Microbial toxins in seafood

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Introduction

According to the Food and Agriculture Organization (FAO, 2020), global fish production has reached to 179 million tonnes in 2018 with a total value of USD 401 billion. Out of that, 156 million tonnes were used for direct human consumption and remaining 22 million tonnes for non-food uses. Global fish consumption has increased from 9.0 kg percapita in 1961 to 20.5 kg in 2018, by about 1.5 percent every year. The fish consumption accounted for 17 percent of total animal protein, and 7 percent of all proteins, consumed globally (FAO 2020). Live, fresh or chilled fish are the most preferred items and utilized maximum (44 percent) for direct human consumption. The rest of production is processed, with 35% frozen, 11% in prepared and preserved forms, and 10% cured (FAO, 2020). Seafood is one of the most traded food commodities (USD 164 billion) in the world. Nearly, 75% of the seafood was imported by the developed countries in international trade and 50% was exported by developing nations.

Fish is considered as safe and healthy food for consumption. However, it is well known those microorganisms are present on fish surface, skin, gills, digestive tract and internal organs. Several outbreaks were reported in association with bacterial pathogens, biotoxins, histamine, viruses, and/or parasites by the consumption of raw or undercooked fish and fish products (Galaviz-Silva *et al.*, 2009). Both pathogenic and spoilage bacteria can be added to fish at any stage of transportation, handling, processing and storage. According to the U.S. Centers for Disease Control and Prevention (CDC), fish was considered as food category commonly implicated in food borne outbreaks involving single food categories (CDC, 2018). A total of 937 food borne outbreaks associated with fish were reported, resulting in 5,011 illnesses, 364 hospitalization, and four deaths in past ten years in United States (CDC, 2018). The fish and fish products have been continuously implicated in food borne outbreaks, contributing 7% of total confirmed food borne-illness outbreaks over recent years (CDC, 2018). The significant increase in food borne outbreaks may be due to the rise of new nutritional trends which supports the consumption of raw or fresh foods. According to CDC 2014, there are 31 major pathogens are reported which can cause 32 diseases in human. The most common outbreaks associated with consumption of fish is scombroid toxin or histamine, *Salmonella* spp. and *Clostridium botulinum*, *Clostridium perfringens.*

Bacterial Toxin

Food borne illness caused by the pathogenic bacteria is an important concern in seafood. The most common types of food borne illness in human are infection and intoxication. Food borne infections are caused by ingesting live pathogens that develop inside the body, generally in the intestine tract. Intoxication is a condition caused by swallowing preformed toxins i.e. toxins created by microorganisms in the food before it is consumed. Furthermore, a toxic-infection (also known as toxin-mediated infections), is caused by the ingestion of pathogens, which produce biologically active toxins in the small or large intestine. Both gram

positive and gram negative bacteria can able to produce toxins. They can produce even single or multiple toxins. Toxin production as a result of (excessive) microbial proliferation can occur at any point in the food production chain. Even though the bacteria were killed during the food processing steps, the toxin remains resident and biologically active. The toxin production in food is influenced by extrinsic (e.g., temperature, humidity, atmosphere) and intrinsic (e.g., pH, aw, nutrients) properties , cell density, growth phase, cell stress, and injury .The ability of toxins production in humans to cause disease symptoms depends on several factors including strain pathogenicity, quality of toxin produced, physic-chemical characteristics of toxins, interactions with food components, metabolites produced by microorganisms, stability in food and in the human gastrointestinal tract, inherent (sub)clinical dose of toxins, mode of action, effect of acute and (sub)chronic exposure, and targets and receptors in the human body (Rajkovic *et al*., 2020).

Types of Toxins

A bacterial toxin is a protein-based macromolecule that can cause toxic harm to a specific organ of the host (Iriarte *et al.,* 2001). Toxins can be divided into endotoxins and exotoxins:

Endotoxins: These are the components of Gram-negative bacteria's outer membrane; they are the most important antigen of the bacteria, and they are released into the medium during various processess such as lysis and cell division. This endotoxin can able to cause endotoxic shock and tissue damage.

