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Invited Review Article

Seafood associated bacterial pathogens of public health significance: A brief review

M. V. Ayyappan[1] and T. C. Joseph[1*]

[1]Microbiology, Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology (CIFT), Cochin – 682 029, Kerala, India

Abstract

Seafood is perishable in nature and offers favourable medium for the growth of microorganism due to the presence of high content of water and nutrients. The absence of hygienic condition during production and processing results in proliferation of microorganisms thereby causing health risk to seafood handlers and consumers. The quality and safety of seafood is directly linked to the consumption of contaminated seafood. The potential hazards of microbial, chemical, or physical origin in farming, production, processing, or distribution stages, can compromise the quality of the product and becomes unacceptable for consumption. This review briefly describes the major seafood borne bacterial pathogens including emerging pathogens that can cause serious threat to food safety.

Key words: Bacteria, Contamination, Emerging pathogens, Quality, Seafood

Highlights

- The consumption of raw or partially cooked seafood causes potential health risk in human.
- Prevalence, pathogenicity and outbreak data of major seafood borne bacterial pathogens is reviewed.
- The emerging bacterial pathogens are enlisted with recent data supporting its occurrence in seafood.
- Preventive measures to illness depend on the extend of adherence to food safety procedures.

Introduction

Seafood is one of the most popular food consumed globally and its increasing demand is expected to grow to a value of $155.32 billion by 2023 (Gbadegesin and Akintola, 2021). The increased demand of seafood had open up the marketing and consumption of ready to eat and ready to prepare products in recent years. The global fish production in 2018 was 178.5 million metric tons (FAO, 2020). The per capita consumption of seafood globally showed a hike from 9.0 kg in 1961 to 20.5 kg in 2018 with an increase of 1.5 percent per year (Sheng and Wang, 2021). It was reported that 88% of the global seafood production were utilized for direct human consumption and over 38% of them were traded internationally (FAO, 2020). Globally seafood provides 20 percent of the per capita intake of animal proteins for around 3.3 billion people (FAO, 2020). Further, the sector provides livelihood to almost 60 million people worldwide (FAO, 2018). The diversified growth in aquaculture production in the Indian seafood sector accelerated the export of marine products to reach a value of Rs. 46662.85 crores during 2019 - 2020 (FAO, 2020). It has been reported that about 60% of the Indian population consumes fish and the per capita fish consumption pattern varies across the states with Tripura, Kerala, and Manipur ranking high with 29.29 kg, 19.41 kg, and 14.1 kg respectively in 2019-2020 (Singh and Basudha, 2021).

*Corresponding Author, E Mail: tomscjoseph@gmail.com

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. (Ronzio, 2003). However, when the seafood is consumed in raw form as fresh, live, partially cooked etc. despite having these advantages, are associated with foodborne illness (Hicks, 2016). The introduction of advanced processing methods and value addition techniques with concomitant improvement in testing and diagnostics of its quality and safety enables the optimum utilization of seafood. The dynamic nature of seafood as well as the hike in seafood consumption increases the risk in foodborne disease outbreak worldwide which further led to the strict compliance of regulatory measures in terms of quality and safety, during marketing of fish and fishery products (Giusti *et al*., 2007). Several authors have reported the role of seafood as one of the major vehicles for the outbreak of foodborne diseases (Angelillo *et al*., 2000; Newell *et al*., 2010; Dumen *et al*., 2020).

There has been an increase in foodborne outbreaks worldwide. Approximately 48 million cases of foodborne diseases were reported annually from USA alone with around 1.28 lakh hospital cases and 3000 fatalities (Scallan *et al*., 2011). Fish was the single food category that was most commonly implicated (17%) in the foodborne outbreaks (Dewey-Mattia *et al*., 2018). In India, out of 37 foodborne outbreaks during 1980 to 2009, 24 of them were related to bacterial contamination in foods (Vemula *et al*., 2012). However, a significant increase in foodborne outbreaks from 2009 to 2018 contributing a total of 2688 cases with 1.5 lakh illness and 572 deaths and with 25 outbreaks due to consumption of seafood (Bisht *et al*., 2020). With the increase in sea food borne outbreaks, awareness regarding hygiene, waste management etc. has increased worldwide. Rapid industrialization has resulted in the release of sewage and other industrial effluents into natural water bodies, increasing the chances of sea food borne diseases (Bukola and Zaid, 2015). The seafood-borne outbreaks are mainly caused by bacteria, viruses and parasites (Iwamoto *et al*., 2010). Developed countries have database on foodborne outbreaks whereas developing countries are yet to have a reliable database due to lack of proper diagnostic facilities, faulty reporting system, inefficient database management and monitoring system (Callejon *et al*., 2015). There are limited reports on sea food borne outbreaks in India and the cases are often underreported (Saraswathi *et al*., 1989; D'Souza *et al*., 2018; Bisht *et al*., 2020).

