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Review Article

Bacterial Pathogens in Seafood – Indian Scenario

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Abstract

Fish and fishery products are frequently contaminated with bacterial pathogens. Common pathogens that are found in Indian seafood are Salmonella, Vibrios, *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*. The consumption of these infected fish and their products can result in mild to chronic illnesses. In addition, the presence of these food borne pathogens causes huge monetary loses to fishermen and exporters. In India a proper system of documentation and reporting of food-borne illness is lacking. Indian seafood are often contaminated by human activities and sewage released into the water bodies. Poor sanitation in fish landing centre and open fish markets also exacerbates the situation. The quality of fish sold in domestic market in India is poor compared to that of export trade. The importance of proper handling and storage of seafood to control the growth of pathogenic bacteria need to be emphasized. Proper reporting and documentation system with strong public awareness programmes can be very effective in management of food safety issues in the future.

Keywords: Seafood, Bacteria, Salmonella, Vibrios, *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*

Introduction

Seafood is a major vehicle for transmission of several bacterial diseases. Human infections due to many pathogenic bacteria are reported to have been transmitted through fin fish, shell fish and other sea food products (Okuda et al., 1997). Fishery products have been recognized as a major carrier of food-borne pathogens like *Salmonella* sp, *Staphylococcus aureus*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Yersinia enterocilitica*, *Listeria monocytogenes*, *Campylobacter jejuni* and *Escherichia coli* (Venugopal et al., 1999). Estuaries and coastal water bodies are the major sources of seafood in India and are often contaminated by the activities of adjoining population and partially treated or untreated sewage released into these water bodies. Seafood harvested from such areas often contains pathogenic microorganisms (Kumar et al., 2005). In addition poor sanitation in landing centres and the open fish markets exacerbates the situation (Kumar et al., 2001). It has been reported that quality of fish sold in domestic market in India is poor compared to that of export trade and are mostly contaminated with pathogenic microorganisms (Nambiar & Iyer, 1990). The microbial quality and presence of food borne bacterial pathogens in fish and fishery products of the Cochin area has been investigated by many authors (Nambiar & Iyer, 1990; Nambiar & Iyer, 1991; Thampuran & Surendran, 1998; Surendran et al., 2002; Lalitha & Surendran, 2002).

In India, the role of seafood sector in providing economic and nutritional security is very large. The rising demand of seafood nationally and internationally further leads to production of unscrupulous, under-processed and unhygienic products that may harbor various species of bacterial and viral seafood-borne pathogens. The presence of these food borne pathogens causes huge monetary loses to the fishermen and the exporters. Often food borne outbreaks are not properly documented in developing countries, unlike the western counterparts; hence, less number of reports are available in these countries.

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Bacterial Pathogens in Seafood

Salmonella

Salmonellosis constitutes a major public health burden and represents a significant cost to society in many countries. The incidence of Salmonella in seafood is a major problem for the seafood industry as international regulatory authorities stipulate a zero tolerance for this organism in seafood. Salmonella serovars are causative agents of the largest number of enteric infections to humans and the incidences of foodborne salmonellosis has been reported worldwide. *S. typhimurium* is the most common serovar in seafoods reported worldwide including India (Saroj et al., 2008). Seafood is a frequent source of Salmonella contamination (D'Aoust et al., 2001). Relatively, high incidence of Salmonella contamination is reported from developing countries. The microbiological testing of food samples for the presence of this pathogen is mandatory (USFDA, 2004). The microbiological criteria for Salmonella and other bacterial pathogens in seafood for different countries are given in Table 1.

In India, a number of investigators have reported Salmonella in seafood (Table 2). The natural habitat of Salmonella is the gastrointestinal tract of animals, including birds and man (Pelzer, 1989). This organism finds its way into the river water, coastal and estuarine sediments through faecal contamination. There are several reports indicating the presence of Salmonella in the shrimp culture pond sediments in India (Iyer & Varma, 1990a; Nayyarahamed et al., 1995; Bhaskar et al., 1995). Salmonella have also been reported in carp and tilapia raised in ponds fertilised with raw sewage in India (Balasubramanian et al., 1992; Iyer & Shrivastava, 1989). Incidence of Salmonella has been reported from aquaculture ponds of Tamil Nadu (Sivakami et al., 1996). Salmonella has been isolated from cultured *P. monodon.* The incidence of Salmonella in the clam meat may be due to its filter feeding behaviour while its incidence in formulated feed may mainly be due to the human handling (Bhaskar et al., 1995). It has been concluded that Salmonella is a part of the natural microfauna of the brackish water ponds. In shrimp processing

Table 1. Microbial criteria of certain bacterial pathogens in Raw and cooked Fish

Countries/ Food Authority	Microbiological criteria/guidelines/specification/maximum limits		References
	Raw Fish (fresh/frozen)	RTE Fish /Cooked Fish	
INDIA	*E. Coli :* <20 g^{-1} *S. aureus*: <100 g^{-1} Salmonella: ND in 25 g Shigella: ND in 25 g *Vibrio cholerae*: ND in 25 g *Vibrio parahaemolyticus*: ND in 25 g	*E.* Coli: ND in 25 gm *S. aureus*: ND in 25 gm Salmonella: ND in 25 g Shigella: ND in 25 g *Vibrio cholerae*: ND in 25 g *Vibrio parahaemolyticus*: ND in 25 g	FSSAI, 2011
EU	*E. Coli :* <230/100 g *S. aureus*: <10^3/g	Salmonella : ND in 25 g *V. parahaemolyticus*: ND in 25 g *L. monocytogenes* : ND in 25 g *S. aureus:* <20 g^{-1}	EU Directives (EC, 2073/2005).
US	*E. Coli :* MPN of 230/100 g Salmonella: ND in 25 g *Listeria monocytogenes:* ND in 25 g *Vibrio cholerae*: ND in 25 g *Vibrio parahaemolyticus:* 1x 10^4/g *Vibrio vulnificus :* Absence *S. aureus*: <10^5−10^6/g *Clostridium botulinum*: Absence of viable spore, vegetative cells, toxin	*E.coli* ETEC: 1x 10^3 /g Salmonella: ND in 25 g *Listeria monocytogenes:* ND in 25 g *Vibrio cholerae*: ND in 25 g *Vibrio parahaemolyticus*: <1x 10^4/g Vibrio vulnificus: Absence *S. aureus*: <10^5−10^6/g *Clostridium botulinum*: Absence of viable spore, vegetative cells, toxin	FDA Compliance Programme 7303.842