Exotoxins: These are protein-derived macromolecules that the bacterium produces and then releases into the media. Depending on their mechanism of action, exotoxins are classified as follows:

Toxins Type I: These toxins alter the cells of the host's without internalizing in the cells; for example, the superantigens produced by *Staphylococcus aureus.*

Toxins Type II: Within this group there are hemolysins and phospholipases; they cause pore formation and/or membrane destruction in the host cells. The pathogen can penetrate the host cell using this virulence factor. Eg: aerolysin and GCAT protein produced by *Aeromonas* spp.

Toxins Type III: These toxins are known as A/B due to their binary structure. Fraction B binds to the receptor of the cell and fraction A has enzymatic activity, which, depending on the toxin and its mechanism of action, will cause cell damage; for example, the Shiga toxin produced by *Escherichia coli* O157:H7, the Cholera toxin (Ctx) produced by *Vibrio cholerae*, and the Anthrax toxin produced by *Bacillus anthracis*

The exotoxins produced by bacteria play an important role in the pathogenesis of diarrheal illness, inducing excessive liquid secretion without the destruction and death of intestinal mucosal cells. These toxins are generically referred to as enterotoxins (Hernández-Cortez *et al.,* 2017)

Toxins produced by pathogens involved in foodborne diseases are as follows:

- *Bacillus cereus,*
- *Clostridium botulinum,*
- *Clostridium perfringens and*
- *Staphylococcus aureus.*
- *Pathogenic Escherichia coli*
- *Vibrio cholera*

- *Shigella* spp.
- *Yersinia enterocolitica*

Bacillus cereus

Bacillus cereus is one among the *Bacillus* spp. that has been identified as the most frequent cause of foodborne illness. *B. cereus* is commonly found in many raw and unprocessed foods and the presence of low numbers of *B. cereus* in raw foods is regarded normal, while the numbers more than 5 log CFU/g (or per mL) are considered as a hazard to food safety (Sanchez-Chica, *et al.,* 2020). *B. cereus* usually found in rice, pasta, dairy, meat and seafoods. Food poisoning due to this organism may occur when foods are prepared and held without adequate refrigeration for several hours before serving. The *B. cereus* spores can withstand heat processes, and germinated vegetative cells can multiply and produce toxins under ideal conditions. Therefore, in order to inactivate *B. cereus,* suitable time/temperature profile must be developed, which will be often specific for specific foods as well as maintain cold chain due to psychotropic character of some strains of *B. cereus* (Webb *et al.*, 2019).

B. cereus toxins cause two distinctly different forms of food poisoning—the emetic or vomiting type and the diarrheal type. The emetic type is an intoxication caused by the presence of emetic toxin, cereulide, in food. Cereulide intoxication is characterized by the quick onset of symptoms (0.5 to 6 hours), which include nausea, vomiting, and occasionally abdominal cramps and/or diarrhoea, which normally resolve within 24 hours. The Intoxication/infection dose is ca. 10 μg/kg−1 bw, 0.01μg/g[1] of food (produced by *B. cereus* of more than 10^5 CFU/g food, depending on the strain, food and condition. The diarrheal type is produced by the synthesis and release of protein enterotoxins in the small intestine after consumption of viable *B. cereus* vegetative cells and/or spores. Hemolysin BL (Hbl), nonhemolytic enterotoxin (Nhe), and cytotoxin K are known to be implicated in this syndrome. They are all heat labile, pH sensitive, and proteases sensitive proteins, which is why preformed toxins in food typically do not result in foodborne intoxication (Rajkovic *et al.*, 2020). The symptoms of diarrheal type are characterized by 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. The heat toxin stability of diarrheal type is 5 min. at 56 ºC whereas emetic type(cereulide): 90 min at 121 ◦C.