Based on the ranking system given by Huss *et al*. (2000), seafood falls under three risk categories; high, medium and low. The high-risk category includes raw fish and shellfishes and frozen bivalves. The medium risk category includes the fresh and frozen fish and shell fishes to be consumed after cooking. Low risk categories include minimally processed fish and fishery products such as pasteurized and heat processed products. One of the major risks recognised for the contamination of seafood by pathogenic bacteria is by the exposure of food chain to contaminated water (Amagliani *et al*., 2012). 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 resulting in the contamination of seafood with pathogens like, pathogenic *E. coli, Salmonella, Campylobacter* etc. or viruses such as Hepatitis A, Norwalk etc. (Chua *et al*., 1989). The consumption of raw or partially cooked seafood especially bivalve molluscs can be one of the major contributing factors for the spread of sea food borne pathogens (Nair *et al*., 2007). Another reason for the spread of contaminating pathogens in seafood is the poor personal hygiene of workers and food handlers (Simon and Sanjeev, 2007). 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 (Kumar *et al*., 2015). Many of the

pathogens grow rapidly at room temperature. Fish or fishery product left at an ambient temperature is easily spoiled and can get contaminated with pathogens (Feldhusen, 2000). This review describes the major microbial pathogens and food safety concerns associated with sea foods.

Sea food borne pathogens

Fish and fish products are rich in protein, essential fatty acids, vitamins and minerals which provide favourable conditions for the growth of bacteria. The microflora of seafood comprises both indigenous bacteria such as *Vibrio*s, *Pseudomanas*, *Shewanella* etc. (Parlapani *et al*., 2013) as well as exogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter aerogenes* etc. (Boziaris and Parlapani, 2017; Gufe *et al*., 2019). The human pathogenic bacteria mainly belong to the second category. However, the native microflora of seafood and aquatic environments are reported as causative agents in diseases (Su and Liu, 2007). The presence of such bacteria in seafood reflects the possible risk of transmission to the higher organisms, including humans.

The introduction of certain bacteria to the aquatic environment through anthropogenic activities is mainly associated with foodborne outbreaks (Rippey, 1994). The factor affecting the presence of such pathogens in fish are farming or capture procedures, ecological conditions, processing and distribution protocols followed etc. (Poli, 2005). Some pathogens such as *Yersinia ruckeri* can cause diseases only in aquatic animals (Fernandez *et al*., 2003). However certain bacteria can cause diseases in both aquatic animals and humans, such as *Vibrio* spp. (Igbinosa and Okoh, 2008), *Aeromonas hydrophila* (Daskalov, 2006) etc. The public health significant bacteria of seafood origin can transmit disease to human through fish and fishery products. The major human pathogenic bacteria associated with sea foods are *Vibrio* spp., *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium botulinum*, *Yersinia* spp., *Bacillus cereus* and *Shigella* spp. (Feldhusen, 2000).

***Salmonella*:** Infection caused by *Salmonella* continues to be the major cause of sea food borne outbreaks globally. The main source of contamination is associated with raw oyster, salmon, tuna, value added products of tuna, sole etc (Amagliani *et al.*, 2012; Kumar *et al*., 2015). Infection due to *Salmonella* causes gastrointestinal disease and typhoid fever in human (Hassan *et al.*, 2018). *Salmonella* induced sea food borne outbreaks are reported from several countries worldwide (Brands *et al*., 2005; Iwamoto *et al*., 2010; Barret *et al*., 2017). Non typhoidal serovars are generally associated with sea food borne outbreaks. It was reported that USA alone contributes about 1 million cases of foodborne non typhoidal *Salmonella* infection globally (Hassan *et al*., 2018). Kumar *et al*. (2015) studied the growth dynamics of *Salmonella* in seafood by evaluating expression of genes related to virulence and stress at varying temperatures and reported that *Salmonella* could multiply at ambient temperature and 45°C with increased expression of virulence genes at room temperature. In India, the prevalence of *Salmonella* is high and ranged between 30.5% in fish to 34.1% in calms (Kumar *et al.*, 2009). Prevalence rates of 20.7% have been reported in fish samples from Mumbai, India (Prabhakar *et al*., 2020). A recent study revealed S. *Typhimurium* in 7.9% of the aquaculture farms in Kerala (Greeshma *et al*., 2021). The prevalence rates were low in temperate countries such as US, Spain and Mexico, ranging from 1.5% to 16.4% (Martinez-Urtaza *et al*., 2004; Simental and Martinez-Urtaza, 2008; Setti *et al*., 2009; DePaola *et al*., 2010). 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* (Kumar *et al*., 2009). *Salmonella* infection occurs either

through the contact with infected animals, or through the consumption of contaminated sea foods. Antony *et al.* (2009) reported *Salmonella* Weltevreden outbreak in 34 students from Mangalore, India with fish being source of infection. In international seafood trade, about 1% of all rejections is due to *Salmonella* contamination in variety of seafood products such as fresh, frozen cooked and ready to eat products (Geetha *et al*., 2020). The refusals of import due to *Salmonella* in seafood formed 66% of the total import rejections to US during 2010-2015 (Rao *et al*., 2017).