ICMSF	*E.coli*: n= 5, c=3, m=11,M=500 *Vibrio parahaemolyticus/g:* n=5, c=2, m=10^2,M=10^3 *S. aureus:* n=5, c=2, m=10^3, M=10^4 Salmonella/25g: n=5, c=0, m= 0	*E.coli* :n= 5, c=3, m=11, M=500 *Vibrio parahaemolyticus/g:* n=5, c=1, m=10^2,M=10^3 Staphylococci= n=5, c=0, m=10^3 Salmonella/25g: n=5, c=0, m= 0	www.icmsf.org
JAPAN	*E. Coli:* Less than 10/g Salmonella: ND in 25 g *S. aureus :* Less than 10^3/g *Vibrio parahaemolyticus:* < 100 MPN count/g (raw consumption): *Clostridium perfringens*: 10^5/g *Bacillus cereus :* 10^5/g	Salmonella: ND in 25 g *Listeria monocytogenes:* < 10^2/g *S. aureus: L*ess than 10^3/g Campylobacter spp: ND in 25 g *Clostridium perfringens*: 10^4/g *Bacillus cereus :* 10^4/g	Japan Food Sanitation Law. MHLW.
HONGKONG	*E. Coli :* <20/g *E. Coli 0157:* ND in 25 g Campylobacter spp.: ND in 25 g Salmonella spp. : ND in 25 g *Vibrio cholerae*: ND in 25 g	*E. Coli :* < 20/g *E. Coli 0157:* ND in 25 g Campylobacter spp.: ND in 25 g Salmonella spp. : ND in 25 g *Vibrio cholerae*: ND in 25 g *Vibrio parahaemolyticus*: <20/g *S. aureus*: <20/g *Clostridium Perfringens:* <20/g *B. cereus:* <10^3 *Listeria monocytogenes: <20/g*	Hong Kong Food and Environmental Hygiene Department, 2007
AUSTRALIA	*E.coli* :n= 5, c=1, m=10^2,M=10^3 *Vibrio cholerae* /g: n=5, c=0, m= 0. Staphylococci/g: n= 5, c=2, m=10^2,M=10^3 Salmonella/25g: n=5, c=0, m= 0	*E.coli*: n= 5, c=1, m=10^2, M=10^3 *Vibrio cholerae*/g: n=5, c=0, m= 0. *Vibrio parahaemolyticus*/g: n= 5, c=2, m=10^2,M=10^3 Staphylococci/g: n=5, c=2, m=10^2, M=10^3 Salmonella/25 g: n=5, c=0, m= 0 *Listeria monocytogenes* /25 g: n=5, c=0, m=0. *Clostridium perfringens :* <10^2/g *Bacillus cereus:* <10^2/g *Campylobacter* spp: ND in 25 g	Australia New Zealand Food Standards Code, (2001)
NEW ZEALAND	*Vibrio cholerae* /g: n=5, c=0, m= 0. Staphylococci/g: n= 5, c=2, m=10^2,M=10^3 Salmonella/25g: n=5, c=0, m= 0	*Vibrio cholerae*/g: n=5, c=0, m= 0. *Vibrio parahaemolyticus*/g: n= 5, c=2, m=10^2,M=10^3 Staphylococci/g: n= 5, c=2, m=10^2,M=10^3 Salmonella/25g: n=5, c=0, m= 0 *Listeria monocytogenes*/25g: n=5, c=0, m=0	New Zealand Food Safety Authority
SOUTH AFRICA	*E.coli Type 1: <10/100g* Salmonella: < 20/g *Shigella:* < 20/g *Vibrio cholerae:* < 20/g *Vibrio parahaemolyticus:* < 20/g *Coagulase-positive S.aureus:* < 20/g	*E.coli Type 1:*< 20/g Salmonella: <20/g *Shigella:* <20/g *Vibrio cholerae:* <20/g *Vibrio parahaemolyticus:* <20/g *Coagulase-positive S.aureus:* <20/g	Department of Health South Africa, 1997.

Specifications and Standards for Foods, Food Additives, etc. Under the Food Sanitation Act 2010 (Abstract). 2011. Japan External Trade organization (JETRO). [cited on 2014 sept 29]
FSSAI-Food Safety and Standards Authority of India.
Available from: https://www.jetro.go.jp/en/reports/regulations/pdf/foodext2010e.pdf

industry, the principal sources of Salmonella contamination are sediments (Iyer & Varma, 1990a) in the untreated animal manure, feeds (Venkateshwaran et al., 1985; Bhaskar et al., 1995) used in the shrimp farm primarily for the purpose of pond fertilization, coastal water used for handling and processing the seafood, rodent, lizard and bird droppings (Kaura & Singh, 1968; Gopalakrishnan & Varma, 1990; Bhaskar et al., 1995; Hatha et al., 2003).

Salmonella sp has been isolated from processed foods, fish and poultry meat from India (Bandekar et al., 2004; Shabarinath et al., 2007). Lakshmanan et al. (1993) reported Salmonella infections in 3.2% of frozen Cephalopods products (*Loligo* sp and *Sepia* sp) from India. A study on Salmonella in imported seafood reported that *Salmonella weltevreden* was the most frequently isolated serovar in the seafood of Indian origin (Heinitz et al., 2000). The various serovars reported in India include *Salmonella chingola*, *Salmonella cerro*, *Salmonella nchanga*, *Salmonella oslo* and *Salmonella mbandaka* (Nambiar & Iyer, 1991). The presences of Salmonella on processed and semi-processed shrimps, catfish and seerfish have been reported (Varma et al., 1985; Iyer & Shrivastava, 1989).