Control measures: Proper hygiene and appropriate temperature control should be maintained throughout the production and storage. Optimization of heat process and temperature control to prevent spore germination and multiplication of vegetative cells of *B. cereus*, quick chilling methods to cool foods below 7.2º C within 4hrs of preparation should be followed.

Clostridium botulinum

Clostridium botulinum is a dangerous food poisoning organism and it produce a very deadly, exotoxin (neurotoxin) when grows in food. The food poisoning caused by this organism is known as 'botulism'. *C. botulinum* is an anaerobic, gram-positive, spore-forming rod-shaped bacteria. The spores of *C. botulinum* are highly heat resistant. Seven different toxins i.e. A to G are known to exist. Nausea, vomiting, fatigue, headache, paralysis, difficulty to talk, double vision and sound in the ear are the usual symptoms. Symptoms develop within 18-36 h of consuming infected food. Death occurs due to respiratory failure. Mortality rate is very high (10 – 50%). This organism is found throughout the environment and found in the intestinal

tract of fish, gills and viscera of crabs and shell fish. It can survive in normal cooking temperature and grows in vaccum packed and MAP. Botulism is the problem in home canned foods or canned foods that are improperly sterilized. Botulism is also reported from smoked, salted and fermented fish.

C. botulinum has four groups, as well as seven antigenic variations of botulinum neurotoxins (A–G). 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. *C. botulinum* type E is most common in seafoods and considered as a major concern because it can grow at very low temperatures 3.3°C and produces little noticeable evidence of spoilage. *C. botulinum*-proteolytic (mesophilic bacteria) belongs to group I, while *C. botulinum*-non-proteolytic belongs to group II (psychrophilic microorganisms). Group I produces heat-resistant spores, which are inactivated by the "Botulinum cook" (121°C/3 min) applied to canned goods with low acid content; neurotoxins generated in this group include A, B, F, and H. Group II produces spores that are moderately heat resistant, and the neurotoxins produced are B, E, and F. Group II can able to grow and produce neurotoxin at refrigeration temperatures, as low as 3.0 ◦C, and is a concern in minimally processed refrigerated foods. Foods involved in botulism are fruits and vegetables, meats, fish, and miscellaneous combined foods (Peck, 2005). Intoxication/Infection dose is 1 µg/kg b.w. orally, for 70 kg man 0.09 to 0.15 µg intravenously or intramuscularly, 0.70 to 0.90 µg inhalationally. The toxin stability is 80ºC for 10 min (function of pH and other factors); exact values are also toxin dependent. Substances in food such as divalent cations and organic acid anions protect the toxin from heat.

Clostridium perfringens

Clostridium perfringens is an anaerobic pathogen which can able to produce several toxins and cause enterotoxic diseases in humans and 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 diarrhea, have been attributed to a protein enterotoxin produced during sporulation of the organism in the intestine. (Toxico-infection)

C. perfringens are estimated to be the second most common bacterial causes of foodborne illness in the US, causing one million illnesses each year. *C.perfringens* strains are classified into seven groups A, B, C, D, E, F and G based on the different toxins it produces (alpha, beta, epsilon, and iota). The alpha, beta, epsilon, and iota, are responsible for the tissue lesions and the host's death and are considered to be major toxins. Alpha toxin: The alpha toxin, found in type A strains of *C. perfringens* causes gas gangrene and also hemolysis in infected species. Beta toxin: This lethal toxin is found in *C. 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. Epsilon toxin: This toxin is produced by type B and type D strains of *C. 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. Iota toxin: The iota toxin is produced solely by type E strain of *C. perfringens* and is known as an AB toxin. The iota toxin can cause

tissue death in infected individuals. Among the seven groups, *C. perfringens* type F is commonly involved in foodborne toxico-infections. *C. perfringens* type F carries the α-toxin gene and the cpe gene and produce CPE (*C. perfringens* Enterotoxin) single polypeptide of approximately 35 kDa upon sporulation, but do not carry the structural genes for β-toxin, ε-toxin, or ι-toxin (Mi, Li and McClane, 2018; Rood *et al.,* 2018). The Infection / Intoxication dose is 10^6 to 10^7 CFU/g of food (ingested vegetative cells produce CPE during intestinal sporulation). The toxins produced usually in the small intestine of the host. The heat stability of toxin is at 60 ◦C for 5 min and pH 5 to 10.

