogenes*
7. *Salmonella* spp.
8. *Shigella* spp.
9. *Staphylococcus aureus*
10. *Faecal Streptococci*
11. *Vibrio cholerae*

12. *V. parahaemolyticus*
13. *V. vulnificus*
14. *Yersinia enterocolitica*
15. *Aeromonas spp.*
16. *Plesiomonasshigelloides*

Bacillus cereus

This is a common food contaminant and well-known causative agent of food-borne illness. Their infection is not commonly reported because of its usually mild symptoms. A fatal case due to liver failure Food poisoning caused by B. cereus may occur when foods are prepared and held without proper refrigeration for several hours before being served.

B. cereus is an aerobic spore-forming bacterium. It is commonly found in soil, on vegetables, and in many raw and processed foods. Consumption of foods that contain 10^6 CFU /g may result in food poisoning. Foods incriminated in food poisoning outbreaks include cooked meat and vegetables, boiled or fried rice, vanilla sauce, custards, soups, and raw vegetable sprouts as well as fish. Two types of illnesses have been attributed to *B. cereus*. The first is characterized by abdominal pain and diarrhoea. It has an incubation period of 4-16 hours and symptoms that last for 12-24 hours. The second is characterized by an acute attack of nausea and vomiting. It has an incubation period of 1-5 hours. Diarrhoea is not common with the second type of illness. The organism has possible severity of the emetic syndrome which emphasise the importance of adequate refrigeration of prepared food. Because the emetic toxin is pre-formed in the food and not inactivated by heat treatment, it is important to prevent growth and the production of cereulide during storage. Some *B. cereus* strains are known to be psychrotrophic and to have the highest emetic toxin production between 12- and 15°C.

Cooking of seafood does not inactivate the spores of *B. cereus* and would not be the recommended means to control this pathogen. Proper hygiene and appropriate temperature control are needed to prevent B. cereus illness. Effective control measures depend on destruction by a heat process and temperature control to prevent spore germination and multiplication of vegetative cells in cooked, ready-to-eat foods. Avoid holding cooked foods at room temperature; Use quick chill methods to cool foods below 45^0F (7.2^0C) within 4 hours of preparation; Hold/store hot foods above 140^0F (60^0C) until served, and Reheat foods rapidly to 165^0F (74^0C) or above.

Campylobacter jejuni and C. coli

These are Gram-negative, microaerophilic, curved thin rods with corkscrew motility. *C. jejuni* is widely distributed in the intestinal tract of poultry, livestock, and warm-blooded domestic animals. It is a very common and important cause of diarrheal illness in humans. Eating raw seafood is risk factor for sporadic Campylobacter infection. Symptoms include profuse diarrhoea (sometimes bloody), abdominal pain (intensity and duration can be somewhat severe), headache, weakness, and fever. Many infections occur without symptoms. *C. jejuni* is transmitted through: contaminated foods, including raw clams, mussels and oysters; person-to-person contact; and contaminated water. Cross-contamination of foods by dirty food-contact surfaces, including cutting boards and hands, may be the most frequent route of transmission. Profuse diarrhoea, abdominal pain, headache and fever and

meningitis in neonates are major symptoms because of this organism. Infective dose ranges from 500 to 10,000 cells. This organism survives refrigeration and freezing.

Hazards from *C. jejuni* can be controlled by thoroughly cooking seafood and by stressing the importance of proper (and frequent) hand and equipment washing and sanitary food-handling practices. Since the infective dose of *C. jejuni* is thought to be small, time/temperature abuse of food products is not necessary to result in this illness. Although the heat resistance of *C. jejuni* in seafood has not been determined, the reported heat resistance of this organism in other products is low (69). Preventing campylobacteriosis from consumption of raw shellfish depends on protecting shellfish growing waters from fecal contamination.

Clostridium botulinum

C. botulinum is a gram positive, spore-forming rod shaped bacteria that grows in the absence of air. *Clostridium botulinum* is a dangerous food poisoning organism and it produce a very deadly, exotoxin when grows in food. The food poisoning is known as 'botulism''. The spores are highly heat resistant. Eight different toxins such as A, B, C1, C2, D, E, F & G are known to exist. Type- E is present in sea mud and is mostly involved in botulism food poisoning in seafood. Food poisoning is due to the ingestion of toxin. Symptoms develop within 12-24 h of consuming infected food. Nausea, vomiting, fatigue, headache, paralysis, difficulty to talk, double vision and sound in the ear are the usual symptoms. Death occurs due to respiratory failure.