The inspection practices for the monitoring of bacterial pathogen uses *Listeria* spp. and *Salmonella* spp. as target bacteria for the food products. The optimum cooking period for food including seafood is set with respect to *Salmonella* spp. as a target pathogen where the cooking period for the destruction of *Salmonella* will be sufficient for killing other suspected pathogens in the products (USFDA, 2008). Several countries have set zero tolerance for *Salmonella* spp. in food products including sea foods (Hastein *et al*., 2006)

***Escherichia coli*:** *Escherichia coli* is a commensal bacterium commonly found in the intestinal tract of warm-blooded animals including humans. Hence, the presence of this bacterium in food products indicates faecal contamination (Visnuvinayagam *et al*., 2017). There are around 186 O-types and 57H-serotypes of *E. coli* that are generally non-pathogenic in nature, however, there are certain pathotypes that are pathogenic to human being; enterohaemorrhagic *E. coli (EHEC)*, entero-pathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *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 (Toma *et al*., 2003). 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 (Beatty *et al*., 2006). EPEC causes infantile diarrhoea and the outbreak is mostly seen in least developed countries due to the poor sanitation and hygiene habits (Barlow *et al*., 1999). The STEC is highly virulent and is grouped under enterohaemorrhagic *E. coli* (EHEC). *E. coli* O157:H7 of EHEC category cause diarrhoea and haemolytic uremic syndrome (HUS) in humans (DeCludt *et al*., 2000) and several infections have been reported in many parts of the world (Manna *et al*., 2006). The incidences of STEC in seafoods from Mangalore, India have been reported by Kumar *et al*. (2001). Thampuran *et al*. (2005) have isolated *E. coli* strains from finfish samples in retail markets in Cochin, India with the ability to produce haemolysis of human blood. However, typical *E. coli* O157 or other ST *E. coli* were not detected. Enterohaemorrhagic *E. coli* (EHEC) O157:H7 was isolated from Indian white shrimp, *Fenneropenaeus indicus* in retail fish markets of Cochin (Surendraraj *et al*., 2010). 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 (Griffin and Tauxe, 1991). Roy *et al*. (2013) have reported the incidence of ETEC and EPEC from samples in shrimp farms of Pakistan and recommended good hygienic practices for controlling the occurrence of pathogenic *E. coli*. Wang *et al*. (2011) reported the presence of *E. coli* in 10·5% of imported samples sold in retail markets in Baton Rouge, Los Angeles, but did not detect the pathogenic strains. Canizalez-Roman *et al*. (2013) isolated EPEC strains from seafood samples from Mexico. Recently, several studies were reported from Cochin estuary regarding the presence of pathogenic and antibiotic resistant *E. coli* (Sukumaran and Hatha 2015; Divya and Hatha, 2019). Relatively high prevalence of ETEC (18.6%) and EPEC (4.0%) with low prevalence of STEC (0.8%) among pathogenic *E. coli* (23.4%) from seafood samples of different fish markets and landing

centres of Kerala were reported (Murugadas *et al*., 2015). Antony *et al*. (2021) isolated of *E. coli* O157 from molluscan growing areas along the Indian coast with high prevalence of enterotoxigenic strains followed by other pathotypes such as EHEC, EPEC and EIEC.

***Staphylococcus aureus*:** Staphylococcal foodborne illness is due to the consumption of food contaminated with membrane-damaging, invasive staphylococcal toxins (Murugadas *et al*., 2017). The presence of enterotoxigenic *S. aureus* in fishery products and fish processing environments have been reported from India (Simon and Sanjeev, 2007; Murugadas *et al*., 2017) and Korea (Rhee and Woo, 2010). 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 (Gould, 2006; Carnicer-Pont *et al*., 2006). MRSA outbreak that resulted in mortalities were reported from Netherlands and banana was implicated as the source of infection (Kluytmans *et al*., 1995). The ingestion of contaminated shredded pork barbeque and coleslaw resulted in food poisoning outbreak due to MRSA in United States (Jones *et al*., 2002). The prevalence of *S. aureus* in Indian seafood ranged from 9 to 23% during the period from 1985 to 2016 (Simon and Sanjeev, 2007; Visnuvinayagam *et al*., 2015; Murugadas *et al*., 2016). The presence of MRSA in Indian seafood was first reported in 2015 in fish samples from Cochin and Mumbai coast by Visnuvinayagam *et al*. (2015). Later on, there were several reports of MRSA in seafood from India with incidence ranging from 6-11% (Kumar *et al*., 2016; Murugadas *et al*., 2016). It is imperative to prevent the seafood contamination with MRSA by following strict personal hygiene and GMP from harvest of the raw material to the consumption of end product by the consumers.