Control measures: Prevention from cross-contamination of cooked foods. Cleaning and sanitizing food contact surfaces after being used for raw products is an effective way to control.

Staphylococcus aureus

Staphylococcus aureus is Gram positive, non-motile, facultative anaerobic, spherical non-sporing cocci, arranged in grape-like clusters. The primary habitat of *Staphylococcus aureus* is man**.** This organism is found in sweat, ear gum, tears, throat, ulcers, boils and nasal cavities**.** Fish caught from the open sea doesn't contain *Staphylococcus aureus* when the material is taken onboard and handled by workers, contamination takes place**.** So, its presence in seafood / food indicates lapse in maintaining personal hygiene

Staphylococcus aureus is considered as one of the major food borne pathogens responsible for food poisoning outbreaks worldwide. They are enterotoxin producing pathogenic bacterium and occurring as commensal flora of humans (Alves *et al.,* 2014). They have a great significance in food industry due to the ability of certain strains to produce heat stable enterotoxin and other virulence factors which are responsible for staphylococcal food poisoning (SFP). (Argudin *et al.,* 2012; Tango *et al.,* 2015). Symptoms of SFP include nausea, violent vomiting, and abdominal cramping, with or without diarrhea within 2-4hr of consumption (Chen *et al.,* 2018). The minimum amount of toxins required to have symptoms is about 1ng/g of food. SFP is widely reported on protein rich foods such as meat, dairy and fish products which have extensive manual handling, inadequate heating and inappropriate storage (Adam and Moss 2007). The bacteria can be killed by heat treatment, but toxin produced is very heat resistant and remain in food even after cooking, which can cause food poisoning.

SEs (Staphylococcus enterotoxins) belongs to a great family of staphylococcal and streptococcal pyrogenic exotoxins, characterized by common phylogenetic relationships, structure, function, and sequence homology. SEs function not only as potent gastrointestinal toxins causing emesis but also as superantigens that stimulate nonspecific T-cell proliferation. (Rajkovic *et al.,* 2020). To date, 26 SEs and enterotoxin-like types have been described: enterotoxins A (SEA), B (SEB), C1 (SEC1), C2 (SEC2), C3 (SEC3), D (SED), E (SEE), G (SEG), H (SEH), I (SEI), J (SElJ), K (SElK), L (SElL), M (SElM), N (SElN), O (SElO), P (SElP), Q (SElQ), R (SER), S (SES), T (SET), U (SElU),W(SElW)), V (SElV), X (SElX), and Y (SElY).Enterotoxins are encoded in prophages, plasmids, or chromosomal pathogenicity islands.

The location of the SE genes on mobile genetic elements presents an additional risk factor in *S. aureus* food intoxication, due to possible horizontal gene transfer (Cafini *et al.,* 2017; Lindsay, 2014). The transfer of genetic elements in *S. aureus* has contributed to strain variability and enhanced virulence. It is well known that *S. aureus* strains usually carry more

than one SE encoding gene. The stability of toxin is SEA: 3 min at 80 ◦C, 1 min at 100 ◦C; SEB 87 min at 99 ◦C. Stable at wide range of pH and resistant to gastric pH.