This organism is found throughout the environment and has been isolated from soil, water, vegetables, meats, dairy products, ocean sediments, the intestinal tracts of fish, and the gills and viscera of crabs and other shellfish. These characteristics allow it to survive normal cooking temperatures and to grow in a vacuum-packaged and modified-atmosphere environment. *C. botulinum* produces a powerful neurotoxin that causes botulism. Growth is necessary for *C. botulinum* to produce toxin. Symptoms include diarrhea, vomiting, abdominal pain, nausea and weakness. These are followed by double, blurred vision and dilated, fixed pupils. In severe cases, paralysis of the muscles responsible for breathing can cause death. The type of *C. botulinum* Type E that is most common in fish and fishery products is of particular concern because it grows at temperatures as low as 38 F and produces little noticeable evidence of spoilage. *C. botulinum* Type A is the form of this bacteria that is most common in land-based products. It is a common contaminant on processing equipment. It will grow at temperatures no colder than 50^0F and produces a putrid odour in products in which it grows. However, its spores are much more heat-resistant than the Type E form of the bacteria.

Because *C. botulinum* produces heat-resistant spores and requires the absence of oxygen for growth, botulism has been most commonly associated with improperly canned food (usually home canned). Semi-preserved seafoods, including smoked, salted and fermented fish, have also been identified as causes of botulism.

Hazards from *C. botulinum* can be controlled by inhibiting growth of the bacteria or by destroying it in seafood. Proper thermal processes for canned seafood destroy the bacteria. Heavy salting or drying to reduce the water activity below 0.93 and fermentation or acidification to below pH 4.6, are effective means of preventing *C. botulinum* growth. Maintaining proper storage temperatures alone is not considered an adequate control measure for *C. botulinum* Type E because of its ability to grow at low

temperatures and because of the severity of the illness. Nonetheless, in many products, it is an important second barrier to growth.

Proper processing under hygienic conditions and adequate retorting are suggested as remedy.

Clostridium perfringens

It is a spore forming, anaerobic (oxygen-free growth conditions) bacterium. *C. perfringens* is commonly found in soil, dust, and the intestinal tract of animals. Food poisoning caused by *C. perfringens* may occur when foods such as meat or poultry are cooked and held without maintaining adequate heat or refrigeration before serving. The illness is a self-limiting gastroenteritis with an incubation period of 8-15 hours and duration of 12-24 hours. The symptoms, which include intense abdominal cramps, gas, and diarrhoea, have been attributed to a protein enterotoxin produced during sporulation of the organism in the intestine.

The presence of small numbers of *C. perfringens* is not uncommon in raw meats, poultry, dehydrated soups and sauces, raw vegetables, and spices. Because the spores of some strains are resistant to temperatures as high as 100ºC for more than 1 hour, their presence in foods may be unavoidable. Furthermore, the oxygen level may be sufficiently reduced during cooking to permit growth of the clostridia. Spores that survive cooking may germinate and grow rapidly in foods that are inadequately refrigerated after cooking. Thus, when clinical and epidemiological evidence suggests that *C. perfringens* is the cause of a food poisoning outbreak, the presence of hundreds of thousands or more of these organisms per gram of food substantiates the diagnosis.

Control measures emphasize proper food preparation and storage techniques, especially temperature control. Control measures include: Rapid, uniform cooling of cooked foods of cooked foods to <10ºC (50ºF) within 2-3 hours; Hot holding of cooked foods at or above 60ºC (140ºF); Preventing cross-contamination of cooked foods with bacteria from raw foods by using separate food-contact surfaces for preparing raw and cooked foods items, or by thoroughly cleaning and sanitizing food contact surfaces after being used for raw products; Maintaining food preparation areas so that they are free of soil and dust; Using good personal hygiene methods, and thoroughly washing hands frequently when handling food products, especially after handling raw products and before handling cooked products.

Pathogenic Escherichia coli

E. coli are Gram-negative, rod-shaped, non-spore forming facultative anaerobic bacteria. E. coli are naturally found in the intestinal tracts of all animals, including humans. Most forms of the bacteria are not pathogenic and serve useful functions in the intestine. Pathogenic strains of E. coli are transferred to seafood through sewage pollution of the coastal environment or by contamination after harvest. *E. coli* food infection causes abdominal cramping, water or bloody diarrhoea, fever, nausea, and vomiting. There are *six categories of diarrheagenic E. coli* which include Enterotoxigenic *E. coli (*ETEC*),* Enteropathogenic *E. coli (*EPEC*),* Enteroinvasive *E.coli (*EIEC*),* Enterohemorrhagic *E. coli (*EHEC*,* Shiga toxin-producing *E. coli or* STEC*),* Enteroaggregative *E. coli (*EAEC *or* EAggEc*)* andDiffusely adherent *E. coli (*DAEC*).*

The primary habitat of the organism is intestinal tract of man and animals and its presence in food in food is generally considered as an indication of faecal contamination. Natural water get contaminated with *E. coli* either by direct contact with faeces or by mixing up with sewage. This water, when used for seafood processing, contaminates the product. Similar possibilities arise when the ice used for preservation or the utensils used for processing are contaminated with *E. coli*.