Many factors including inadequate supplies of clean water, inadequate sanitary measures, lack of food hygiene and food safety measures have been

Table 2. Studies on Salmonella in Seafood

Location	Fish/shellfish	Wild/Farmed	Source of contamination	Reference
Mumbai	Shellfish	Wild	Market	Valsan et al., 1985
Mangalore	NA	Wild	Sediment soil	Srikantaiah et al., 1985
Cochin	Clam (*Villorita cyprinoides*)	Wild	Processing plant	Varma et al., 1988
Cochin, Mumbai	Fish, Shrimp	Wild	Processing plant, Rodents, Market	Iyer & Shrivastava, 1989
Cochin	Fish	Wild	Retail Market	Nambiar & Iyer, 1990; Nambiar & Iyer, 1991
Coimbatore	Fish, Shell Fish	Wild, Farmed	Market	Hatha & Lakshmanaperumalsamy, 1997
Karnataka	Shrimp (*Penaeus monodon*)	Farmed	Water, Feed	Bhaskar et al., 1998
Mumbai	Fish, Prawns	Wild	Landing Centre, Market	Panda, 2002
Kakinada, Mumbai	Prawns, Scampi	Farmed	Water, Feed	Bandekar et al., 2004
Mangalore	Fish, Shellfish	Wild	Landing Centre, Market	Shabarinath et al., 2007
West Coast of India	-	Wild	Water, Sediments	Abhirosh et al., 2008; Abhirosh et al., 2010
Cochin	Fish, Shellfish	Farmed, Wild	Landing centres, Retail markets	Kumar et al., 2009d
Mumbai	Fish	Farmed, Wild	Retail Market	Kakatkar et al., 2010
Chennai	Fish (*Priacanthus hamrur*, *Megalopsis cordyla*)	Wild	Water	Sujatha et al., 2011
Andra Pradesh	Fish (*clarias butrachus*), Prawns (*Macrobrachium rosenbergii*)	Wild/farmed	Retail Market	Bujjamma et al., 2015
West Bengal	Fish, Shellfish	Wild, Farmed	Markets	Dutta et al., 2015

responsible for increased incidence of food borne salmonellosis (Gopalakrishnan & Joseph, 1980; Gopalakrishnan & Damle, 1981; James et al., 1985).

The study conducted by the National Salmonella and *Escherichia* Centre (NSEC), Kasauli, India, during the period 2001-2005 identified the presence of seven prominent serovars of *Salmonellae* in seafood samples (Kumar et al., 2009a). In India, an outbreak of foodborne illness due to *S. weltevreden* was recorded as early as in 1985 (Aggarwal et al., 1985). In 2009 an outbreak of gastroenteritidis among 34 female nursing students due to *S. weltevreden* has been reported in Mangalore, India (Antony et al., 2009) due to the consumption of fish.

The exponential use of antibiotics in agriculture, aquaculture and poultry for growth, promotion and prophylaxis in intensive animal farming has led to the development of antibiotic resistance in Salmonella. Multidrug resistant Salmonella has gained prominence in many parts of the world and has become a cause of concern (Helms et al., 2005; Deekshit et al., 2012). There are many reports from India about emergence of antibiotic resistant Salmonella (Anon, 1990; Aggarwal et al., 1991; Kumar et al., 2009b; Rao et al., 2014). Presence of multi drug resistant *S. oslo* and *S. bareily* has been reported from the sea-foods exported from India and Vietnam (Khan et al., 2006). Kakatkar et al. (2011) have compared the heterogeneity in the antibiotic resistance pattern of *S. Weltevreden* serovars isolated from fish samples with the diverse origin of Salmonella contamination such as feed, water, soil or post-harvest handling and marketing.

During recent years, molecular techniques have been increasingly used for detection of pathogens in food. The effective control of Salmonella in seafood can be implemented through the prompt identification of the pathogen based on PCR and Real-Time PCR (Shabarinath et al., 2007; Kumar et al., 2008; 2010). The use of PCR for the detection of Salmonella would reduce the detection time since the time required for selective plating and biochemical identification is much longer. The prevalence of Salmonella in seafood is much more than that reported by conventional isolation technique. In 2003, Kumar et al., demonstrated the presence of Salmonella serovars in 30% finfish, 20% clams and 5% shrimp by hns-based PCR technique. Recently it was reported that PCR assay was found to be the most sensitive in the detection of Salmonella in seafood when compared to culture, ELISA methods (Kumar et al., 2008). PCR was also used to study the prevalence of virulent lac+ and lac- Salmonella serovars in seafood collected from Cochin (Kumar et al., 2009c). In 2010, highly sensitive Real Time PCR assay was proposed to quantify Salmonella in seafood (Kumar et al., 2010). Bhowmick et al. (2011) used PCR to study the distribution of genes present in SPI-2 in serovars of Salmonella associated with seafood in India. A combination of RAPD, ERIC-PCR and SDS-PAGE to investigate the genetic diversity among seafood associated non-typhoidal Salmonella serovars were used in 2012 (Bhowmick et al., 2012). Jeyasekaran et al. (2012) developed a rapid and sensitive multiplex PCR (MPCR) based assay for the detection of Salmonella serovars in shrimps within 8 h of pre-enrichment.

Along with the antibiotic resistance, the pathogenicity islands also play a prominent role in the virulence characteristics of Salmonella. Salmonella pathogenicity Islands (SPI) are large clusters of virulence gene in Salmonella which determines their invasive phenotypes (Bhowmick et al., 2011). Among the 17 pathogenicity islands of Salmonella identified, SPI-1 and SPI-2 play a major role in adhesion, invasion and survival in the host cells (Karunasagar et al., 2012a). Deekshit et al. (2013) developed an mPCR method for the detection of antibiotic resistance genes as well as Salmonella pathogenicity island 2 genes in Salmonella from seafood.

Vibrios

Vibrios of seafood origin have attracted increasing attention from time to time as it is found to be one of the most important causes of human food poisoning. In India the incidence of this bacterium was reported to be doubled in the last 5 years (Chowdhury et al., 2000). The occurrence of various Vibrio species in water, sediment, Fish and shrimp samples from the east and west coast of India has been studied and the details are given in Table 2. Vibrios are frequently isolated from seafood and in particular from shellfish.