Control measures: Adequate control over the health and hygiene of fish handlers. The fish has to be maintained at low temperature (below 5°C) during handling and processing. Minimize time/temperature abuse of seafood, especially after cooking

Pathogenic Escherichia coli

E. coli is Gram-negative, rod-shaped, non-spore forming facultatively anaerobic bacteria. It is commonly found in the gut of humans and warm-blooded animals. Pathogenic strains of *E. coli* are transferred to seafood through sewage pollution of the coastal environment or by contamination after harvest. Similar concerns occur if contaminated ice used for preservation or the utensils contaminated with *E. coli.* Improperly cleaned boat deck, and containers used in onboard trawlers can also act be source of contamination. There are six categories of pathogenic *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) and Diffusely adherent *E. coli* (DAEC). Among these Shiga toxin-producing *E. coli* (STEC) has been associated with severe foodborne outbreaks of major public health importance in the last years. STEC produces toxins, known as Shiga-toxins because of their similarity to the toxins produced by *Shigella dysenteriae.* Shiga toxins (Stx) can be divided into two categories: Stx1, which is identical to the toxins produced by *Shigella dysenteriae* 1, and Stx2, which is around 60 % similar to Stx1. Production of one or more Shiga toxins is essential to cause disease, but the production of Stx2 is more closely linked to the severity of the disease such as hemolytic uremic syndrome (HUS) and HC (Farrokh, *et al.,* 2013). STEC strains can be classified as O157 and non-O157. Serotype O157:H7 is the most common serotype involved in severe infections resulting to HUS and HC, and it has been linked to the majority of large-scale outbreaks of STEC infections. Symptoms of STEC are severe diarrhea, stomach cramps, and vomiting. Diarrhea is often bloody without fever. Symptoms typically appear 3-4 days after eating contaminated product, but can range from 1-10 days. STEC can grow in temperatures ranging from 7 °C to 50 °C. A recent study found that *E. coli* O157 strains possess inherent genetic mechanisms which enable growth at low temperatures (< 15 °C), compared to non-pathogenic *E. coli* (Vidovic *et al.*, 2011). Some STEC can grow in acidic foods, down to a pH of 4.4, and in foods with a minimum water activity (a_W) of 0.95.

Control measures: The only effective method of eliminating STEC from foods is to introduce a bactericidal treatment, such as heating (for example, cooking or pasteurization) or irradiation. Basic good food hygiene practices have to be followed during handling and processing of foods.

Vibrio cholerae

V. cholerae are Gram-negative, coma shaped, aerobic, motile rods, non-spore forming bacteria. *V. cholerae* can be divided into two major groups: the cholera-causing strains of serogroups O1 and O139, and non-O1/non-O139 *V. cholerae.* The non-O1 strains do not cause diarrhoea as severe as cholera but they frequently cause extraintestinal infections. The main virulence factor of *V. cholerae* O1 (Ogawa, Inaba, and Hikojima serotypes, Classical and El Tor biotypes) and O139 is CTX toxin (Cholera toxin). It is a potent enterotoxin and causes toxico-infections in humans. It activates the adenylyl cyclase; increases the levels of

intracellular cAMP promoting fluid and electrolytes secretion in the intestinal epithelium, causing diarrhea. This toxin can be identified by the presence of the ctxAB gene. Symptoms includes profuse diarrhea, after an incubation period from 2 h to 5 days; stools have the appearance of rice water, there is dehydration and electrolyte imbalance, which can lead to death. The pathogen is shed in their feces for 7–14 days, which is a very serious source of contamination since it is possible to infect others. The disease is occasionally spread through eating raw or undercooked shellfish that are naturally contaminated.

Control measures: Proper disinfection of contact surfaces. Avoid cross contamination of cooked products and strictly maintain the personal hygiene of seafood/food handlers

***Shigella* spp.**

Shigella belongs to the family Enterobacteriaceae. They are gram-negative, non-motile, and facultative anaerobic bacteria and classified in four serogroups, A (*Shigella dysenteriae*), B (*Shigella flexneri*), C (*Shigella boydii*) and D (*Shigella sonnei*). The disease caused by *shigella* is known as 'shigellosis', and S. *dysenteriae* is responsible for the more severe forms of shigellosis. *Shigella* can be transmitted through direct contact (person-to-person) or indirectly through contaminated food and water, ice, contact surface, files or food handlers who are carriers of this organism. Shigella is naturally found in the intestinal tract of humans. The virulence factor found in Shigella spp., is shiga toxin (Stx), which is commonly found in *S. dysenteriae* serotype 1 and closely resembles Stx in Shiga toxin-producing *Escherichia coli* (STEC). It is a heat labile exotoxin. It acts by inhibition of protein synthesis causing the death of susceptable cells.