Off-shore water is generally free from E. coli, but its incidence is noted in coastal waters. Fishing in these waters or washing the boat deck and fish containers using coastal water or harbour waters are known to be the major source of contamination with E. coli. -Inadequately cleaned boat deck and containers used onboard trawldrs also act as source of contamination. If the temperature is favourable, the contaminating organism multiplies rapidly and further aggravates the situation. Generally this organism is harmless: Pathogenic strains of E.coli are considered to be harmful.

Hazards from E. coli can be prevented by: heating seafood sufficiently to kill the bacteria, holding chilled seafoods below 40 F, preventing postcooking cross-contamination, and prohibiting people who are ill from working in food operations. The infective dose of E. coli is dependent upon the particular strain from only a few organisms to millions. For this reason, time/temperature abuse of food products may or may not be necessary to result in illness.

a. Maintenance of proper hygiene and sanitation ;

b. Chlorination of process water;

c. Adoption of scientific system or cleaning and disinfection;

d. Avoid fishing from near-shore waters;

e. Avoid washing the catch with coastal/harbour waters;

f. Avoid delay in processing;

g. Reduce the temperature of the material at every stage ie. <4°C.

Listeria monocytogenes

This organism is Gram-positive, micro-aerophilic, non-spore forming, and motile rods. It can survive freezing and thawing. *L. monocytogenes* grows in refrigerated temperatures (even 1°C) and it can survive both acidic and alkaline pH. This is the most heat resistant pathogenic bacteria among non-spore formers.

L. monocytogenes is widespread in nature and has been isolated from soil, vegetation, marine sediments and water. About 1% of human population is known to carry *L. monocytogenes.* Studies have indicated that *L. monocytogenes* is present in various kinds of foods including fish and shellfish. It causes listeriosis in humans. Most healthy individuals are either unaffected by *L. monocytogenes* or experience only mild flu-like symptoms. Victims of severe listeriosis are usually immune-compromised. Those at highest risk include: cancer patients, individuals taking drugs that affect the body’s immune system, alcoholics, pregnant women, persons with low stomach acidity and individuals with AIDS. Severe listeriosis can cause meningitis, abortions, septicemia and a number of other maladies, some of which may lead to death.

The greatest threat of listeriosis is from ready-to-eat products that do not require further cooking at home. *L. monocytogenes* in raw food that will be cooked before consumption is less of a concern to the food industry since the bacteria are killed during cooking. *L monocytogenes* has been isolated from raw fish, cooked crabs, raw and cooked shrimp, raw lobster, surimi and smoked fish. One of its most significant characteristics is its ability to grow at temperatures as low as 31ºF. Hazards from *L. monocytogenes* can be prevented by thoroughly cooking seafood and by preventing cross-contamination once the seafood is cooked. Since the infective dose of *L. monocytogenes* is thought to be small, time/temperature abuse of food products may not be necessary to result in illness.

***Salmonella* spp.**

Salmonella are non-spore forming, mostly motile (exception *S. pullorum* and *S. gallinarum*) facultative — anaerobic, Gram-negative rods. More than 3000 serotypes of this organism are known to exist at present. All serotypes of *Salmonella* can survive freezing at -40°C and also survive for months together at frozen condition (-18°C).

Salmonella is naturally found in the intestinal tracts of mammals, birds, amphibians and reptiles but not in fish, crustaceans or mollusks. *Salmonella* is transferred to seafood through sewage pollution of the harvest environment or by contamination after harvest.

Salmonella are enteric organisms producing enteric fever and food borne gastroenteritis. Food poisoning due to *Salmonella* is known as "Salmonellosis" infants, elderly and the under nourished are more susceptible to the disease and in such individuals salmonellosis is known to occur even from one single cell of *Salmonella*. *Salmonella* food infection causes nausea, vomiting, abdominal cramps and fever. Outbreaks of *Salmonella* food infection have been associated with raw oysters, salmon, tuna salad, shrimp cocktail, stuffed sole and gefilte fish.

Hazards from *Salmonella* can be prevented by: heating seafood sufficiently to kill the bacteria, holding chilled seafood below 40 F, preventing post-cooking cross-contamination and prohibiting people who are ill or are carriers of *Salmonella* from working in food operations. The infective dose of *Salmonella* is thought to be extremely variable, relatively high for healthy individuals and very low for at-risk individuals, such as the elderly or medically compromised. For this reason, illness could result even without time/temperature abuse, but abuse has been a contributing factor in many outbreaks.

Preventive measures are Avoid fishing from polluted waters; Avoid washing with coastal/harbour waters; Avoid sorting the catch on sea beaches; Use only chlorinated water for processing and for ice manufacture; Periodical medical check-up for workers; All contact surfaces/utensils meant for processing need be cleaned and disinfected before and after use; Avoid the workers who are suffering from diarrhoea/vomiting; Before starting the work, all fish handlers may wash and disinfect their hands; Entry of rodents, wall lizards, flies etc., to the processing hall may be avoided

Shigella spp.

