

Seafood-borne Bacterial Pathogens

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The increasing demand for fish and fishery products and the development in international fish trade have raised major concern about the quality and safety of the products internationally traded. The safety of seafood products varies considerably and is influenced by a number of factors such as origin of fish, microbiological ecology of the product, handling and processing practices and preparations before consumption. Worldwide, food poisoning outbreaks have increased continuously. Expansion of international trade, consumer demand for "lightly processed" foods, mass production of foods, and changes in eating habits have all contributed to these food poisoning outbreaks. Seafood may be a vehicle for many bacterial pathogens. These seafood diseases caused by bacterial pathogens or their toxins, are associated with improper food preparation, handling and storage. This paper reviews the present scenario on occurrence of seafood borne pathogens in India and discusses in the light of international data.

Key words : Bacterial pathogens, prevalence, seafood, food -borne diseases, fishery products

Seafood is an important part of diet in many parts of the world and there is an ever increasing demand for fish and fish products around the world. The seafood trade is one of the world's largest and fastest growing international commodity industries. With increasing the seafood consumption—especially in developing countries – the amount or the value of the seafood products traded in this region are being expanded over time. World fish production (catches of wild fish plus production in aquaculture) has increased steadily to 132 million tons in recent years (FAO, 2000; MPEDA, 2003). India is the sixth-largest producer of fish in the world and the second-largest producer of freshwater fish. Of the total fish production of 5.9 million mt in 2002, about 36% is accounted by aquaculture in India. There

is substantial evidence that seafoods are high on the list of foods associated with outbreaks of foodborne diseases (Huss *et al.* 2003).

Seafood-borne outbreaks

Fish, shellfish, and other marine organisms are responsible for at least 1 in 6 food poisoning outbreaks with a known etiology in the United States. In other parts of the world, the impact of seafood poisoning is even greater. In the period 1971-1990 seafood was the single most important vehicle in food poisoning outbreaks in Korea (32%) and Japan (22%), where seafood was responsible for 43% and 62%, respectively, of outbreak-related fatalities (Chan 1995; Lee *et al.* 1996). Worldwide, food poisoning outbreaks have increased continuously. Expansion of international trade,

consumer demand for "lightly processed" foods, mass production of foods, and changes in eating habits were the main reasons for this. It has been estimated that as few as 1% of the actual cases of food-borne diseases are reported because many go undetected or unreported (Mossel 1982). This is because neither the true vehicle for the disease agent is identified nor the victim/ the physician are aware of the etiological role of foods. Thus, continued vigilance to protect the public from food-borne disease remains a necessity.

Disease outbreaks from fish and shellfish in Japan, US, and Europe are shown in table 1. In United States, crabs, shrimp and oysters are

Table 1. Etiological agents of food-borne illness outbreaks associated with fish and shellfish.

Pathogenic bacteria	Seafoodborne outbreaks		
	UK ^a 1992-1999	Japan ^a 1987-1996	US ^a 1990-1998
<i>Salmonella</i> spp.	14	69	17
<i>Vibrio</i>			
<i>parahaemolyticus</i>		707	18
<i>cholerae</i>		3	1
<i>vulnificus</i>			1
<i>Escherichia coli</i>		19	1
<i>Shigella</i>			2
<i>Campylobacter</i>			
<i>jejuni</i>	3	4	2
<i>Clostridium botulinum</i>			
Type E		9	14
<i>Clostridium perfringens</i>	3	9	2
<i>Staphylococcus aureus</i>	1	71	1
<i>Bacillus cereus</i>	1	2	3

^aCaico 1998, ^a Huss *et al.*, 2003

the most often implicated sources of pathogens that naturally occur in marine or freshwater environments and most illnesses are associated with eating under-cooked or raw shellfish, particularly raw molluscan shellfish (Huss *et al.* 2003). Other seafood problems are normally due to recontamination or cross-contamination of cooked product with raw product, followed by time/temperature abuse. In Canada, 56 percent of the outbreaks were attributed to bivalves and fish. In Japan, for products of fish and shellfish,