Control measures: *Shigella* contamination can be controlled by strictly maintaining the personal hygiene of workers. Good sanitary and handling practice has to follow during food processing or storage. Avoid time/temperature abuse and cold chain should be maintained. Identify and avoid carriers from food operation and monitor for exclusion of pest.

Yersinia enterocolitica

Yersinia enterocolitica is naturally found in a wide range of foods, water, animals, and soil. They are a biochemically diverse group capable of surviving and developing in refrigerated temperatures. In terms of food safety, the ability to multiply at refrigeration temperatures is quite important. It is a gastrointestinal pathogen and cause illness in humans particularly in in young children, are fever, abdominal pain, and diarrhea, which is often bloody. In adults, in addition to symptoms resembling appendicitis, severe parenteral forms may appear, such as erythema nodosum, or micro abscesses in internal organs. It is transmitted via the feco-oral route by the consumption of contaminated food or water. *Y. enterocolitica* can able to produce heat-stable enterotoxins and play a key role in the pathogenesis of yersiniosis (Samoraj, 2022). The invitro conditions required to produce enterotoxin in *Y. enterocolitica* strains are 26 ºC and 37 ºC, pH7-7.5. *Y. enterocolitica* produce enterotoxins after reaching the final part of the small intestine. The Yersinia stable toxins (enterotoxins) produced by *Y. enterocolitica* are biologically and antigenically similar to STX1 (Shiga Toxin I) enterotoxins produced by *E. coli*. Enterotoxins provoke diarrhea, which is the main cause of mortality in yersiniosis

Detection Methods

The toxins produced by the bacteria are the most important virulence factor of foodborne pathogens and a major contributor of foodborne related diseases. They are proteins

or peptides that vary from one another in terms of their size, structure, toxicity, toxicological end points, solubility, and stability, primarily in relation to the types of food matrix. These differences influence the characteristics of required detection methods. The commonly used methods used for detection and quantification methods for toxins in foods are bioassay method (whole animal assay and cell culture assay), immunological method (Enzyme-linked immunosorbent assays and reversed passive latex agglutination assay), mass spectrometry, and molecular assays.

References

Adams, M, R., Moss, M. O., Food Microbiology, third ed., RSC, Cambridge, 2007.

Alves, D, R., Gaudion, A., Bean J, E., Perez Esteban, P., Arnot, T, C., Harper, D, R., Kot, W., Hansen, L. H., Enright, M, C., Tobias, A., Jenkinsa, A. 2014. Combined use of bacteriophage K and a novel bacteriophage to reduce *Staphylococcus aureus* biofilm formation. Appl Environ Microb 80:6694–703.

Argudın MA, Mendoza MC, Gonzalez-Hevia MA, Bances M, Guerra B, Rodicioa MR. 2012. ´ Genotypes, exotoxin gene content, and antimicrobial resistance of *Staphylococcus aureus* strains recovered from foods and food handlers. Appl Environ Microb 78:2930–5.

Cafini, F., Thi Le Thuy, N., Román, F., Prieto, J., Dubrac, S., Msadek, T., & Morikawa, K. (2017). Methodology for the study of horizontal gene transfer in Staphylococcus aureus. Journal of Visualized Experiments 121, 1–7.

Centers for Disease Control and Prevention (CDC). (2018). National Outbreak Reporting System (NORS). Retrieved from https://wwwn. cdc.gov/norsdashboard/

Chen, D., Song, Q., Xu, Z., Zhang, D., 2018. Characterization of enterotoxin A-producing Staphylococcus aureus., Infection and Drug Resistance, Page 531–538.