Shigella are Gram-negative, facultatively anaerobic, non-sporulating, non-motile, rod shaped bacteria. They are the most difficult enteric pathogens to isolate as not indigenous in foods. The disease caused by *Shigella* is generally known as `shigellosis', which causes mild diarrhea, fever, abdominal cramps and severe fluid loss. This organism are transmitted through food or water contaminated with human

excreta. Shigella is naturally found in the intestinal tract of humans.The organisms pass the acid barrier of the intestine, multiply in the gut and produce ulceration of large intestine followed by dysentery. *S. dysenteriae*causes the most severe illness. Shigella is transferred to seafood through sewage pollution of the coastal environment or by contamination after harvest. The transmission of the organism from one individual to another is by means of contaminated food, water, ice, flies, contaminated contact surface or food handlers who are carriers of this organism. They survive longest when food holding temperatures are 25°C or lower.

Shigella contamination can be prevented by eliminating human waste contamination of water supplies and by improved personal hygiene for people who are ill or are carriers of Shigella and work in food operations. Avoidance of time/temperature abuse, pest control, and Use properly chlorinated water for processing will be help to overcome this organism.

Staphylococcus aureus

Humans and animals are the primary reservoirs for *S. aureus*. *S. aureus* can be found in the nose and throat and on the hair and skin of 50 percent of healthy individuals. However, the bacteria can be found in air, dust, sewage and surfaces of food-processing equipment. *S. aureus* can produce a toxin if allowed to grow in food. The toxin is not destroyed by the cooking or canning processes. *S. aureus* has the ability to grow and produce toxins in food with very little available water (0.85 aw, 10% salt), which would prevent the growth of other pathogens. *S. aureus* food poisoning causes nausea, vomiting, abdominal cramping, watery or bloody diarrhea, and fever.

Hazards from *S. aureus* can be prevented by: minimizing time/temperature abuse of seafood, especially after cooking, and requiring that food handlers engage in proper hygiene.

It is known that contamination of food with coagulase-positive staphylococci could cause food poisoning, as the organism growing in food materials in considerable numbers, secretes exotoxin.

Habitat and source of contamination

The primary habitat of *Staphylococcus aureus* is man. This organism is present in sweat, ear gum, tears, throat, ulcers, boils and nasal cavities. It is known that 50% of human beings are carriers of this organism. Investigations carried out by CIFT have shown that 45% of seafood handlers in the processing units at Cochin are carriers of *S. aureus*.

Fish caught from the open sea doesn't contain *S. aureus*. When the material is taken onboard and handled by workers, contamination takes place. So its presence in seafood indicates lapse in maintaining personal hygiene.

Toxin production

Few Staphylococci per gram of food material may be harmless, but food poisoning outbreak may occur if the product is handled carelessly during processing, so as to allow multiplication of the organism in dangerous proportions. The organism can multiply vigorously and produce toxin at temperature near about room temperatures i.e. 30-37°C. Therefore refrigeration of the material during handling and processing is highly essential in preventing further multiplication and toxin production. The toxin once formed will not be destroyed even at 100°C even though the organisms are killed at this temperature.

It is thus evident that, once sufficient quantity of toxin is formed in food material before its consumption, food poisoning can follow even though the food material is cooked. Foods most likely to be involved in staphylococcal food poisoning are cooked and processed foods having low number of competing micro-organisms. Large number of *Staphylococcus aureus*, usually more than one million organism/g of food material must be present at one time to produce enough enterotoxin. Less than one microgram of toxin is sufficient to cause illness in a sensitive individual. Staphylococcal food poisoning is caused only by certain well defined strains of *S. aureus*; such strains are known as enterotoxigenic strains. Food-borne out breaks due to coagulase-negative strains of Staphylococci are seldom reported. *S. aureus* are known to produce 9 different types of enterotoxins designated as enterotoxin A, B, C1, C2, D, E, F, G and H. This is the most drought resistant pathogenic bacteria and they cannot compete with general bacterial flora.

Disease symptoms

Nausea, vomiting, abdominal pain, diarrhoea and subnormal blood pressure are some of the usual symptoms. It may appear within 1 - 4 h of consumption of contaminated food. Complete cure is possible within 48h.

Effect of freezing and frozen storage

The studies have shown that only 5 - 15% of *S. aureus* cells inoculated to cooked shrimps are killed during freezing at -40°C. However, during frozen storage at -18°C, there is gradual reduction and in 7 months the viability is completely lost.

Preventive measures

a. Adequate control over the health and hygiene of fish handlers

b. Refrigeration (below 4°C) of the material during handling and processing

c. Minimise time/temperature abuse of seafood, especially after cooking.

Faecal *Streptococci*

Faecal streptococci are Gram-positive, facultative anaerobic, non-spore forming non-motile and catalase negative cocci. An emerging zoonotic pathogen which infections generally occur through injuries associated with preparing whole fresh fish for cooking.

Primary habitat and source of contamination are same as in the case of E.coli. One gram of faeces contains 10^8 faecal streptococci, therefore their presence in food product is generally regarded as an indication of faecal contamination. Just like E.coli, faecal streptococci are absent in off-shore water but are present in considerable numbers in coastal waters. Unclean boat deck, utensils, water and ice are the major source of contamination.