Vibrio parahaemolyticus, *Salmonella*, *Staphylococcus aureus*, *Escherichia coli* and *Clostridium perfringens* are the leading causes of illness (Table 1). The Food and Veterinary Office of the European Union has also initiated a Rapid Alert System for food to inform member states about problems or risks concerning foods which do not meet food safety requirements. In 1999, 107 seafood products were involved in Rapid Alerts (out of 295 total). Chilled and Frozen fish/ fish products were implicated in 75 of these Alerts (Huss *et al.* 2003) and the reason was the presence of pathogenic bacteria (*Vibrio* spp., *Salmonella*, *L.monocytogenes*, *Staphylococcus*). Shrimp, crab tails, cray-fish tails were implicated in 30 Alerts and the reason was always the presence of pathogenic bacteria (pathogenic *Vibrio* spp., *Salmonella*, *Staphylococcus*). The number of Alerts has increased steadily during 1999-2001 and basically exploded in 2002 (Huss *et al.* 2003). In 39.7% of Alerts, microbial contaminants (mainly *V.parahemolyticus*, *Salmonella* and *V. cholerae*) were the main cause. Exporting countries from Asia, accounted for 69.8% of the Alert Cases, followed by Africa (17.8%), the Americas (8.8%), Europe (2.7%) and Oceania (0.9%).

Seafood borne bacterial pathogens

Seafood may be a vehicle for many bacterial pathogens (Feldhusen, 2000). At least ten genera of bacterial pathogens have been implicated in seafood-borne diseases. Shellfish, especially the filter feeding bivalve mollusks (oysters, scallops, mussels, clams, cockles) can accumulate pathogenic bacteria in the alimentary tract. Since the alimentary tract of these bivalves forms the major edible portion for humans, these mollusks can serve as extremely effective vehicles for a wide range of organisms pathogenic to humans.

Seafood-borne pathogenic bacteria may conveniently be divided into 3 groups (Table 2) according to their ecology and origin as those

Table 2. Bacterial pathogens associated with seafoods

Group	Bacterial pathogens
I. Pathogenic bacteria indigenous to aquatic environment	<i>Vibrio cholerae</i> , <i>Vibrio parahaemolyticus</i> , <i>Vibrio vulnificus</i> , <i>Clostridium botulinum</i> types B,E,F, <i>Plesiomonas shigelloides</i> , <i>Aeromonas hydrophila</i>
II. Pathogenic bacteria indigenous to general environment	<i>Listeria monocytogenes</i> , <i>Clostridium botulinum</i> types A and B, <i>Clostridium perfringens</i> , <i>Bacillus cereus</i> (only toxigenic strains).
III. Pathogenic bacteria in the animal/ human reservoir	<i>Salmonella</i> spp., <i>Shigella</i> spp., Pathogenic <i>Escherichia coli</i> , <i>Campylobacter jejuni</i> , <i>Yersinia enterocolitica</i> (very few pathogenic serotypes), <i>Staphylococcus aureus</i>

who are indigenous to: 1. the aquatic environment. 2. the general environment. 3. the animal/human reservoir (Huss *et al.* 2003). The level of indigenous (aquatic and general) bacterial pathogens in fish is generally quite low, and when fish products are adequately cooked, food safety hazards are insignificant. Pathogenic bacteria can be introduced into marine waters mainly by commercial shipping activity through fouling, ballast water from ship tank systems, oil fall, discharge of sewage and sediment. Pathogens can also be introduced to coastal waters through sewage (raw or partially treated) from industrial and domestic outfalls, disposal of domestic waste, recreational activities and sediment discharge from rivers. Animal manure is the most significant vector of introduction of bacterial pathogens to aquaculture ponds and aquatic environment. The organisms associated with faecal contamination of seafood continue to pose a large-scale health threat through seafood consumption. Food safety issues associated with cultured fish and shrimp differ from region to region and from habitat to habitat and vary according to the method of production, management practices and environmental conditions (Reilly, 1998).

The safety concerns related to bacterial pathogens in seafood is shown in Table 3 and 4. The mere presence of pathogens from the aquatic and general environment is of no safety concern, not even in ready-to-eat (RTE) products

(Huss *et al.* 2003) because they occur in low numbers and their infectious dose is generally high ($>10^3$ CFU). In contrast, the presence of pathogens from the animal/ human reservoir is a serious safety concern for products to be eaten without (further) cooking as their infectious dose is low. Growth of pathogens is likewise a serious safety concern for most RTE products. For raw fish products to be eaten raw the safety concern is limited. Growth of these pathogens is only possible at elevated temperatures ($>5^{\circ}\text{C}$) and at this condition spoilage will proceed very rapidly and the fish will probably be rejected due to off-odours and off-flavours long before being either toxic or infective organisms reach high numbers.

