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BACTERIA OF PUBLIC HEALTH SIGNIFICANCE

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Seafood is generally considered microbiologically safe when cooked and offers several health benefits including reduction of cardiovascular diseases, contribution to improving bone strength and congenital developments in infants, reduction of joint pains and inflammations etc. However, when the seafood is consumed in raw form such as fresh, live, partially cooked etc, despite having these advantages, are associated with foodborne illness. Rapid industrialization has resulted in the release of sewage and other industrial effluents into natural water bodies, increasing the chances of seafood borne diseases. The seafood-borne outbreaks are mainly caused by bacteria, viruses, and parasites. The major risk recognised for the contamination of seafood by pathogenic bacteria is by the exposure of food chain to contaminated water. The water runoff from polluted areas such as waste waters from agricultural, industrial and sewage will significantly change the microbial flora of the harvesting water bodies and culture ponds which will result in the contamination of seafood with pathogens like*, pathogenic E. coli,* S*almonella, Campylobacter etc.* or viruses such as Hepatitis A, Norwalk etc. The consumption of raw or partially cooked seafood especially bivalve molluscs can be one of the major contributing factors for the spread of seafood borne pathogens. Another reason for the spread of contaminating pathogens in seafood is the poor personal hygiene of workers and food handlers. Inadequate storage temperature and use of poor-quality raw material in the preparation of seafood etc will increase the risk of illness due to bacteria. Many of the pathogens grow rapidly at room temperature. Fish or fishery product left at ambient temperature is easily spoiled and contaminated with pathogens. This chapter covers the details of major seafood borne bacterial pathogens including emerging pathogens that are causing serious threat to food safety measures.

Salmonella

Infection caused by *Salmonella* continues to be the major cause of seafood borne outbreaks globally. The main sources of contamination are associated with raw oyster, salmon, tuna, value added products of tuna, sole etc. Infection due to *Salmonella* causes gastrointestinal disease and typhoid fever in human. *Salmonella* induced seafood borne outbreaks are reported from several countries worldwide. Non typhoid serovars are generally associated with seafood borne outbreaks. It was reported that USA alone contributes about 1 million cases of

food borne non-typhoid disease globally. In India, the prevalence of *Salmonella* is ranged between 30.5% in fish to 34.1%. The prevalence rates were low in cold temperate regions such US, Spain and Mexico, ranging from 1.5% to 16.4%. The major serovars of *Salmonella* reported from seafood samples of fishing harbours and fish markets in Cochin (India) were *S. Weltevreden, S. Rissen*, *S. Typhimurium* and *S. Derby. Salmonella* infection occurs either through the contact with infected animals, or through the consumption of contaminated seafoods.

Pathogenic *Escherichia coli*

Escherichia coli are a commensal bacterium commonly in the intestinal tracts of warm-blooded animals including humans. Hence, the presence of this bacterium in food products indicates faecal contamination. There are around 700 serotypes of *E. coli* that are generally non-pathogenic in nature, however, there are certain pathotypes that are pathogenic to human being; entero-hemorrhagic *E. coli (EHEC),* enteropathogenic *E. coli* (EPEC), entero-toxigenic *E. coli* (ETEC), enter invasive *E. coli* (EIEC) entero-aggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and Shiga toxin-producing *E. coli* (STEC). This classification is based on their O:H antigen types, virulence characteristics and clinical syndromes. ETEC causes gastroenteritis in humans and low dose of toxin production is sufficient for the excessive fluid secretion and diarrhoea in humans as well as in infants. EPEC causes infantile diarrhoea and the outbreak is mostly seen in least developed countries due to the poor sanitation and hygiene habits. The STEC is highly virulent and is grouped under enterohemorrhagic *E. coli* (EHEC). *E. coli* O157:H7 of EHEC category cause diarrhoea and hemolytic uremic syndrome (HUS) in humans and several infections have been reported in many parts of the world. Virulence in STEC is due to the presence of virulence genes such as either *stx1,* or *stx 2,* and both*, ehxA* and *eae* genes. The minimal dose of less than 100 cells is able to cause food poisoning in humans.