Vibrios constitute a major portion of the microbiota in brackishwater pond ecosystem. Otta et al. (1999) and Vaseeharan & Ramasamy (2003) noted that Vibrio species accounted for 38–81% of the bacterial biota in shrimp farms from India. The most common Vibrio species found in farming phases of black tiger shrimp in India were *V. alginolyticus*, *V.*

Table 2. Distribution of Vibrios in marine and estuarine waters of India

Location	Vibrio species	Fish/ Shellfish	Wild/ Farmed	Source of contamination	Reference
Different parts of India	*V. parahaemolyticus*/ *V. cholera*/ *V. alginolyticus*	F/SF	W	F/SF samples	Singh et al., 1996
East Coast	*V. alginolyticus*/ *V. parahaeomolyticus*	SF	Farmed	Water Sediment	Gopal et al., 2005
West Coast	*V. vulnificus*			Shrimp	
South West Coast	*V. cholerae*	SF	W	SF/ Water/ Seawater samples	Saravanan et al., 2007
Cochin	*V. parahaeomolyticus*	F/SF	-	Market	Chakraborty & Surendran, 2008
South West coast of India	*V. parahaeomolyticus*	F/SF	Wild	F/SF Samples	Reghunath et al., 2008; 2009
West Coast	*V. parahaeomolyticus*	-	Wild	Water/ sediments	Abhirosh et al., 2008; 2010
Mumbai	*V. cholerae*	F	Farmed	Fish sample	Kakatkar et al., 2010
Cochin	*V. alginolyticus* *V. parahaeomolyticus* *V. vulnificus*	F/SF	Wild	Shrimp / Fish samples	Smitha et al., 2011
Chennai	*Vibrio cholerae*	F	Wild	Water	Sujatha et al., 2011
Cochin	*V. parahaeomolyticus*	F	W/F	Retail Outlet	Sudha et al., 2012
East Coast of India	*V. cholera* *V. parahaeomolyticus* *V. alginolyticus*	SF	Farmed	Pond water/ Pond Sediment/ Shell fish/ Hatchery water/ Post Larvae	Rao & Surendran, 2013
Cochin	*Vibrio cholera* 0139	SF	Farmed	SF sample	Joseph et al., 2015
West Bengal	*Vibrio cholerae*	V/SF	W/F	Markets	Dutta et al., 2015

parahaemolyticus and *V. vulnificus* (Bhaskar & Setty, 1994). Not all vibrio species are pathogenic. Out of the 65 species of the genus Vibrio, only 3 species *V. parahaemolyticus*, *V. cholera* and *V. vulnificus* are most important and are responsible for most cases of food borne illnesses (Gopal et al., 2005; Sudha et al., 2012). Other *Vibrio* sp that can cause illness though less frequently, among seafood consumers are *V. mimicus*, *V. fluvialis*, *V. damsella*, *V. hollisae*, *V. alginolyticus*, *V. furnissi*, *V. metschnikovii* and *V. cincinnatiensis.*

Vibrio parahaemolyticus

Vibrio parahaemolyticus has been identified as a common cause of seafood-borne illness in many Asian countries (Deepanjali et al., 2005). *V. parahaemolyticus* is a halophilic gram negative, motile, oxidase positive, straight or curved rod-shaped, facultative anaerobic bacteria that occur naturally in the marine environment. They form part of the indigenous microflora of aquatic habitats of different salinity and are the major causative agents for some of the most serious diseases in fish, shellfish and penacid shrimp (Nelapati et al., 2012). *V. parahaemolyticus* causes three major syndromes of clinical illness, *i.e.* gastroenteritis, wound infections, and septicaemia (Nair et al., 2007).

V. parahaemolyticus occurs in estuarine environments worldwide (Joseph et al., 1983; Karunasagar et al., 1986; Karunasagar et al., 1987a). Even though this organism is considered as halophilic in nature, its seasonal distribution in fresh water environments and association with fresh water fishes of Calcutta were reported (Sarkar et al., 1985; Nair et al., 2007).

Many studies from India reported the isolation of these organisms from different fresh and frozen fish and shellfish (Bandekar et al., 1982; Karunasagar et al., 1990; Prasad & Rao, 1994; Thampuran et al., 1996; Sanjeev, 1999; Sanjeev et al., 2000). In India the incidence of *V. parahaemolyticus* causing food poisoning due to the consumption of contaminated seafoods was reported to be doubled in the last 5 years (Choudhury et al., 2000). The largest incidence of food borne illness due to *V. parahaemolyticus* is reported from Calcutta in 1996 (Nair et al., 2007). Food Borne infection due to the *V. parahaemolyticus* at the Christian Medical College Hospital, Vellore emphasized the public health hazard of this organism in India (Lalitha et al., 1983). Early investigations in Calcutta revealed the dominance of serotype O1:K56 among 12 diarrheal cases (Chatterjee & Sen, 1974). The isolation of *V. parahaemolyticus* from market samples of freshwater fishes was attributed to cross-contamination due to mishandling at fishmongers' stalls (Sarkar et al., 1985). Contamination of freshwater fish by seawater fish at the fish market and secondary contamination of other foods in the kitchen by *V. parahaemolyticus*-contaminated fish brought from markets are thought to be the most likely routes of transmission in this setting (Pal et al., 1984). Nithya et al. (2008b) reported that 13.33% of the stool samples collected from ailing fish handlers gave positive results for *V. parahaemolyticus* in Calcutta. Epidemiological studies revealed higher incidence of *V. parahaemolyticus* in human carriers in Kolkata (Deb et al., 1975). A pandemic clone of *V. parahaemolyticus* belonging to serogroup O3: K6 which was first detected in Kolkata (Okuda et al., 1997) has been responsible for many outbreaks in Asia (Chowdhury et al., 2004).

Deepanjali et al. (2005) observed high levels of *V. parahaemolyticus* during the dry season b*et*ween January and May and decreased during post monsoon months Abraham et al. (1997); Sanjeev (2002) reported that the incidence of *V. parahaemolyticus* in fresh, marine and brackish water fish in India varied from 35 to 55%. The incidence of this organism was reported to be high in faeces, least in external surface and moderate in gills of the fish (Sarkar et al., 1985; Sanjeev & Stephen, 1995). Nithya et al. (2008a) observed that the incidence of *V. parahaemolyticus* contamination ranges from 15 to 46.66% of fish samples collected from different fish mark*et*s of West Bengal, India. Sarkar et al. (1985) reported that the incidence and counts of *V. parahaemolyticus* were consistently higher in association with plankton than with water and sediment samples.

The hemolytic activity of TDH, named the Kanagawa phenomenon, has been reported to be commonly associated with strains isolated from humans with gastroenteritis but were rarely observed in environmental isolates (Joseph et al., 1983). Sanjeev (1999); Sudha et al. (2002) reported higher positives from environmental strains of Cochin. Sanjeev & Stephen (1995) reported that 22.9% of the isolates from shell fish were found to be kanagawa positive *V. parahaemolyticus*. They also reported that all strains of *V. parahaemolyticus* isolated from cooked, shucked clams were Kanagawa negative and 50% of the isolates from mussels were kanagawa positive. Detection of pathogenic *V. parahaemolyticus* is traditionally done by Wagatsuma agar test for the Kanagawa reaction, which requires fresh human or rabbit blood and tends to give false positive reaction (Raghunath et al., 2008). Haemolysins are related to virulence in many Vibrio species. Haemolysin production by clinical and environmental strains from Indian coastal water has been studied (Karunasagar, 1981; Malathi et al., 1988; Karunasagar et al., 1989).