Pathogens indigenous to aquatic and general environment

Vibrio spp.

Vibrio spp. are frequently isolated from seafood particularly, shellfish, and a number of these organisms are human pathogens (Huss 1994). Infections caused by *Vibrio* species are largely classified into 2 distinct groups: *Vibrio cholera* infection and noncholera *Vibrio* infections. Historically, the noncholera *Vibrio* species were classified as halophilic or nonhalophilic, depending on their requirement of sodium chloride for growth. *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* are universally recognised as important human pathogens; with *V. hollisae*, *V. alginolyticus*, *V. fluvalis* and *V. mimicus* each responsible for a smaller, but substantial, number of infections (Morris, 1999).

V. cholerae is the aetiological agent of cholera (Greek term meaning "flow of bile"), the most feared epidemic diarrheal disease that can cause death in healthy people. *V. cholerae* is noninvasive, affecting the small intestine through secretion of an enterotoxin. Only two cholera serotypes (*V. cholerae* 01 and 0139) have been shown to cause the disease. *V. cholerae* is generally associated with freshwater and

Table 3. Seafoodborne illness associated with bacterial pathogens indigenous to aquatic and general environment

Pathogenic bacteria	Seafood transvector	Minimal infectious dose	Clinical presentation	Importance
<i>Vibrio parahaemolyticus</i>	Crustaceans, fish,	10^5 - 10^6 cfu/g	Diarrhoea, nausea, Vomiting.	Gastroenteritis Bivalve molluscs high risk.
<i>Vibrio cholerae</i> Serovar O1 and O139	Prawn, squid, shellfish, seafoods	10^2 to 10^8 - 10^9 cfu/g	vomiting, Diarrhoea,	cholera, epidemic in Asia, Africa, South America, small hazard for healthy people.
Non- O1 strains <i>Vibrio vulnificus</i>	Fish, mussels, oysters, Clams, shrimp	10^5 - 10^6 cfu /g	Weak Diarrhoea Wound infection, death by septicaemia	highest mortality rate, leading cause of seafood related infections and shellfish associated deaths in the US.
<i>Clostridium botulinum</i> Type E	Fish, shellfish, smoked / VP fish products	0.1- 1m g toxin lethal dose	weakness, paralysis, death due to respiratory paralysis	Botulism, fatal food poisoning, Greater than 65% of cases fatal, little risk for products stored < 3°C
<i>Aeromonas</i> spp.	Shellfish seafood	10^6 - 10^7 cfu/g	Diarrhoea, vomiting	Gastroenteritis (at risk immunocompromised)
<i>Plesiomonas shigelloides</i>	Fish, shellfish	> 10^6 cfu/g	watery diarrhoea	Gastroenteritis mild self limiting disease
<i>Listeria monocytogenes</i>	Raw seafood, Smoked / salted fish	> 10^2 cfu/g	diarrhea, vomiting and nausea.	listeriosis
<i>Bacillus cereus</i>	Seafood, squid, prawn	10^6 - 10^9 cfu/g	diarrhea nausea, Vomiting	Cause either Diarrheal illness or Emetic illness
<i>Clostridium perfringens</i> Japan.	sporadic, fish/ shellfish	10^6 - 10^8 cfu/g	Diarrhoea, seldom lethal	some diarrheal illness cases in USA,

Table 4. Seafoodborne illness associated with bacterial pathogens animal/ human reservoir

Pathogenic bacteria	Seafood transvector	Minimal infectious dose	Clinical presentation	Importance
<i>Salmonella</i> spp.	Prawn, fish, molluscs	10^2 cfu/g depends on species	Diarrhoea, vomiting, fever.	Salmonellosis. Normally small risk.
<i>Shigella</i>	Fish, molluscs	10^1 - 10^2 cfu/g	Severe watery / bloody diarrhea, fever, cramping.	Shigellosis (Gastroenteritis) Shellfish related infections in the US
<i>Yersinia enterocolitica</i>	Fish/ shellfish	10^7 - 10^9 cfu/g	Diarrhoea, Vomiting. Fever	Yersiniosis. Shellfish and fish related outbreaks
<i>Campylobacter jejuni</i>	molluscs	10^1 - 10^2 cfu/g	Diarrhoea	Gastroenteritis, Infections in USA, small hazard.
<i>Escherichia coli</i>	Fish/ shellfish contamination by food handlers	< 10^1 - 10^{10} depends on strain	Diarrhoea, Fever depends on strain	Vomiting Infectious dose low in certain strains, therefore their mere presence in the product must be avoided. Children are more susceptible.
<i>Staphylococcus aureus</i>	Seafood contamination from infected persons	10^5 - 10^6 cfu /g 0.14 -0.19 m g toxin /kg Wt	Vomiting, diarrhea and abdominal cramps.	enterotoxins cause intoxication - gastroenteritis Frequency of intoxication not known