Staphylococcus aureus

Staphylococcal food borne illness is due to the consumption of food contaminated with membrane-damaging, invasive, one or more staphylococcal toxins. The presence of Enterotoxigenic *S. aureus* in fishery products and fish processing environments have been reported from India (Murugadas, 2017). Infection due to Methicillin resistant *Staphylococcus aureus* (MRSA) is mostly hospital acquired and the high prevalence of this bacterium in health care sector is reported from all over the world. There are only two incidences of food borne outbreaks due to MRSA. MRSA outbreak that resulted in mortalities was reported from Netherlands where banana was implicated as the source of infection. The ingestion of contaminated shredded pork barbeque and coleslaw resulted in food poisoning outbreak due to MRSA in United States. The prevalence of *S. aureus* in Indian seafood ranged from 9 % to 23 % during the period from 1985 to 2016.

Vibrio parahaemolyticus

The food borne outbreaks caused by *Vibrio parahaemolyticus* are associated with consumption of raw, partially cooked seafood especially bivalve mollusc. This bacterium was first reported as an entero-pathogen in a food borne diseases in Japan in 1950 due to the consumption of partially cooked sardine. It has been considered as the one of the leading causes of food poisoning agent globally. Food borne illness due to the presence of these bacteria has been frequently. It has been detected in many seafood samples including eel, octopus, squid, shrimp, oyster, sardine, tuna, mackerel, perch, pompano, etc. Most of the environmental strains are non-pathogenic and does not cause any infections. Pathogenic strains are characterized by the presence of haemolysin genes such as *tdh* and/ or trh gene (Okuda, 1997). Most of the pathogenic environmental strains carry *trh* gene whereas presence of *tdh* gene is more in clinical strains that cause infection. Main symptoms of infection include gastroenteritis, wound infection and in rare cases, septicaemia can occur. No dominant serovars were involved in food poisoning until the appearance of O3:K6 pandemic serotype in India in 1996.

Vibrio cholerae

The transmission route of *V. cholerae* to human occurs mainly through aquatic environments particularly water. There are reports of this pathogen in fish and fishery products from several parts of the world. Several cases of rejections of consignments of seafood in international trade due to the presence of *V. cholerae* have been reported. Generally environmental strains are non-pathogenic and do not possess any virulence related genes such as *ctx, zot, ace*, and *tcp*A. The survival and evolutionary dynamics of *V. cholerae* in water causes the emergence of diverse Sero and bio variants of *V. cholerae* due to the gene transfer mechanisms. The horizontal and lateral gene transfer mechanism causes the acquisition of virulence genes, antigenic types such as O1 and O139 etc. Toxigenic *V. cholerae* of classical biotype, had been responsible for infections previously and many epidemic outbreaks were reported in the 19th century which was gradually replaced with an emerging strain of the El Tor biotype in 20th century. Re-emergence of classical biotype together with El Tor strains were reported in Bangladesh during 1982 and these strains were frequently reported in gastroenteritis and diarrhoea from this area until 1993. Another epidemic strain of *V. cholerae* carrying O139 antigen was first reported in 1992 in Southern Asia. The incidence of cholera due to O139 and O1 Biotype El Tor strains gradually increased thereafter in India and Bangladesh. Subsequently, the variant of O1 El Tor (hybrid) which carries *tcp*A classical genes or classical *ctx* A or *ctx* B genes have been reported from clinical cases of cholera from Bangladesh. The non-toxigenic strains of O1 are different in terms of its biochemical and serological properties. Clinical and environmental origin of non-toxigenic strains of O1 has been reported from several countries. However, the non-toxigenic strains lacking toxigenic genes also have the

potential of causing diarrhoea in human. The mechanism of virulence and pathogenicity of this strain remains unknown.