The PCR amplification of a cloned fragment sequence of chromosomal DNA specific for *V. parahaemolyticus* could be used to detect *V. parahaemolyticus* in fish and shellfish (Karunasagar et al., 1997). Studies revealed detection of this pathogen by PCR using species specific, tl gene was found to be rapid and sensitive (Chakraborty et al., 2008). Banerjee et al. (2002) developed a rapid DNA probe method for detecting *V. parahaemolyticus* grown on hydrophobic grid membrane filters (HGMF). *V. parahaemolyticus* is detected through hybridization between *V. parahaemolyticus* DNA immobilized onto HGMF and DIG-labeled probes specific for the tlh gene.

PCR employed for the detection of *V. parahaemolyticus* target the genes encoding virulence determinants and also species specific markers that include tdh, trh, tlh and toxR (Karunasagar et al., 1996a; Bej et al., 1999; Dileep et al., 2003). trh- bearing V. parahaemolyticus are more frequently distributed in tropical seafood than tdh-bearing *V. parahaemolyticus. (*Deepanjali et al., 2005; Parvathi et al., 2006). The presence of the tdh, trh, toxR genes

and a chromosomal locus of unknown function in *V. parahaemolyticus* was studied by PCR (Gopal et al., 2005). The toxR targeted PCR method was found to be sensitive and rapid in the detection of *V. parahaemolyticus* from environmental samples as well as atypical colonies (Chakraborty & Surendran, 2008). Both tdh and trh genes could be detected in higher number of samples after 18 h enrichment in APW and ST broth and hence demonstrates the need for enrichment for detection of pathogenic *V. parahaemolyticus* in seafood by PCR (Raghunath et al., 2009). Kumar et al. (2011) developed a monoclonal antibody based ELISA for the rapid detection of pathogenic *V. parahaemolyticus* in seafood. The presence of T3SS2β positive *V. parahaemolyticus* was confirmed from seafood harvested along the Mangalore coast by PCR (Kumar et al., 2013). The toxins secreted by the T3SS machineries are believed to have a profound role in pathogenesis of the organism (Karunasagar et al., 2012b).

Vibrio cholerae

V. cholerae causes Cholera, a potentially epidemic and life-threatening secretory diarrhea. It is characterized by numerous, voluminous watery stools, often accompanied by vomiting and resulting in hypovolemic shock and acidosis. Cholera has been classified as category B bioterrorism by Centre for Disease Control and Prevention (WHO, 2008). *V. cholerae*, the causative agent of cholera in humans, is classified into two serotypes: O1 and non O1 (Chatterjee & Maiti, 1984). Epidemics of cholera caused by toxigenic *V. cholerae* O1 and O139 serotypes represent a major public health problem in most of the developing countries. Since late 1992, *V. cholerae* serogroup O139 has emerged as a second etiologic agent of cholera in the Indian subcontinent (Nair et al., 1994). Outbreaks of seafood borne cholera have been noted quite often in the past few decades. In 1993, V. cholera serogroup O139 made an explosive appearance and caused a severe epidemic in the Indian continent (Ramamurthy et al., 1993).

Fish and other seafood have been implicated as a source of cholera outbreaks (Joseph et al., 1965). Many studies have reported the incidence of *V. cholerae* in fresh and frozen seafood in India. (Table 2). Varma et al. (1989) has reported the presence of *V. cholerae* 01 in 0.2% of raw fishery products and *V. cholerae* non 01 in 26.3% of raw and 12.14% of frozen products in Kerala and Tamil Nadu during 1986-1987. *V. cholerae* non 01 have been isolated from seafoods (Karunasagar et al., 1988; Mathew et al.,1988). The possibility of contamination of fishery products with *V. cholerae* non O1 during different stages of handling and processing has been studied (lyer & Varma 1990b; lyer et al., 1990). Water is recognized as the most important vehicle for cholera transmission. The seafood-borne non O1 *V. cholera* possess genetic attributes essential for the organism to cause gastroenteritis of a less severe type (Saravanan et al., 2007). The prevalence of *V. cholera* and other Vibrios from environmental and other seafood samples has been reported by Sathiyamurthy et al. (2013). The incidence of *V. choIerae* in molluscan shellfish like oysters and clams has been reported. Molluscan shellfishes are generally filter feeders. Higher Vibrio counts were also reported in bivalves (Aiyanperumal et al., 1995). *V. cholerae* has been found to be associated with pond mud, water and shrimp samples in India. In shrimp farms, non O1 and non-O139 strains are more frequent (Karunasagar et al., 2003). The higher prevalence of *V*. *cholerae* in formulated feed may be due to the excessive human handling on the pond site, for obvious reasons. (Bhaskar & Setty, 1994). Formulated feed was found to be the principal sources of *V. cholera* contamination in the shrimp processing industry in Karnataka (Bhaskar et al., 1998).

The choleragenic *V. cholerae* are characterized by their ability to produce cholera toxin encoded by the ctx gene (Saravanan et al., 2007). Testing the strains for the presence of gene encoding the cholera toxin can be used to confirm the choleragenic nature of Vibrio cholera strains associated with seafoods (Karunasagar et al., 1995). Kumar et al. (2009e) investigated the antibiotic resistance pattern of *V. cholerae* non 01 and Non 0139 species isolated from seafood samples. The *V. cholerae* samples displayed multiple antibiotic resistance. The prevalence of *V. cholerae* in seafood in the Mangalore region were carried out using conventional microbiological techniques by Karunasagar et al. (1992a); Ahamed et al. (1995). Molecular techniques such as PCR are very useful tools for the detection of pathogenic strains of *V. cholerae* and *V. parahaemolyticus* in aquaculture systems (Gopal et al., 2005). Saravanan et al. (2007) studied the prevalence of *V. cholerae* in the aquatic environment and seafood in and around Mangalore in southern India using conventional microbiological methods and PCR. A highly sensitive multiplex PCR was

developed to detect genus-specific rpo A gene and cholera toxin-producing ctxA and Rtx gene from the toxin-producing strains of *V. cholerae* in the environmental samples (Jeyashekharan et al., 2011). It can be concluded that the presence of *V. cholerae* in fish and fish products are inevitable. Only way out is the hygienic handling, processing and proper cooking of fish and fishery products.