estuarine environments. A cholera epidemic caused by *V. cholerae* O139 serogroup erupted in Madras, southern India and these strains were later isolated from Calcutta and Bangladesh (Albert *et al.*, 1993; Ramamurthy *et al.*, 1993b).

Marinated raw fish dish (ceviche), crabs and undercooked seafood or shellfish were implicated epidemiologically as a vehicle of

transmission of cholera cases (Lin *et al.*, 1986). *V. cholerae* non-O1, strains that lack the genetic potential for causing epidemic disease, were isolated from 14% of freshly harvested oyster lots in the US (Twedt *et al.* 1981., Wallace *et al.* 1999). *V. cholerae* non-O1 was isolated from fish from Cochin and Mangalore in India (Table 5). *V. cholerae* O1 and non-O1 was isolated in

Table 5. Indigenous bacterial pathogens prevalent in fresh and processed fish/ shellfish from tropical Indian waters

Pathogenic bacteria	Fresh		Processed		Reference
	Sample analysed	% samples positive	Sample analysed	% samples positive	
<i>Vibrio parahaemolyticus</i>	Fish/shellfish Shrimp oysters	5- 35 83 10	Frozen fish Iced shellfish	4 —5 28-32	Karunasagar <i>et al.</i> 1984; Sanjeev and Iyer 1986; Prasad and Rao 1994; Sanjeev <i>et al.</i> 2000; Sudha <i>et al.</i> 2003; Varma <i>et al.</i> 2003 Deepanjali <i>et al.</i> 2005; IEAE 2005.
<i>Vibrio cholerae</i> O1 non-O1	- Fish/shellfish	- 8 - 23	Frozen fish	ND- <1 ND - 27	Mathew <i>et al.</i> 1989; Sindhu and Surendran 2005; Prasad and Rao 1994; Varma <i>et al.</i> 2003; IEAE 2005.
<i>Vibrio vulnificus</i>	Fish/shellfish oysters	5-18 43	Frozen fish/ shellfish Iced shellfish	4-7 7-26	Prasad and Rao 1994; Thampuran and Surendran 1998; Parvathi Ammini <i>et al.</i> 2005 Sanjeev <i>et al.</i> 2000; Sanjeev and Mukundan, 2003
<i>Clostridium botulinum</i>	Fish/shellfish	17-24	Frozen fish Cured fish	15 10	Lalitha 1999; Lalitha and Gopakumar 2000; Lalitha and Surendran, 2003.
<i>Aeromonas</i>	Fish/shellfish	100	Frozen fish	66	Thampuran and Surendran 1998
<i>Plesiomonas shigelloides</i>	shellfish	present	-	-	Annual report , Central Institute of Fisheries Technology 2003-2004
<i>Listeria monocytogenes</i>	Fish/shellfish	ND- 17	Frozen fish	ND	Fuchs and Surendran 1989; Jayasekharan <i>et al.</i> 1996; Karunasagar and Karunasagar 1999; Anu Kamat and Dilip Bongirwar 2001; Varma <i>et al.</i> 2003; IEAE 2005.
<i>Bacillus cereus</i>	Fish/shellfish	19- 24	Frozen fish	0-11	Nambiar and Surendran 2003; Sanjoy Das <i>et al.</i> 2005
<i>Clostridium perfringens</i>	Fish/shellfish	4 -65	Prawn -Frozen Canned	11ND	Nambiar and Iyer 1973; Iyer <i>et al.</i> 1986 Lalitha <i>et al.</i> 1990; Lalitha and Surendran, 2003.

Table 6. Pathogenic bacteria in the animal/ human reservoir prevalent in fresh and processed fish / shellfish from tropical Indian waters.