***Vibrio parahaemolyticus*:** The foodborne 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 foodborne outbreak in Japan in 1950 due to the consumption of partially cooked sardine (Fujino *et al*., 1953). Foodborne illness due to the presence of these bacteria has been frequently reported (Harakudo *et al*., 2003; Nair *et al*., 2007; Peng *et al*., 2010; Xu *et al*., 2016). The bacteria were detected in many seafood samples including eel, octopus, squid, shrimp, oyster, sardine, tuna, mackerel, perch, pompano etc. (Beuchat, 1982; Oliver and Kaper, 1997; Anjay *et al*., 2016).

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 *et al*., 1997). Most of the pathogenic environmental strains carry *trh* gene whereas presence of *tdh* gene is more in clinical strains that cause infection (Nair *et al*., 2007). Main symptoms of infection include gastroenteritis, wound infection and in rare cases, septicaemia can occur (Su and Liu, 2007). No dominant serovars were involved in food poisoning until the appearance of O3:K6 pandemic serotype in India in 1996. Pandemic strains possess *tdh* and are negative for *trh* and urease. Till now, around 75 different combinations of O and K serotypes of *V. parahaemolyticus* are reported (Ishibashi *et al*., 2000). Frequently isolated serovariants includes O4:K68, O1: KUT and O1:K25 (Matsumoto *et al*., 2000).

The incidence rate of *V. parahaemolyticus* in seafood ranges from 35 to 100% (Ayyappan *et al*., 2018; Narayanan *et al*., 2020a). Although the occurrence of O3:K6 in seafood in India has not been reported, its serovariants such as O10: KUT have been detected in seafood (Pal and Das, 2010). Recently, pandemic strains were isolated from clinical samples from Kolkata, India (Pazhani *et al*., 2014). Anjay *et al*. (2014) reported a high rate of prevalence of *V. parahaemolyticus* (75.5%) in seafoods collected from different fish markets

in Kolkata. Sudha *et al.* (2014) reported the presence of antibiotic resistant pathogenic *V. parahaemolyticus* strains from seafoods in Cochin, Kerala. Recent study suggested that marine water fish and shellfish samples from Kolkata harbors considerable percentages of pathogenic and pandemic strains of *V. parahaemolyticus* (Anjay *et al.*, 2016). Ayyappan *et al.* (2018) have reported the presence of pathogenic *V. parahaemolyticus* with characteristics of pandemic clones from seafood and environmental samples of Mumbai, India. Multidrug resistant pathogenic *V. parahaemolyticus* carrying *tdh* and *trh* genes were prevalent in shrimp aquaculture farms (Narayanan *et al.*, 2020b). Presence of potentially pathogenic *V. parahaemolyticus* harbouring *tdh* gene was reported from marine fishes sold in Kerala, India (Narayanan *et al.*, 2020a).

***Vibrio cholerae*:** The transmission route of *V. cholerae* to human is mainly through aquatic environments particularly water. There are reports of this pathogen in fish and fishery products from several parts of the world (Kumar and Lalitha, 2013; Joseph *et al.*, 2015; Azarian *et al.*, 2016). 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 *tcpA* (Sechi *et al.*, 2000). The survival and evolutionary dynamics of *V. cholerae* in water causes the emergence of diverse sero and biovariants of *V. cholerae* due to gene transfer mechanisms (Azarian *et al.*, 2016). The horizontal and lateral gene transfer mechanism causes the acquisition of virulence genes, antigenic types such as O1 and O139 etc. (Faruque *et al.*, 2004). 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 (Nair *et al.*, 2002). Re-emergence of classical biotype together with El Tor strains were reported in Bangladesh during 1982 (Samadi *et al.*, 1983) and these strains were frequently reported in gastroenteritis and diarrhoea from the area until 1993. Another epidemic strain of *V. cholerae* carrying O139 antigen was first reported in 1992 in Southern Asia (Albert, 1994). The incidences 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 carry *tcp*A classical genes or classical *ctx*A or *ctx*B genes have been reported from clinical cases of cholera from Bangladesh (Safa *et al.*, 2006). 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 (Faruque *et al.*, 1998; Wang *et al.*, 2020). However, the non-toxigenic strains lacking toxigenic genes also have the potential of causing diarrhoea in human (Azarian *et al.*, 2016). The mechanism of virulence and pathogenicity of this strain remains unknown. Joseph *et al.* (2015) reported the cause of infection in moribund shrimps, *P. monodon*, from a cultured farm is due to *V. cholerae* O139 strain, capable of infecting human via contact. Azarian *et al.* (2016) have identified two environmental non-toxigenic *V. cholerae* O1 strains lacking *ctx* gene. However, these strains possess classical *tcpA* genes. Genetic analysis revealed that these strains are phylogenetically comparable with clinical and environmental strains of O1, O139 and non-O1 *V. cholerae* strains. Saravanan *et al.* (2007) reported the presence of *V. cholerae* O139 in shrimp from local fish market in southern India. The detection of choleragenic *V.cholerae* O1 Ogawa, El Tor strains in shrimp and fish samples from Cochin by Kumar and Lalitha (2013) suggested that the seafood pose a serious risk to the consumers.