Effect of freezing and frozen storage

Faecal streptococci are comparatively resistant to many adverse conditions. About 30% reduction of faecal streptococci takes place during freezing at -40°C, during subsequent storage at -18°C not much of reduction in count takes place even after 2 years of storage. This organism is considered as a better indicator or sanitation than E.coli, because of their resistance to sub-zero temperature.

Symptoms: causes gastrointestinal and respiratory diseases.

Preventive measures: similar to E.coli

Vibrio cholerae

V. cholerae are Gram-negative, coma shaped aerobic, non-spore forming, and motile rods. *V. cholerae* is found in estuaries, bays, and brackish waters. It is naturally occurring and is not necessarily related to sewage contamination. *V. cholerae* tends to be more numerous in the environment during warmer months.

There are a number of types of *V. cholerae*, and these produce very different symptoms. One type, *Vibrio* cholerae 01, initially causes abdominal discomfort and mild diarrhea. As the illness progresses, the symptoms may include: watery diarrhea, abdominal cramps, vomiting and dehydration. Death can occur. Susceptibility to cholera is enhanced in people who have had gastric surgery, take antacids or have type O blood. Outbreaks of this type of cholera have been associated with oysters, crabs and shrimp from the Gulf of Mexico. V. cholerae 01 has also been recovered from Chesapeake Bay waters, although no illness has been reported from that area.

Another type of *V. cholerae* non-01 causes diarrhea, abdominal cramps and fever. Nausea, vomiting and bloody diarrhea have also been reported. The severity of the symptoms is dependant, in part, upon the specific strain. In its most severe form, *V. cholerae* non-01 has resulted in septicemia (blood poisoning) in individuals with medical conditions that weaken their immune systems. The illness has been associated with consumption of raw oysters, but the bacterium has also been found in crabs.

Hazards from *V. cholerae* can be prevented by cooking seafood thoroughly and by preventing cross-contamination once the seafood is cooked. It is the causative agent of cholera. The current definition of V *cholerae* consists of the classical (non-hemolytic) and El Tor (hemolytic) biovars. The El Tor *vibrios*are generally more infectious than the classical *V. choleraeserotypes*and it can survive longer in the environment.

Serotypes

V. cholerae 01 Ogawa, Inaba and Hikojimaand V. cholerae 0139 Bengal

Those strains which are biochemically identical to *V. cholera* 01, but don't posses the 01 somatic antigens are collectively known as non-01 *V. cholerae,* previously known as non-agglutinable *vibrio*s (NAG'S) or non-cholerae *vibrio*s (NCVs). Non-01 *V. cholerae* produce mild diarrhoea. Further, this organism appears to be a hybrid of the 01 strain and the non-01 strain. Hence other serotype is named as "Bengal" for the recognition of the origin of this strain. Unlike *V. cholerae* 01, O139 organism produces a polysaccharide capsule.

Habitat

The only natural habitat of *V. cholerae* is man.

Source of contamination

Contamination occurs through food, water, flies and contaminated hands.

Symptoms

Nausea, vomiting, profuse diarrhoea, etc., develop after an incubation period of 1 – 4 days. The stool resembles 'rice water' and contains mucus, epithelical cells and a large number of *Vibrios.*

Preventive measures

a. Proper disinfection of contact surface.

b. Avoid cross contamination of cooked products

c. Strict personal hygiene of seafood handlers.

Allowable limit - should be absent.

Vibrio parahaemolyticus

V. parahaemolyticus is naturally occurring in estuaries and other coastal areas throughout most of the world. In most areas, *V. parahaemolyticus* is more numerous in the environment during the warmer months and, as a result, most outbreaks in the United States occur during the summer.

The most commonly experienced symptoms of *V. parahaemolyticus* illness include: diarrhea, abdominal cramps, nausea, vomiting and headache. Fever and chills are less frequently reported. The illness has been associated with consuming contaminated crabs, oysters, shrimp and lobster.

Hazards from *V. parahaemolyticus* can be controlled by thoroughly cooking seafood and preventing cross-contamination after cooking. Control of time/temperature abuse is also an important preventative measure.

V. parahaemolyticus is a marine pathogen present in marine and brackish-water environments. It can cause food poisoning when it is consumed in large numbers (more than 10^5/g of Kanagawa-positive strains), along with food materials. This type of food poisoning is more in countries like Japan, where there is a habit of eating un-cooked seafood. In recent years, the incidence of *V. parahaemolyticus* infection has been increasing in many parts of the world, and this has been attributed to the emergence of a new clone of the 03:K6 serotype carrying only the *tdh*gene. The onset of symptoms is within 12 h of eating infected food. They are Gram-negative, rod shaped bacteria which are non-sporulating, halophilic, motile, and oxidase-positive.