Pathogenic bacteria	Fresh		Processed		Reference
	Sample analysed	% samples positive	Sample analysed	% samples positive	
<i>Salmonella</i>	Fish/shellfish Crustaceans	ND -17 17	Frozen fish/ shellfish	<1 -15	Lakshmanan <i>et al.</i> 1984; Iyer <i>et al.</i> 1986; Nambiar and Iyer 1990; Hatha and Lakshmanaperumalsamy 1997; Nambiar and Surendran 2003.
<i>Shigella</i>	Fish/shellfish	ND - 4	Frozen Iced/ dried fish/ shellfish	ND	Varma and Iyer 1987; Singh and Kulshrestha 1993; Sanjeev and Mukundan 2003.
<i>Yersinia enterocolitica</i>	Fish/shellfish	ND - 15%	Frozen / Dried fish/ shellfish/	ND-8	Anu Kamat and Dilip Bongirwar 2001; Nambiar and Surendran 2003 ; IEAE 2005
<i>Escherichia coli</i> O157:H7	Fish/shellfish	30- 48 ND	Frozen fish/ prawn Squid/ cuttle fish	ND - 49 20	Kumar <i>et al.</i> 2001; Nambiar and Surendran, 2003; Rekha Devi <i>et al.</i> 2003; Thampuran <i>et al.</i> 2005
<i>Staphylococcus aureus</i>	Fish/shellfish	9	Frozen fish / shellfish Cooked Frozen crab Dried Fish Frozen squid/ cuttle fish	5-14 100 47 10- 23	Sanjeev <i>et al.</i> 1985; Nambiar and Iyer 1990; Rekha Devi <i>et al.</i> 2003; Nambiar and Surendran 2003; Varma <i>et al.</i> 2003; IEAE 2005. Sindhu and Surendran (In press)

brackish water cultured shrimp in Southeast Asia (2 and 33%) and in cultured shrimp in India (Reilly, 1998). Hazards from *V.cholerae* can be prevented by cooking seafood thoroughly and by preventing cross-contamination once the seafood is cooked.

V.parahaemolyticus is a halophilic bacterium native to marine or estuarine environment. It is a well known enteropathogen responsible for food-borne gastroenteritis worldwide. *V.parahaemolyticus* is an invasive organism affecting primarily the colon.

V. parahaemolyticus is associated with the consumption of inadequately cooked or refrigerated crustaceans and fish. In recent years, the incidence of *V. parahaemolyticus* infection has been increasing in many parts of the world, and this has been attributed to the emergence of a new clone of the O3:K6 serotype carrying only the *tdh* gene. (Matsumota *et al.* 2000). In Japan, it has been implicated as a cause of at least a quarter of total foodborne diseases. In India about 10% of the cases of gastroenteritis in patients admitted to the Infectious Diseases Hospital in Kolkata are due to *V. parahaemolyticus* (Deb *et al.* 1975). In the United States, oyster-associated *V. parahaemolyticus* outbreaks are more common than other shellfish-associated *V. parahaemolyticus* outbreaks (Huss *et al.* 2003). There have been reports of incidence (Baffone *et al.* 2000) of virulent strains of *V. parahaemolyticus* in seafood, including crayfish, lobster, shrimp, and crab. In India, there are very few reports on the disease outbreak from *V. parahaemolyticus*. Morris (1999) reported the isolation of this organisms from 3.5–23.9% of patients with diarrhoea. According to Karunasagar *et al.*, (1991) certain food poisoning outbreak cases, has been linked with consumption of seafood carrying *V. parahaemolyticus*.

The level of *V. parahaemolyticus* in Indian fishery products varies considerably. *V. parahaemolyticus* has been isolated from fresh (10–35%) and processed seafood (5%) in India (Table 5). In the study carried out in India (IAEA, 2005), 28% of the shrimp samples from the Non-EU plants were also positive for *V. parahaemolyticus*.

V. parahaemolyticus is sensitive to low temperatures. The minimum temperature limiting growth is 5°C and growth is possible at temperatures >15 °C. Therefore, by keeping food at temperatures below 5°C (cold holding/chilling), is the best way to prevent or slow down the growth. But there is also report on

the survival of *V. parahaemolyticus* at 3.5°C under starvation conditions (Jiang & chai, 1996). Hazards from *V. parahaemolyticus* can be controlled by thoroughly cooking seafood and preventing cross-contamination after cooking. Control of time/temperature abuse is also an important preventive measure.