Listeria monocytogenes

Listeria monocytogenes is major concern in lightly preserved food products and the prevalence of this bacterium in considerably increased in ready to eat fishery products. Seafood has the highest risk among the minimally processed products. *L. monocytogenes* enters into seafood by cross-contamination and the presence of this pathogen in seafood has been reported from different seafood products. Prevalence rate of this pathogen in seafood products varies from 0 to 17 %. However, the prevalence in seafood is relatively low compared to other food products such as dairy and other animal products. The mortality rate due to *L. monocytogenes* infection is very high ranging from 20% to 30% in immuno-compromised patients and hence an important public health concern. The symptoms of infection include septicaemia, meningitis, gastroenteritis, pneumonia, and spontaneous abortion. Regulatory agencies such as FDA, ISO, WHO, etc. have included this pathogen in zero tolerant categories in processed food products due to its survivability in wide environmental conditions. This pathogen is able to withstand high NaCl concentration of up to 20%, pH range of 4.3 to 9.8, temperature range of 0.5 to 45°C, and low water activity of 0.91. This pathogen is very well adapted to grow in refrigerated condition, and pose serious risk to the chilled and frozen products once it is contaminated.

***Yersinia* spp.**

The genus *Yersinia* belongs to *Enterobacteriaceae* family. Presently, it comprises of 16 species and two species (*Y. enterocolitica* and *Y. pseudotuberculosis)* are pathogenic to human. *Y. enterocolitica* is widely distributed in aquatic and animal reservoirs with swine serving as a major reservoir. Yersiniosis is caused by *Y. enterocolitica* of which virulence biotypes associated with infections are biotypes 1B, 2, 3, 4, and 5. The spectrum of disease ranges from mild diarrhoea to acute gastroenteritis, enterocolitis and pseudo appendicitis in humans. *Y. enterocolitica* is able to withstand freezing for long period of time and remain viable after extended frozen storage which raises public health concerns in the low temperature preservation and processing of seafood.

Clostridium botulinum

C. botulinum is grouped under Gram positive bacteria, and are anaerobic spore producing bacilli of important public health concern in seafood industry. This bacterium is autochthonous to the aquatic environment and aquatic sediments and forms major reservoir of this pathogen. The toxigenic types of *C. botulinum* belong to type A, B, E and F. The major risk factors in seafood are due to the presence of these toxigenic types. Botulinum food poisoning is due to the consumption food contaminated with preformed toxins of *C. botulinum* and low oral dose of 70 µg is sufficient to causes illness in human. Its prevalence in seafood depends upon

several factors such as topographical location, culture practices, detection methods etc. The fish poses serious risk due to its direct contact with sediment and the ingestion of spores through contaminated feed/sediment. This bacterium is a major concern in packaged seafood products where cold chain is not maintained during storage, transport and distribution chain. The favourable condition for the growth of *C. botulinum* in preserved products such MAP or vacuum-packed products include, pH of about 4.6, water activity of 0.93%, low salt up to 3%, temperature range of 3°C to 50°C.

Emerging pathogens in seafoods

Apart from the well reported seafood borne pathogens, several other pathogens are also emerging throughout the world irrespective of the geographical conditions, and able to cause infectious diseases in the current century. It is not always true that emerging pathogens are a new category of microorganisms; instead it can be already established pathogens in which the virulence or resistance to disease characteristics is high as a result of stressful conditions such as changes in the habitat, climate, overdose of antibiotics etc. It is important to study the time of emergence of particular bacteria of infectious category to the food chain via source tracking and establishment of national network of surveillance system, so that the epidemic spread can be controlled by effective implementation of the mitigation measures and re-emergence can be prevented.

Vibrio vulnificus

Vibrio vulnificus a halophilic bacterium belonging to *Vibrionaceae* and widely distributed in brackish water and marine environments. High concentration of these bacteria can be seen in filter feeding bivalves that inhabits coastal polluted waters. So, the major risk factor for the food borne outbreak is the consumption of contaminated raw or partially cooked shellfishes. Infection can also occur through open wounds and may lead to septicaemia in fatal cases. The fatality rate of *V. vulnificus* infection ranges from 20 to 60%. Recently, this bacterium has emerged as public health significant bacteria due to its high fatality rate all over the world.