Symptoms

Abdominal pain, vomiting, diarrhoea, and fever are the usual symptoms.

Effect of freezing and frozen storage

99% of the organisms are destroyed during freezing at - 40°C and during storage at -

18°C complete destruction takes place within few days. The generation time of these organisms is only 7-10 minutes;

There are two types of *V. parahaemolyticus* are known to exist.

- Kanagawa - positive (Pathogenic)
- Kanagawa-negative (Non-pathogenic)

Isolates from clinical sources are Kanagawa- positive whereas those from marine sources are Kanagawa - negative.

Icing the material immediately after catch, washing with potable water and improvement of hygiene are considered as remedial measures.

Vibrio vulnificus

V. vulnificus is a naturally occurring marine bacterium. *V.vulnificus* requires salt for survival and is commonly isolated at salinities of 7 ppt to 16 ppt. It is primarily found in the Gulf of Mexico, but it has also been isolated from the Atlantic and Pacific oceans. The numbers of the bacterium in the environment are highest during the warmer months of April through October.

The most common symptoms include: skin lesions, septic shock, fever, chills and nausea. Abdominal pain, vomiting and diarrhea are less frequently reported. Death occurs in about 50 percent of the cases. A number of medical conditions make individuals more susceptible to the life threatening effects of this bacterium, including: liver disease, alcohol abuse, cancer, diabetes, chronic kidney disease, immunosuppressive drug or steroid usage, low stomach acidity and AIDS. V. vulnificus sepsis has been associated with the consumption of certain molluscan shellstock.

Hazards from V. vulnificus can be controlled by thorough cooking of shellfish and by preventing cross-contamination once the seafood is cooked. The risk of V. vulnificus infection may also be reduced by rapidly refrigerating oysters from the Gulf Coast during warm-weather months. Individuals in the “high risk” groups should not consume raw molluscan shellfish.

V*ibrio vulnificus*

It is an emerging pathogen, phenotypically similar to *V. parahaemolyticus.*

And is the etiological agent for 3 syndromes.

a. primary septicemia b. skin infections c. acute diarrhoea

Entry through two portals

a. Ingestion of raw sea foods

b. Exposure of skin lesions

Mortality is up to 60%. It is the part of the normal bacterial flora of estuarine and marine waters.

V. vulnificus is Gram-negative, halophilic, lactose-positive, rod shaped bacteria. All strains are pathogenic; infection dose is not known! Infection is associated with the consumption of raw seafood particularly oysters. Ten minutes treatment at 50°C proved adequate to reduce *V. vulnificus* to a non-detectable level. Icing is very effective to reduce the load of the organism. This organism is closely associated with oyster tissues and is not removed fully by controlled purification methods such as UV light assisted depuration No effective means commercially exist for elimination of the health hazard in oyster intended for raw consumption and so, it is advised to avoid raw seafood completely.

Yersinia enterocolitica

Y. enterocolitica is naturally found in soil, water and domesticated and wild animals. Yersiniosis causes diarrhea, vomiting, abdominal pain and fever, often mimicking appendicitis. Outbreaks have been associated with oysters and fish.

Hazards from *Y. enterocolitica* can be prevented by: heating seafood sufficiently to kill the bacteria, holding chilled seafoods below 40 F and preventing post-cooking cross-contamination.

***Aeromonas* spp.**

The genera *Aeromonas* comprise Gram-negative, facultatively anaerobic, oxidasepositive, glucose-fermenting rod-shaped bacteria, generally motile. *Aeromonas* species viz. *A. hydrophila, A. sorbia*and *A. caviae*has been described as emerging food-borne pathogens. Besides gastroenteritis *A. hydrophila*may cause cholera like infections. *Aeromonas spp.* Are natural members of aquatic environments and is commonly found in fish and fish products of all aquatic environments. *A. hydrophila*is very resistant organism and it can survive in food items stored in cold for long period. Oysters have been implicated in food-borne disease. *Aeromonas* associated diarrhoea has been reported from different parts of India. Some *Aeromonas* spp. are psychrotrophs and some others are enteropathogenic. Studies have shown that very high percentage of the isolates from seafood produced hemolysin (79.2%) and cytotoxin (91.7%). Psychrotrophic*Aeromonas* strains are able to grow at 4-5°C and produce toxin in oysters at 5°C. Combination of chilling, salting and/or acidification is effective means of preventing the growth of *Aeromonas.*

Plesiomonasshigelloides

The genera *Plesiomonas*comprise Gram-negative, facultatively anaerobic, oxidase positive, glucose fermenting, rod shaped bacteria, generally motile. It is an emerging pathogen, mostly associated with fresh water and seawater in warm months. This organism is predominantly associated with seafood. *P. shigelloides*was implicated as the causative agent for diarrhoea after consumption of seafood in Hong Kong and USA. It cannot grow at chilled condition, but can survive. Growth can be prevented by chilling, moderate salting/acidification.