***Listeria monocytogenes*:** *Listeria monocytogenes* is major concern in lightly preserved

food products and the prevalence of this bacterium is considerably increased in ready to eat fishery products (Jami *et al*., 2014). Seafood has the highest risk among the minimally processed products (Rocourt *et al*., 2003). *L. monocytogenes* enters into seafood by cross-contamination and the presence of this pathogen in seafood has been reported from different seafood products (Jami *et al*., 2014; Basha *et al*., 2019). Prevalence rate of this pathogen in seafood products varies from 0 to 17% (Fallah *et al*., 2013; Momtaz and Yadollahi, 2013). However, the prevalence in seafood is relatively low compared to other food products such as dairy and other animal products (Basha *et al*., 2019). 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 septicemia, meningitis, gastroenteritis, pneumonia and spontaneous abortion (Vazquez-Boland *et al*., 2001). Regulatory agencies such as Food and Drug Administration (FDA), International Standard Organization (ISO), World Health Organization (WHO) etc. have included this pathogen in zero tolerant categories in processed food products due to its survivability in wide environmental conditions (O'Connor *et al*., 2010). This pathogen is able to withstand high NaCl concentration of upto 20%, pH range of 4.1 to 9.8, temperature range of 0.5 to 45°C and low water activity of 0.91 (Lungu *et al*., 2009). 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. There has been an increase in the incidence of this pathogen in Indian seafood especially from Mangalore, Mysore and Goa region since 1992 (Norhana *et al*., 2010). The prevalence of this pathogen in fresh and processed fishery products from Cochin region ranged from 1.2 to 2.7% (Das *et al*., 2013; Basha *et al*., 2019). Moharem *et al*. (2007) reported an incidence of 1.83% from fresh fish samples. Incidence of *L. monocytogenes* was reported in seafood from fish markets of Goa (Parihar *et al*., 2008; Gawade *et al*., 2010). CDC (2011) have reported that raw, smoked, cooked, lightly processed products of fish, meat and milk are more likely to be contaminated by this pathogen, causing serious health issues to people who consumed it.

***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 (Simonova *et al*., 2007). *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 (Bottone, 1997). *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. A study of various water sources in Brazil confirmed the presence of pathogenic strains of *Y. enterocolitica* belonging to virulence biotype 2 and 3 (Falcão *et al*., 2004). Cheyne *et al*. (2009) found that the *Yersinia* spp. recovered from water sources were non-pathogenic in nature. Sinha *et al*. (2000) reported non-pathogenic biotypes of *Y. enterocolitica* from Indian waters. However, there was no correlation between the presence of faecal pollution and occurrence of *Yersinia* spp. in water. The prevalence of *Yersinia* species in Indian seafood samples were reported by Kishore *et al*. (2012) with 54% of *Y. intermedia*, 19% *Y. aldovae* and low prevalence (0.03%) of *Y. enterocolitica*. Later Akhila *et al*. (2013) and Shanmugapriya *et al*. (2014) have reported the prevalence of 20% and 75% of *Y. enterocolitica* in fish samples.

***Clostridium botulinum*:** *C. botulinum* is grouped under Gram positive bacteria, and is

anaerobic spore producing bacilli of important public health concern in seafood industry (Lalitha and Gopakumar, 2000). This bacterium is autochthonous to the aquatic environment and aquatic sediments forms major reservoir of this pathogen. The toxigenic types of *Clostridium botulinum* belong to type A, B, E and F (Lindstrom *et al*., 2001). The major risk factors in seafood are due to the presence of these toxigenic types. Botulinum food poisoning is due to the consumption of food contaminated with preformed toxins of *C. botulinum* and low oral dose of 70 µg is sufficient to causes illness in human (González-Escalona *et al*., 2014). 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 as in modified atmospheric packaging or vacuum-packed products include, pH of about 4.6, water activity of 0.93%, low salt upto 3%, temperature range of 3°C to 50°C (Genigeorgis, 1985). It is reported that the non-pathogenic types belonging to type C and D are more prevalent in sea foods from India (Lalitha and Gopakumar, 2000). *C. botulinum* was detected in fresh and salted fish sold in retail markets in Cochin, Kerala (Lalitha and Surendran, 2002). It was found that, *C. botulinum* types A-E were able to grow and produce toxin at 15°C and 30°C stored in modified atmospheric seafood products (Lalitha and Gopakumar, 2001). The fatality rate is reported to be high as compared to other foodborne pathogens (Shukla and Sharma, 2005). It was reported that about *C. botulinum* contributes 0.2% of the total food outbreaks in different states of the European Union in 2017 with 7.7% mortality cases (EFSA / ECDC, 2019).