Vibrio vulnificus

Vibrio vulnificus is seafood borne pathogen. The pathogenic bacterium *V. vulnificus* is widely distributed in estuarine waters throughout the world (Parvathy et al., 2005). The species *V. vulnificus* has been divided into three sub groups based on their biochemical properties; the biotype three causes wound infection in humans during handling of fish and it causes mortality to persons with liver diseases and hemochromatosis (Rajapandian et al., 2009). It also causes high mortality rate when compared with other species (Gopal et al., 2005). The seasonal distribution of *V. vulnificus* in Indian tropical waters has been studied, studies suggests that the density of *V. vulnificus* is controlled more by salinity than temperature and is higher during summer (Parvathi et al., 2004; Rajapandian et al., 2009).

The prevalence of *V. vulnificus* in cultured shrimps on the farming phase has been studied (Bhaskar & Setty, 1994). There are only limited reports available in the world regarding the pathogenicity of *V. vulnificus* (Thampuran & Surendran, 1998). The prevalence of this organism in coastal waters and shellfish in India has been reported earlier (Karunasagar et al., 1987b; 1990) and a clinical case has also been documented (Saraswathi et al., 1989). Several PCR-based methods have been described for the detection of *V. vulnificus* in seafood. Parvathy et al. (2004; 2005) developed an 18 h enrichment method in APW followed by nested PCR for the detection of *V. vulnificus*. A PCR system that uses gyrB sequences to detect *V. vulnificus* in tropical Seafood has been reported (Venkateswaran et al., 1998; Kumar et al., 2006). RAPD and *gyrB* sequencing has been used to study the presence of genetically distinct groups of *V. vulnificus* in tropical waters (Parvathy et al., 2005). The health risks caused by the widespread presence of *V. vulnificus* in Indian waters are not known as only very few epidemiological studies have been conducted in India, Further studies are needed to understand the ecology and virulence of *V. vulnificus* strains in Indian waters.

Listeria monocytogenes

Listeriosis is an important bacterial disease caused by the intracellular pathogen, *Listeria monocytogenes* and has been recognized as a food-borne disease since 1981. *L. monocytogenes* is Gram positive, facultative food borne pathogen of humans and animals (Dhama et al., 2013) capable of surviving under refrigeration conditions, low pH and in high salt concentration (Gandhi & Chikindas, 2007). In human, the early stage of infection by *L. monocytogenes* generally displays initial flu–like symptoms such as chilling, nausea, fever and gastroenteritis. Untreated cases may lead to septicemia, meningitis, encephalitis, abortion and occasionally death (Barbuddhe et al., 2008). Most susceptible persons for listeriosis are immunocompromised hosts such as pregnant women, and elderly people. The presence of *L. monocytogenes* has been documented in the tropical environment (Parihar et al., 2008; Das et al., 2013). Earlier reports suggested the absence of *L. monocytogenes* in tropical fish (Fuchs & Surendran, 1989; Manoj et al., 1991; Karunasagar et al., 1992b; Kamat & Nair, 1994). The results by Jeyashekaran et al. (1996) suggest that the absence of *L. monocytogenes* in tropical fish reported earlier could be due to inadequate methodology used. Although India has not experienced any major outbreak of listeriosis, sporadic cases in human have been reported and *L. monocytogenes* has been previously isolated from different food products including seafood. The details of *L. monocytogenes* isolated from various seafood samples and environments in different parts of India are given in Table 3.

Biofilms of *L. monocytogenes* are of particular concern; since the biofilm form makes elimination of *L. monocytogenes* from the food processing plants difficult (Tirumalai, 2013). Rapid detection of even low levels of this organism in fish can be achieved with PCR (Kumar et al., 2012). PCR has been used to confirm *L. monocytogenes* in seafood from Goa using hlyA gene (Gawade et al., 2010). PCR has also been used for RAPD analysis of *L. monocytogenes* in fresh dry and smoked seafood (Dhanashree et al., 2003)

L. monocytogenes has several important virulence markers. Among them, Listeriolysin O (LLO) is one of the important marker encoded by hlyA gene and is essential for disruption of phagocytic vacuole and release of bacteria into cytoplasm (Kumar et al., 2012). In particular, ELISA-based formats designed to detect its major virulence factor listeriolysin

Table 3. *Listeria monocytogenes* isolated from various samples and locations in India

Location	Fish/Shellfish	Wild/Farmed	Source of contamination	References
Mangalore	F	-	Fish samples/ Handling areas	Manoj et al., 1991
Mangalore	F/SF	W-	Fish samples Environment Samples	Jeyasekharan et al., 1996; 2003
Mangalore	F/SF	W/F	Fish/Clam samples	Dhanashree et al., 2003
Goa	F/SF	W/F	Bivalves/Prawn/Finfish	Gawade et al., 2010
Nagpur	F	W	Fish Samples	Jallewar et al., 2007
Mysore	F	-	Fish Samples	Moharem et al., 2007
Goa	F	W	Fish samples	Parihar et al., 2008
Hyderabad	F	-	Fish Samples	Kumar et al., 2012
Hyderabad	F	-	Fish Samples	Kumar et al., 2012
Mangalore	F	W	Fish samples	Vinothkumar et al., 2013
Kerala	F/SF	W/F	Seafood samples/ Mud/ Sand/Ice	Das et al., 2013
Tuticorin	F	W	Environment samples	Selvaganapathi, 2018

(LLO), have been developed for diagnosis of listeriosis in animals (Barbuddhe et al., 1999a) and humans (Barbuddhe et al., 1999b). Jallewar et al. (2007) amplified the virulence associated genes of *L. monocytogenes* like plcA, actA, hlyA and iap for its rapid detection. Multiplex PCR have been using in the simultaneous detection of *E. coli* O157: H7 and *L. monocytogenes* (Mukhopadhyay & Mukhopadhyay, 2007).