Fungal hazards

The fungi associated with foods are generally yeasts and moulds. The greatest concerns for food safety are mycotoxins e.g. aflatoxin, fusarin, patulin, etc. which are produced by moulds and may be associated with chronic illness, such as cancer. Fungi need lesser moisture for growth compared to bacteria. If the water activity (aw) is less than 0.60 there will not be any growth of fungi or other microorganisms. Water activity of biscuits is 0.30 and sugar is 0.10.

Viral Pathogens

Viral hazards

Viruses contaminate the foods same way as bacteria. It reproduces only within susceptible living cells. A ready to eat food containing a pathogenic virus is a health hazard. Viruses don't reproduce in food; it exists in foods without growing, so they need no food, water or air to survive. Viruses don't cause spoilage but may cause illness. It can survive in human intestine, water, frozen foods etc. for months. Viruses can be found in people who were previously ill. Adequate cooking can destroy it.

Viral Pathogens:

• Hepatitis A Virus

• Norwalk Virus

Hepatitis A

Virus Viruses survive better at low temperatures and are killed at high temperatures. As a result, most outbreaks of hepatitis occur during winter and early spring. Viruses can remain alive for long periods of time in seawater and have been shown to survive over one year in marine sediments.

Both raw and steamed clams, oysters, and mussels have been implicated in outbreaks of hepatitis A. Symptoms of hepatitis A include weakness, fever and abdominal pain. As the illness progresses, the individual usually becomes jaundiced. The severity of the illness ranges from very mild (young children often experience no symptoms) to severe, requiring hospitalization. The fatality rate is low, and deaths primarily occur among the elderly and individuals with underlying diseases.

Hazards from hepatitis A can be prevented by thoroughly cooking seafood and by preventing cross-contamination of cooked seafood. But hepatitis A appears to be more resistant to heat than other viruses. A laboratory study showed that hepatitis A viruses in infected oysters were inactivated after heating at 140 F for 19 minutes. Therefore, mollusks steamed only until the shells open (a common cooking practice) are not exposed to heat long enough to inactivate hepatitis A viruses.

Norwalk Virus

Norwalk virus is considered a major cause of nonbacterial intestinal illness (gastroenteritis). Illness from Norwalk virus has been associated with eating clams (raw and steamed), oysters and cockles. Norwalk virus causes nausea, vomiting, diarrhea, abdominal cramps, and occasionally fever in humans.

Hazards from Norwalk virus can be prevented by thoroughly cooking seafood and by preventing cross-contamination of cooked seafood. Additionally, a recent outbreak has demonstrated that controlling overboard discharge of untreated sewage from shellfish harvesting vessels would reduce the incidence of illness attributable to Norwalk virus.

1. Hepatitis A virus

This virus can survive in seawater and sediments for long periods (over a year). It can also survive better in lower temperature and killed in higher temperature.

Symptoms

Weakness, fever, abdominal pain and the patient gradually get jaundiced.

Thorough cooking is the preventive measure.

2. Norwalk virus

These are considered to be the major cause of nonbacterial intestinal illness (gastroenteritis). They are associated with eating raw and steamed clams or oysters etc. cause nausea, vomiting, diarrhoea, abdominal cramps and fever. This hazard can be prevented by thorough cooking and preventing cross contamination of cooked foods.

Parasites

• *Anisakis simplex*

• *Pseudoterranovadecipiens*

• *Diphyllobothrium latum*

Anisakis simplex

Anisakis simplex, commonly called herring worm, is a parasitic nematode or roundworm. Its final hosts are dolphins, porpoises and sperm whales. The larval (wormlike) stage in fish and squid is usually 18 to 36 millimeters in length, 0.24 to 0.69 millimeters in width and pinkish to whitish in color.

Anisakiasis, the human illness caused by Anisakis simplex, is associated with eating raw fish (sushi, sashimi, lomilomi, ceviche, sunomono, Dutch green herring, marinated fish and cold-smoked fish) or undercooked fish.

Parasites in fish are considered a hazard only in fish that the processor knows or has reason to believe will be served raw or undercooked. In other products, parasites are considered filth but not hazardous. The FDA has established three freezing processes to kill parasites. Freezing and storing at -4ºF (-20ºC) or below for 7 days (total time), or freezing at -31ºF (-35ºC) or below for 15 hours, or freezing at -31ºF (-35ºC) or below until solid and storing at -4ºF (-20ºC) or below for 24 hours is sufficient to kill parasites. FDA's Food Code recommends these freezing conditions to retailers who provide fish intended for raw consumption. Note: these conditions may not be suitable for freezing particularly large fish (e.g. thicker than six inches).

Pseudoterranovadecipiens

Pseudoterranovadecipiens, commonly called "codworm" or "sealworm," is another parasitic nematode or roundworm. The usual final hosts of Pseudoterranova are gray seals, harbor seals, sea lions and walruses. The larval stage in fish are 5 to 58 millimeters in length, 0.3 to 1.2 millimeters in width and yellowish, brownish or reddish in color.