***Bacillus cereus*:** *Bacillus cereus* is spore forming, Gram positive, motile bacteria and is widely distributed in the environment (Das *et al.*, 2009). Even though this bacterium is autochthonous to the natural environment including aquatic environment, it is considered as human pathogenic bacteria due to its ability to produce heat resistant endospores, which can withstand extreme environmental conditions and capable of producing toxins in wide variety of foods (Ankolekar, 2009). The minimum level of 10^3CFU/g *B. cereus* in food is sufficient to produce toxin (Ankolekar, 2009). It produces heat, acid resistant emetic toxin called celuride, which causes emetic illness. Based on the production of enterotoxin, *B. cereus* is classified as haemolytic *B. cereus* (HBL) encoded by enterotoxin genes such as *hblA, hblC, hblD* and non-haemolytic *B. cereus* (NHE) encoded by *nheA, nheB, nheC*. These enterotoxins are responsible for diarrhoeal illness in human. The other enterotoxins reported in food poisoning are Ent FM, Cytotxin K (Schmid *et al*., 2021). The non-seasonal occurrence of *B. cereus* food poisoning has been reported Tewari *et al.*, 2015). In Europe, the first report of *Bacillus* food poisoning was from vanilla sauce which caused diarrhoeal type illness in 61 persons (Mortimer and McCann, 1974). Rahmati and Labbe (2008) reported 48% haemolytic *B. cereus* in fresh and processed seafood samples from USA. Gdoura-Ben Amor *et al.* (2018) reported *B. cereus* in 32.3% of seafood. In India, incidence rate of 36.2-40% were reported in seafood (Kamat *et al.*, 1989; Das *et al.*, 2009). The first outbreak of seafood associated *B. cereus* food poisoning from Calvia (Spain) where the insufficient cooking of ready to eat tuna steaks was reported in emetic illness (Doménech-Sánchez *et al.*, 2011). Recently, Schmid *et al.* (2021) confirmed the presence of *B. cereus* strains in fibre-based packaging materials that shares same phylogenetic characteristics of the strains found in food and environment.

***Shigella* spp.:** *Shigella* spp. belongs to *Enterobacteriaceae* family causes shigellosis in humans. This bacterium is recognized as a major public health threat in most of the

developing countries (Nadella *et al.*, 2019). The main symptoms include watery diarrhoea, fever and abdominal pain. The pathogenic species identified are *S. dysenteriae*, *S. flexineri*, *S. boydii and S. sonnei* (Niyogi, 2005). Fatality rate is more in children of age less than five years (Nadella *et al*., 2019). The main route of infection is through contaminated water and foods (Sujatha *et al*., 2011). A low dose of even 10 numbers of bacteria can sufficient to multiply inside the intestinal mucosa and cause dysentery (Nadella *et al.*, 2019). The main virulence factors described in *Shigella* spp. are *ial* (invasion associated locus) and *ipa BCD* (invasion plasmid antigen) (Malau *et al*., 2018). The occurrences of *Shigella* spp. in seafood have been reported in India using conventional plating techniques (Sujatha *et al*., 2011; Obaidat and Salman, 2017). Improved method using enrichment PCR along with traditional methods may be opted for the better recovery of these bacteria in seafood with a view of improving its detectability in the presence of other background flora (Nadella *et al*., 2019).

Emerging pathogens in seafoods

Apart from the well reported sea food borne pathogens, several other pathogens are also emerging throughout the world irrespective of the geographical conditions that are able to cause infectious diseases. It is not always true that emerging pathogens are a new category of microorganisms instead, it can be an already established pathogen in which the virulence or 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. The pathogens of emerging category in seafood include *Vibrio vulnificus*, *Vibrio mimicus*, *Cronobacter, Campylobacter* spp., *Arcobacter* spp. etc. The occurrence of these pathogens in disease outbreaks in human have been reported from all over the world.