Since fish and fishery products may be a vehicle for *L. monocytogenes*, it is important to have information on the incidence of this pathogen. In India, only a few surveys have been conducted to assess the presence of *Listeria* sp in seafood. Isolation of *L. monocytogenes* from seafood suggests that there is a risk of acquiring listeriosis through seafoods in India. However, the epidemiological data available on listeriosis in India to date is not adequate for assessing the extent of infection in human beings and animals. The disease largely remains undiagnosed and under reported, largely due to the lack of a reliable, rapid and simple diagnostic test (Barbuddhe et al., 2004) and also a lack of a mandatory notification of listeriosis.

L. monocytogenes will be killed by cooking and raw or semi-raw seafood (graved or cold-smoked) are not consumed in India. However, *L. monocytogenes* in raw seafood may pose a health risk in kitchen if contaminating cooked food or other ready-to-eat food. Considering outbreaks avoidance of consumption of insufficiently cooked seafoods by at-risk populations is recommended. Diligent enforcement of sanitary conditions of food contact surface and handling areas and personal hygiene practices should reduce the potential contamination of fishery products *by L. monocytogenes* at the retail level (Parihar et al., 2008).

Escherichia coli

Fish and fishery products are frequently contaminated with aerobic enteropathogens (Singh & Kulshrestha (1993; 1994) and Sharma et al. (1995; 2006) and on few occasions the consumption of these infected fish and their products have resulted in serious diarrheal illnesses (Saxena et al., 1987). *Escherichia coli* serogroup 0157 is one of the most important emerging foodborne pathogens worldwide (WHO, 1997). Members of Enterobacteriacae especially *E. coli* are frequent causes of food poisoning outbreaks due to their ability to produce one or more enterotoxin (Sharma et al., 2005). Infections due to *E. coli* 0157:H7 can result in severe bloody diarrhoea (Haemorrhagic Colitis, HC) and in life-threatening complications such as (HUS) Haemolytic Uraemic Syndrome (Kumar et al., 2001). Though *E. coli* contamination of tropical seafood is quite common (Kumar et al. 2001; 2005; Thampuran et al., 2005), the distribution of different pathogenic types in seafood is poorly studied except for a

document on the presence of Shiga toxin-producing *E. coli* (Kumar et al., 2001) in Indian seafood.

Saxena et al. (1987) detected O: 8 *E. coli* serotype from fresh water fish. Sharma et al (2006) has detected two different serotypes of *E. coli* O: 8 and O:106 from fish market in Ludhiyana. Out of the different *E. coli* strains isolated from fresh water fish by Singh & Kulshrestha (1994), 30.7% were enterotoxigenic. The highest percentage of *E.coli* 0157 isolated from India during 1996-2005 were from Seafood (8.4%) (Sehgal et al., 2008).

The incidence of *E. coli* in fresh finfish has been reported by many workers (Lakshmanan et al., 1984; Rao & Gupta, 1978; Anand et al., 2002). Anand et al. (2002) reported an *E.coli* count in the range of 0-l00 cfu g^{-1} in fresh finfishes landed in Tuticorin fishing harbour. Thampuran et al. (2005) isolated *E. coli* in finfish samples acquired at the retail market in Cochin. Kumar et al. (2001) investigated the occurrence of STEC in fresh fish, shellfish and meat sold in open markets in Mangalore, India. Two out of 60 fish samples and two out of the 48 clam samples were positive for stx and hlyA genes by PCR. STEC strains belonged to non- O157 serogroups. They concluded that the seafood could be a vehicle for transmission of STEC even in tropical countries. Kumar et al. (2005) determined the prevalence of *E. coli* in tropical seafood and documented a prevalence of 47% for faecal coliforms including *E. coli. E. coli* was isolated from fishes grown in sewage-fed farms and also from retail market fishes of Kolkata that were contaminated with faecal matter of animal and human origin (Manna et al., 2009)

Enterohemorrhagic *E. coli* O157:H7 in shrimp from India was first reported by Surendraraj et al. (2010). Hatha et al. (2003) evaluated the microbiological quality of shrimp products for export trade produced from aquacultured shrimp, observing the high pre-valence of *E. coli* in headless shell-on shrimps. *E. coli* was also detected in cooked, peeled tail-on shrimp samples. Antibiotic resistant *E. coli* have been isolated from seafood in Tuticorin coast. (Jeyasanta et al., 2012).

The presence of pathogenic *E. coli* in seafood reflects secondary contamination, as *E. coli* are known to be associated with the gastrointestinal tract of warm-blooded animals and not known to be present in the environment as a natural flora. Sewage contamination of fish harvesting areas is the major reason for the presence of *E. coli*, but contamination can occur through the use of non- potable water or ice in the landing centers or fish markets, unhygienic handling and storage leading to off-smell, physical damage, building up of bacterial load and contamination with dirt and objectionable microorganisms (Sugumar et al., 2004; Kumar et al., 2005). Testing of seafood for the presence of *E. coli* is still a gold

Table 4. *Escherichia coli* found in different seafood samples in India

Location	Fish/shellfish	Wild/Farmed	Source of contamination	Reference
Cochin	F/SF	-	Processing Plant	Rekha Devi et al., 2002
Cochin	Fish	Wild	Retail Market	Thampuran et al., 2005
Cochin	F/SF	Wild	Retail market	Surendraraj et al., 2005
Kolkata	Fish	Wild	Fish market	Manna et al., 2008
Cochin	F/SF	Farmed	Ponds	Surendraraj et al., 2010
Tuticorin	F	W	Landing centre/ Markets	Jeyasanta et al., 2012
Punjab	F	-	Retail Markets	Gupta et al., 2013
West Bengal	F/SF	W/F	Markets	Dutta et al., 2015
Navi Mumbai	F	-	Markets	Visnuvinayagam et al., 2016
Kerala	F/SF	-	Retail markets	Murugadas et al., 2016a
Mumbai	F/SF	W	Retail market/ landing centre	Prabhakar et al., 2017
Gujarat	F	-	Market/ Processing centre	Sivaraman et al., 2017b
Mumbai	F/SF	-	Retail Market	Visnuvinayagam et al., 2017
North East India	F	F	Aquaculture Farms	Siddhnath et al., 2018

standard used to assess the faecal contamination of seafood processing plants in India and elsewhere (Jeyasekaran et al., 1990). High level of faecal indicator bacteria was also reported both in fish and in other samples from Cochin fisheries harbour area and retail markets of Mumbai (Iyer et al., 1986). Kumar et al. (2004) characterized STEC strains isolated from seafood and beef in Mangalore by bead-ELISA, vero cell cytotoxicity assay, PCR and colony hybridization for the detection of stx1 and stx2 genes. STEC has not been yet recognized as major human pathogen in India (Wani et al., 2004).