These nematodes are related to Anisakis simplex and the disease associated with infections is also termed anisakiasis. These nematodes are also transmitted to humans through raw or undercooked fish. Control of Pseudoterranova is the same as for Anisakis simplex.

Diphyllobothrium latum

Diphyllobothrium latum is a cestode, or tapeworm, that parasitizes a variety of fish-eating mammals of the northern latitudes. A similar species is found in the southern latitudes and is associated with seal hosts. Cestodes have a structure that allows them to attach to the intestinal wall of their host and have segmented bodies. Cestode larvae found in fish range from a few millimeters to several centimeters in length and are white or gray in color.

Diphyllobothrium tapeworms primarily infect freshwater fish. But salmon and related fish can also carry the parasites. Diphyllobothrium tapeworms are usually found unencysted and coiled in musculature or encysted in viscera. These tapeworms can mature and cause disease in humans. These cestodes are

also transmitted to humans through raw or undercooked fish. Control of Diphyllobothrium is the same as for Anisakis simplex.

Parasitic Hazards

Parasites are organisms that need 'a host to survive, living on or within it. Human unknowingly consume microscopic and small macroscopic animals with their food. The intestinal tract is inhospitable to most of these organisms, which are either digested or evacuated in the faeces. There are two types of parasites that can infect people through food or water. There are generally (a) parasitic worms and (b) protozoan parasites.

Parasitic worms include

1. Round worms (nematodes)

2. Tapeworms (cestodes)

3. Flukes (trematodes)

For most food borne parasites, the food is part of their life cycle. Some parasites may be transmitted through food or water that is contaminated by faecal material shed by infected host.

a. Parasitic Worms

1. Anisakis simplex

A nematode commonly called as herring worms, its final hosts are dolphins, porpoises and sperm whales. Larval stages are in fish and squid. Parasites in fish are considered to be a hazard only in case the fish is eaten raw or undercooked. "Anisakiasis" is the human illness caused by this parasite. In other products parasites are considered as filth but not hazard.

2. Pseudoterranovadecipiens .

Another parasitic nematode commonly called as cod worm or seal worm. The disease caused by this parasite is also known as "Anisakiasis". The transmission and control of this organism is same as that of *Anisakis simplex.*

3. Diphyllobothrium latum

This parasitic cestode primarily infects freshwater fish. This tapeworm can mature and cause disease in human beings. These worms are transmitted to humans through raw or undercooked fish. Proper cooking can destroy it.

4. Clonorchi sinensis, Metagonimusyokagawai and Heterophyesheterophyes

These are members of a large group of trematode parasites with common characteristic of being acquired through eating raw or insufficiently cooked fish. *C. sinensis* can cause inflammatory and fibrotic changes in the bile duct, gall bladder and liver. *M. yokagawai*and H. *heterophyes*are very small flukes (1-2.5 mm in length). They burrow into the mucosa of the small intestine and produce irritation, inflammation, mucus formation and symptoms of diarrhoea and abdominal pain.

b. Protozoan Parasites

These are single celled animals.

1. Entamoeba histolytica can cause severe disease as classical amoebic dysentery which may be fatal if the parasites invade extra-intestinal tissues, such as liver, lungs, or brain.

2. Giardia lamblia associated with diarrhoea, constipation and gastrointestinal pains, rarely invade the tissues.

3. Toxoplasma gondii: Illness caused is "Toxoplasmosis", symptoms are hydrocephalus, blindness in children. These are less severe in adults. Source of infection are under cooked or raw meats i.e. pork, lamb, beef, poultry & cats. Intermediate host is domestic cat.

***4. Cryptosporidium* spp.**

Most frequently diagnosed opportunistic pathogens associated with diarrhoea and wasting syndrome in patients with AIDS. *Cryptosporidium* produces a life-threatening, prolonged cholera like illness in immuno-compromised patients. `Cryptosporidiosis' is acquired after ingesting food or water contaminated with infective *cryptosporidium* oocytes. The mean prevalence of *C. parvum* in Europe and the U.S. is between 1 and 3% and it is considerably higher in developing countries.

Proper food handling can prevent most foodborne illness and diseases. Follow WHO’s five keys to safer food:

1. Keep clean:
   - Thoroughly wash raw fruits and vegetables with tap water.
   - Keep clean hands, kitchen and chopping board all the time.
2. Separate raw from cooked:
   - Do not mix raw food and ready-to-eat food.
   - Do not mix raw meat, fish and raw vegetables.
3. Cook thoroughly:
   - Thoroughly cook all meat, poultry and seafood, especially shellfish.
   - Reheat all leftovers until they are steaming hot.
4. Keep food at safe temperatures:
   - Refrigerate cooked food within two hours of preparation
   - Never defrost food at room temperature. Defrost frozen food in the refrigerator, cold water or in the microwave.
5. Use safe water and raw materials.
   - Use safe drinking water for food preparation.

Check use-by dates and labels while buying packed food