***Vibrio vulnificus*:** *Vibrio vulnificus* is 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 foodborne outbreak is the consumption of contaminated raw or partially cooked shellfish (Karunasagar and Rohit, 2012). 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% (CDC, 2014; Pei *et al*., 2017). Recently, this bacterium has emerged as significant public health bacteria due to its high fatality rate all over the world (Pei *et al*., 2017). This bacterium is considered as a most fatal foodborne pathogen in USA (Pei *et al*., 2017). Out of the three biotypes known, biotype 1 is responsible for human infections (Thiaville *et al*., 2011). The major virulence factors in *V. vulnificus* are *RtxA1*, haemolysin (*VvhA*) and metalloprotease like *VvpE* and *VvpM* gene which are responsible for haemolytic, cytotoxic and iron acquisition activities (Lee *et al*., 2014). Wide variety of seafoods have been implicated in *V. vulnificus* contamination (Karunasagar and Rohit, 2012; Phillips and Satchell, 2017). The prevalence of this bacteria in seafood were 3.5 to 8% in Europe, 2.4% in South East Asia, 75 to 80% in oysters from India, 100% oysters from USA (Jones *et al.,* 2014). The incidence of this pathogen in fish samples from India is about 16.6% (Thampuran and Surendran, 1998). However, incidence of this pathogen in oyster is high (Karunasagar and Rohit, 2012). Seafoodborne outbreaks were mostly reported from South American countries such as Peru (Ibarra *et al*., 1999), Chile (Poblete *et al*., 2002), Equador (Villacrés *et al*., 2013) Brazil (Costa *et al*., 2013; Franca *et al*., 2013). In India, *V. vulnificus* infection was reported in a case

of gastroenteritis with septicaemia in a child, who had a prior history of contact with seawater (Saraswathi *et al*., 1989). Madiyal *et al.* (2016) reported rare cause of necrotising fasciitis in person who had a history of exposure to sea water due to *V. vulnificus* infection. Later, Bhat *et al*. (2019) reported necrotising fasciitis from India where contaminated seawater is most probable cause of infection. Recently, two cases of seafood related *V. vulnificus* infection were confirmed by PCR targeting virulence genes *rtxA*, *vvhaA* from Mangalore, India (D'Souza *et al*., 2018).

***Campylobacter* spp.:** *Campylobacter* spp. causes gastrointestinal disease termed campylobacteriosis and one of the leading causes of foodborne 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* (EFSA, 2010). USA alone reports 8.45 lakh cases of *Campylobacter* infection per year (CDC, 2011). The outbreak is mainly due to ingestion of contaminated food products, where the chicken alone contributes to about 25% of the infections (Wingstrand *et al.*, 2006). The incidence of *Campylobacter* spp. has been reported in other types of food animals such as cattle, pig, cows, sheep etc (Stanley and Jones, 2003). The *Campylobacter* spp. is a commensal bacterium to poultry and the intestinal tract carry huge amount of these bacteria. The rupture of intestinal tract while processing can disseminate the content to skin. Cross contamination with shellfish harvesting area and handlers can result in seafoodborne outbreak. Human campylobacteriosis are mainly due to *C. jejuni* and it causes 12 times more cases than *C. coli* in England (Friedman *et al*., 2000). Shellfish associated campylobacteriosis was first reported during 1980s where 28 persons were infected after eating raw clams (Griffin *et al.*, 1983). After this, few shell fish outbreaks were reported (Abeyta *et al*., 1993; Wilson and Morre, 1996) and the occurrence of this bacterium in sea waters are frequently reported in recent years (Wilkes *et al*., 2011). After a long period of absence, this enteric pathogen was again isolated from shellfish from France by Rincee *et al*. (2018) with a lower prevalence rate of 27.8% in contrast to the prevalence rate of 42% reported by Wilson and Morre (1996). The evidence of this pathogen in shellfish and shell fish harvested area points to the need to include this bacterium in general microbiological monitoring programme.

***Cronobacter* spp.:** *Cronobacter* species belongs to the family *Enterobacteriaceae* and is considered as an opportunistic pathogen in neonates (Das *et al.*, 2021). 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 (Miranda *et al*., 2017). This bacterium has been isolated from wide range of food sources such as dairy products, plant-based products, dried fish, shrimp, seaweeds and minimally proceeds products (Singh *et al*., 2015; Das *et al.*, 2021). This bacterium is considered as an emerging pathogen of seafood recently due to its survivability in low moisture foods such dried fish product (Das *et al.*, 2021). However, the seafoodborne outbreak due to this bacterium has not been reported so far. Recently, incidence study of *Cronobacter* in different food products from 44 countries by Miranda *et al*. (2017) reported an isolation rate of 9.1% in seafood. The presence of *Cronobacter* spp. in Indian seafoods was first reported from Mumbai, where the highest incidence was in dried and dehydrated fish (55.6%); followed by shellfishes (33.3%), fresh fish (6.3%) (Das *et al*., 2021). The pathogenicity of this bacterium is due to the presence outer membrane protein A (*ompA*), plasminogen activator (*cpa*) and haemolysin (*hly*) gene (Singh *et al.*, 2015). These putative virulence factors were widely distributed in plant-based products and clinical samples. The

*omp*A gene is thought to be present in all *Cronobacter* species and this gene is also present in invasive *E. coli* causing neonatal meningitis (Kim *et al*., 2000). However, the presence of *cpa* and *hly* genes are not reported in seafood products which suggest the presence of specific genetic factors in seafood associated *Cronobacter* spp. (Das *et al*., 2021).