***Campylobacter* spp.**

Campylobacter spp. causes gastrointestinal disease termed campylobacteriosis and one of the leading causes of food borne outbreaks in developed countries. Since 2005 to 2019, this bacterium has been implicated in gastrointestinal disease of more than 2, 20,000 people in EU and ranks first in foodborne outbreak followed by *Salmonella* and *Yersinia*. The USA reports 8.45 Lakh cases of *Campylobacter* infection per year. The outbreak is mainly due to ingestion of contaminated food products, where the chicken alone contributes to about 25% of the infections. The incidence of *Campylobacter* spp. have been reported in other types of food animals such as cattle, pig, cows, sheep etc. *Camplyobacter pleridis* and *C. lari* subsp.*concheus* were isolated from shell fish. The *Campylobacter* spp. is a commensal bacterium to poultry and the intestinal tract carry huge amount of this bacterium. The rupture

of intestinal tract while processing can disseminate the content to skin. Cross contamination with shellfish harvesting area and handlers can result in seafood borne outbreak. Shellfish associated campylobacterosis was first reported during 1980s where 28 persons were infected after eating raw clams.

***Cronobacter* spp.**

Cronobacter species belongs to the family *Enterobacteriaceae* and is considered as an opportunistic pathogen in neonates. Among 7 species of *Cronobacter*, three species are pathogenic to human, namely C. *sakazakii*, *C. malonaticus and C. turicensis*. Out of these, *C. sakazakii* causes high mortality rate of about 40-80% in neonates. This bacterium has been isolated from wide range of food sources such as dairy products, plant-based products, dried fish, shrimp, seaweeds and minimally precedes products. This bacterium is considered as an emerging pathogen of seafood recently due to its survivability in low moisture foods such dried fish product. However, the seafood borne outbreak due to this bacterium was not reported so far.

***Arcobacter spp*.**

Arcobacter is an emerging zoonotic pathogen, belongs to *Campylobacteraceae* and is closely related to the Genus *Campylobacter.* They are able to survive in low oxygen condition, and well adapted to temperature of less than 30°C. *Arcobacter* causes bacteraemia, gastroenteritis and diarrhoea. Out of 27 species, three species are major pathogenic strains causing disease, namely *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*. Food borne infection associated with chicken and vegetables have been reported. Seafood borne outbreak due to *Arcobacter* was not reported so far, however reports of isolation of *Arcobacter* from fish, shellfish, and seawater are available.

Vibrio mimicus

Vibrio mimicus is an important emerging zoonotic pathogen in seafood that causes disease in aquaculture fishes as well as gastroenteritis in human. Major reservoir of this pathogen is raw oysters, fish, turtle eggs, shrimps, and cray fish. Davis *et al*. (1981) studied the biochemical characteristics of atypical *V. cholerae* by biochemical tests revealed new species of sucrose negative strain for which the name *Vibrio mimicus* sp. nov. Strain was proposed. *V. mimicus* carrying ctx gene is reported as pathogenic strain that can cause severe watery diarrhoea and gastrointestinal disorders. In India, there were only few reports of this organism from seafoods.

Food safety with respect to seafood pathogens is an important in terms of public health perspectives as over 200 types of diseases are due to the consumption of contaminated foods. (To ensure food safety, routine microbiological screening tests should be validated in real time so that the contaminated food products get detected. National regulations shall be enforced for ensuring food safety that includes the strict implementation of food hygiene and sanitation programme through Hazard analysis and critical control point (HACCP), together with Good

management practices (GMP), standard operating Procedures (SOPs), Sanitation standard operating procedures (SSOPs) practices from production to consumption stages, there by the product becomes safe at all stages of production, processing and distribution levels. The harmonization of these practices in international trade ensures the safety of seafood products, globally.

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