FAO. 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. Page: 2-8. https://doi.org/10.4060/ca9229en

Farrokh, C., Jordan, K., Auvray, F., Glass, K., Oppegaard, H., Raynaud, S., Thevenot, D., Condron, R., De Reu, K., Govaris,A., Heggum, K., Heyndrickx, M., Hummerjohann,J., Lindsay, D., Miszczycha, S., Moussiegt, S., Verstraete, K.and Cerf, O. (2013) Review of Shiga-toxin- producing *Escherichia coli* (STEC) and their significance in dairy production. Int. J. Food Microbiol., 162: 190-212

Galaviz-Silva, L., Goméz-Anduro, G., Molina-Garza, Z. J., & Ascencio-Valle, F. (2009). Food safety Issues and the microbiology of fish and shellfish. Hoboken, NJ: John Wiley & Sons

Hernández-Cortez, C., Palma-Martínez,I., Uriel Gonzalez-Avila, L., Guerrero-Mandujano, A., Colmenero Solís, R. and Castro-Escarpulli., G. 2017 Food Poisoning Caused by Bacteria (Food Toxins). Poisoning-From Specific Toxic Agents to Novel Rapid and Simplified Techniques for Analysis (eds. Ntambwe malangu).Pp 33-72 Intech open UK http://dx.doi.org/10.5772/intechopen.69953

Iriarte MJ, Ugarte JC, Salazar L. Utility of the airbone endotoxins assay in different occupational environments as a risk indicator by biological agents. Mapfre Medicina. 2001;**12**(4):234-240

Lindsay, J. A. (2014). Staphylococcus aureus genomics and the impact of horizontal gene transfer. International Journal of Medical Microbiology, 304(2), 103–109.

Mi, E., Li, J., & McClane, B. A. (2018). NanR regulates sporulation and enterotoxin production by Clostridium perfringens type F strain F4969. Infection and Immunity, 86, e00416–18.

Peck, M. V. (2005). Clostridium botulinum. In M. Griffiths (Ed.), Understanding pathogen behaviour: Virulence, stress response and resistance (pp. 531–548). Amsterdam, The Netherlands: Elsevier. https://doi.org/10.1533/9781845690229.4.531

Platt-Samoraj, A. ToxigenicProperties of Yersinia enterocolitica Biotype 1A. 2022. Toxins, 14, 118.https://doi.org/10.3390/ toxins14020118

Rajkovic, A., Jovanovic,J., Monteiro, S., Decleer, M., Andjelkovic, M., Foubert, A., Beloglazova, N., Tsilla, V., Sas, B., Madder, A., Saeger, S ., Uyttendaele, M., (2020) Detection of toxins involved in foodborne diseases caused by Gram-positive bacteria *Compr Rev Food Sci Food Saf.* 2020;19:1605–1657.

Rood, J. I., Adams, V., Lacey, J., Lyras, D., Mcclane, B. A., Stephen, B.,Immerseel, F. Van (2018). Expansion of the Clostridium perfringens toxin-based typing scheme. Anaerobe, 53, 5–10.

S. Vidovic, A.K. Mangalappalli-Illathu, D.R. Korber Prolonged cold stress response of Escherichia coli O157 and the role of rpoS International Journal of Food Microbiology, 146 (2011), pp. 163-169

Sánchez-Chica, J., Correa, M. M., Aceves-Diez, A. E., & Castañeda- Sandoval, L. M. (2020). A novel method for direct detection of Bacillus cereus toxin genes in powdered dairy products. International Dairy Journal, 103, 104625

Tango, C. N., Hong S., , Jun Wang, and Deog-Hwan Oh., (2015). Assessment of Enterotoxin Production and Cross-Contamination of Staphylococcus aureus between Food Processing Materials and Ready-To-Eat Cooked Fish Paste. Journal of Food Science. Vol. 80, (12). 2015 page no. 2911-2916.

Webb, M. D., Barker, G. C., Goodburn, K. E., & Peck, M. W. (2019). Risk presented to minimally processed chilled foods by psychrotrophic Bacillus cereus. Trends in Food Science and Technology,93(9), 94–105.