***Arcobacter* spp.:** *Arcobacter* is an emerging zoonotic pathogen, belongs to *Campylobacteraceae* and is closely related to the Genus *Campylobacter* (Zhang *et al*., 2019). They are able to survive in low oxygen condition, and well adapted to temperature of less than 30°C (Ramees *et al*., 2017). *Arcobacter* causes bacteraemia, gastroenteritis and diarrhoea (Collado and Figueras, 2011). Out of 27 species identified, only three species are pathogenic causing disease; *A. butzleri*, A. *cryaerophilus* and *A. skirrowii*. Foodborne infection associated with chicken and vegetables have been reported (González and Ferrus, 2011; Ramees *et al*., 2017). Seafoodborne outbreak due to *Arcobacter* has not been reported so far; however reports of isolation of *Arcobacter* from fish, shellfish, and seawater are available (Romero *et al*., 2002; Laishram *et al*., 2016; Zhang *et al*., 2019). The genus *Arcobacter* was first recognized during 1991 and the main reservoir identified was poultry (Ramees *et al*., 2017). Since 2005, many of the new species have been first identified from shell fishes, mussels, and seawater. The first report of *Arcobacter mytili* and *A. molluscorum* were from mussels (Figueras *et al*., 2011) and *A. bivalviorum, A. venerupis* and *A. venerupis* from shellfishes (Levican *et al*., 2012; Levican *et al*., 2013). The high prevalence and emergence of new species in shellfishes were attributed to the presence of faecal pollution in shellfish growing areas.

This bacterium has been frequently isolated from different animal sources such as cattle, pig, plants, humans etc. (Ramees et *al*., 2017). The transmission route for *Arcobacter* infection is polluted water and food sources. Fisher *et al*. (2014) reported that *Arcobacter* contributes 5-11% of the bacterial population in sewage. Consumption of contaminated products such as raw or partially cooked chicken meat, pork and seafoods are main sources of infections in humans (Collado *et al*., 2009). The pathogenicity of *Arcobacter* to human is unclear till now. The epidemiological surveillance studies and source tracking are essential to know the disease burden of *Arcobacter* contaminated food including seafood.

***Vibrio mimicus*:** *Vibrio mimicus* is an important emerging zoonotic pathogen in seafood that causes disease in aquaculture fishes as well as gastroenteritis in human (Nilavan *et al*., 2021). Major reservoirs of this pathogen are raw oysters, fish, turtle eggs, shrimps, cray fish etc. Davis *et al*. (1981) while studying the biochemical characteristics of atypical *V. cholerae* by biochemical tests revealed a new species that was sucrose negative for which the name *Vibrio mimicus* sp. nov. was proposed. *V. mimicus* carrying *ctx* gene is reported as pathogenic strain that can cause severe watery diarrhoea and gastrointestinal disorders (Nair *et al*., 2012). An asymptomatic infection of V. *cholerae /V. mimicus* was reported in 52.6% of fecal microbiota of healthy children in an urban slum in Kolkata stating a risk of spreading the contamination among the population (Nair *et al*., 2012). In India, there were only few reports of this organism from seafoods. In a prevalence study of pathogenic *Vibrios* from shellfish of Cochin market, Sudha *et al*. (2014) reported 1% *V. mimicus* among potentially pathogenic *Vibrio* species. Recently, Nilavan *et al*. (2021) reported a prevalence of 5.6% in seafood with maximum prevalence in fresh water (18%) and brackish water (19%) fish samples compared to marine fish samples (2%). *V. mimicus* haemolysin gene was present in 12% of the raw and RTE seafood in northwest Mexico indicating potential risk to consumers (Guardiola-Avila *et al*., 2015). The consumption of raw or partially cooked fish pose major risk in seafood related outbreak by this

bacterium. Further there is urgent need for implementation of effective preventive measures in the food supply and value chain.

Conclusion

In recent years, the increased use of fresh, iced and processed seafood demands improved surveillance on seafood microbiological quality to control the foodborne pathogens. Modern facilities in fish markets offer good quality seafood for domestic consumption. The thumb rule in maintaining safety in seafood includes time and temperature control, hygiene and cleanliness of the workers, production area, utensils and avoidance of cross contamination. Increased awareness programme and effective governance and management strategies for assuring the quality of seafood is the need of the hour.

Food safety with respect to seafood pathogens is important in terms of public health perspective as over 200 types of diseases are due to the consumption of contaminated foods (WHO, 2015). Control of pathogens in fishery products can be achieved by following either or the combination of preservation techniques such as storing the products at low temperature preferably at chilled/frozen condition, use of heat treatment etc. One of the food safety concerns of Vibrios is the ability of viable but non culturable (VBNC) state of *Vibrio* species which is very difficult to detect in food by routine microbiological tests. To ensure food safety, routine microbiological screening tests should be validated in real time so that the contaminated food product gets 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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