Staphylococcus aureus

Staphylococcal food poisoning due to the consumption of fish and its products has been reported from all parts of the world. In India, where a proper system of reporting of foodborne illness is non-prevalent there have been few reports of staphylococcal food poisoning (Sajeev & Surendran, 1996). Staphylococcal food poisoning is one of the most prevalent causes of gastroenteritis worldwide, which is caused by the ingestion of food that contains preformed toxins (Jablonski & Bohach, 2001). Of the many extracellular toxins, staphylococcal enterotoxins (SE's) pose the greatest risk to consumer's Health (Simon & Sanjeev, 2007). The foods involved in outbreaks are canned, smoked and salted products, frozen fishery product, boiled fish paste and fish sausages which inhibit the growth of competing organisms (Sanjeev et al., 1986). Simon & Sanjeev (2007) reported the presence of SEA and SEC toxin types in fishery products collected from Cochin, Kerala.

In India various authors have reported the prevalence of enterotoxigenic *S. aureus* in fresh fish and shellfish and various fishery products (Visnuvinayagam et al., 2015; 2016; Bujjamma & padmavathi, 2015; Kumar et al., 2016, Murugadas et al., 2016b). The presence of *S. aureus* has been reported during the different stages of seafood processing at the seafood plants in Cochin (Rekha et al., 2002). The presence of *S. aureus* has been reported in fermented fish products of North-East India (Thapa et al., 2004). Multiple antibiotic resistant *S. aureus* strains have been isolated from different seafood samples (Sanjeev et al., 1985; Manoharan et al., 2008; Visnuvinayagam et al., 2015; Sivaraman et al., 2017a). Only limited data is available about the incidence of enterotoxigenic *S. aureus* strains in fishery products. Staphylococci and especially coagulase positive staphylococci do not constitute the normal flora of fresh marine fish, but only get contaminated either from handlers or from the surface with which they come in contact (Sanjeev et al., 1985; Sanjeev & Iyer, 1988). The presence of virulent Methicillin Resistant *S. aureus* (MRSA) in seafood and aquatic environment are well documented (Sindhu & Surendran, 2006; 2008; Murugadas et al., 2016a; Sivaraman et al., 2016). Murugadas et al., 2017 used Staphylococcal Protein Typing and Multilocus Sequence Typing (MLST) to isolate Methicillin Resistant *S. aureus* (MRSA).

As *S. aureus* is an indicator of hygiene and sanitary conditions the presence of this organism indicates the unhygienic condition during processing, storage etc. The contamination of the product could be the result of combination of improper handling, improper storage and cross contamination. Therefore, the importance of proper handling and storage of seafood as well as the need to control the growth of enterotoxigenic strains of *S. aureus* needs to be emphasized.

Conclusion

Seafood is a major vehicle for transmission of several bacterial diseases. Human infections due to many pathogenic bacteria are reported to have been transmitted through fin fish, shell fish and other sea food products. The role of seafood sector in providing economic and nutritional security is very large in India. The rising demand for seafood nationally and internationally further leads to production of unscrupulous, under-processed and unhygienic products that may harbor various species of bacterial and viral seafood-borne pathogens. The presence of these food borne pathogens causes huge monetary loses to the fisherman and the exporters.

Major bacterial pathogens that are present in the Indian seafood are Salmonella, Vibrios, *L. monocytogenes*, *E. coli*, *S. aureus.* Salmonella serovars are causative agent of the largest number of enteric infections to humans. Many factors including inadequate supplies of clean water, inadequate sanitary measures, lack of food hygiene and food safety measures have been responsible for increased incidence of food borne salmonellosis. Table 1 summarizes the microbiological criteria of certain bacterial pathogens in raw and cooked fish in various countries. Food borne outbreaks due to

V. parahaemolyticus have been reported in many parts of the country. In 1993, *V. cholera* serogroup O139 made an explosive appearance and caused a severe epidemic in the Indian continent. The pathogenic bacterium *V. vulnificus* is widely distributed in estuarine waters throughout the world. The health risks caused by the widespread presence of *V. vulnificus* in Indian waters are not known as only very few epidemiological studies have been conducted in India. Sporadic cases of Listeriosis in humans have been reported in India. In India, only a few surveys have been conducted to assess the presence of *Listeria* sp in seafood. Since fish and fishery products may be a vehicle for *L. monocytogenes*, it is important to have information on the incidence of this pathogen. The epidemiological data available on listeriosis in India to date is not adequate for assessing the extent of infection in human beings and animals. Members of Enterobacteriacae especially *E. coli* are frequent causes of food poisoning outbreaks due to their ability to produce one or more enterotoxin. In India, the distribution of different pathogenic types of *E. coli* in seafood is poorly studied.

The presence of pathogenic *E. coli* in seafood reflects secondary contamination, as *E. coli* are known to be associated with the gastrointestinal tract of warm-blooded animals and not known to be present in the environment as a natural flora. Testing of seafood for the presence of *E. coli* is still a gold standard used to assess the faecal contamination of seafood processing plants in India and elsewhere. In India, where a proper system of reporting of foodborne illness is non-prevalent there have been few reports of staphylococcal food poisoning. As the *S. aureus* is an indicator of hygiene and sanitary conditions the presence of this organism indicates the unhygienic condition during processing and storage. Therefore, the importance of proper handling and storage of seafood as well as the need to control the growth of enterotoxigenic strains of *S. aureus* needs to be emphasized.

Even with the rising importance of fish food in human diet, studies related to prevalence of common foodborne pathogens in fish have been relatively less explored in India. Scarcity of data added to the lack of public awareness and dearth of resources for diagnosis and documentation of fish foodborne pathogens has made matter worse. In this context, a need for enforcement of disease surveillance and monitoring programmes is immediate. Technological advancements in the diagnostics especially the molecular techniques have made the detection of the bacterial pathogens in seafood easier. Strict adherence to the food hygiene practices from catch, storage, processing and also during transportation and preparation of food should be mandated to minimize the growth and spread of pathogens. Strenthening the public health education system and promoting the mass awareness programmes can act as effective mechanisms to achieve the goal.

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