V. vulnificus is a potentially lethal foodborne pathogen found in marine or estuarine environments, causing a rapidly fatal infection in persons with pre-existing liver disease, hemochromatosis (iron overload) or compromised immune systems. *V. vulnificus* can cause wound infections and primary septicaemia. Biotype 1 of this bacterium can be lethal to humans, but biotype 2 may be an opportunistic pathogen for humans.. The organism is the leading cause of seafood-related infections and of shellfish-associated deaths in the US (Morris, 1999). In Denmark, 11 clinical cases of *V. vulnificus* infection were reported during the warm summer (Hoi *et al.* 1998). It is implicated in infections due to consumption of raw or undercooked oysters, raw clams, octopus and other marine fish and shellfish Said *et al.* (1998) reported a wide variety of wound infections due to *V. vulnificus* in Israel.

Several studies have reported the presence of *V. vulnificus* in fresh and processed fish/shellfish from Mangalore and Cochin along the West Coast and in seafood from Kakinada in the East Coast of India. (Table 5). Hazards from *V. vulnificus* can be controlled by thorough cooking of shellfish and by preventing cross-contamination once the seafood is cooked.

Clostridium botulinum

Clostridium botulinum is an anaerobic pathogen, associated with foodborne botulism, a potentially lethal neuroparalytic disease caused by ingesting preformed botulinum neurotoxin (Hauschild, 1989). The disease caused by *C. botulinum* is intoxication. Symptoms of botulism may include visual

impairment (blurred or double vision), loss of normal mouth and throat functions, weakness or total paralysis, respiratory failure, which is usually the cause of death. The organism produces seven neurotoxins that are designated as types A through G based on their serological specificity. The types pathogenic to humans (types A, B, E and F) can conveniently be divided into two groups namely 1). the proteolytic types A, B and F, which are also heat resistant, mesophilic, and have the general environment as the natural habitat, 2). the non-proteolytic types B, E and F, which are heat sensitive, psychrotolerant, and have the aquatic environment as the natural habitat.

C.botulinum is widely distributed in ocean sediments, the intestinal tracts of fish, and the gills and viscera of crabs and other shellfish (Hauschild, 1993). *C.botulinum* type E is most common on fish and fishery products. An outbreak of fish-borne (ribbetz or kapchunka, faseikh) botulism caused by *C.botulinum* type E was reported in Israel, New York and Cairo (Hauschild, 1993). Hot-smoked Canadian whitefish produced in Finland were implicated in botulism outbreaks in 1997 (Korkeala *et al.* 1998). Five percent of 214 vacuum-packaged, and 3% of 123 air-packaged fishery product samples were positive for the neurotoxin type E gene. Semi-preserved seafoods including smoked, salted and fermented fish, have also been identified as causes of botulism. *C.botulinum* has been isolated from fresh (17-24 %) and processed (10-15%) fish and shellfish (Table 5) from India (Lalitha, 1999; Lalitha & Gopakumar, 2000; Lalitha & Surendran, 2002, 2003a).

The ability of *Clostridium botulinum* to survive under adverse conditions in the environment, adapt and eventually multiply in foods makes it a major food safety hazard. Psychrotrophic *C.botulinum* is of particular concern in chilled fishery products because it grows at temperatures as low as 3-5 °C and

produces little noticeable evidence of spoilage. Proteolytic *C.botulinum* may still be able to grow if temperatures are >10°C. At temperatures below 10°C there is no risk of toxin production by proteolytic *C. botulinum* types A and B. Proper thermal processes for canned seafood destroy the spores of non -proteolytic type E (90 °C, 10 min.) proteolytic (121°C, 10 min) and the heat-labile toxin (80 °C, 6 min.). Normal household cooking and frying of raw fish products are therefore sufficient to destroy any pre-formed toxin. This may be one of the reasons for the excellent safety record of unprocessed fish with respect to problems from *C.botulinum*. Heavy salting or drying to reduce the water activity below 0.93 and fermentation or acidification to below pH 4.7 are effective means of preventing *C. botulinum* growth.

Aeromonas spp.

Aeromonas species such as *A. hydrophila*, *A. sobria* and *A. caviae* has been described as an emergent food-borne pathogen of increasing importance causing gastroenteritis. *Aeromonas* spp have been linked to two major group of human diseases: extra intestinal infections in patients having chronic underlying disorders (mainly by strains of *A.veronii* subsp. *sobria* and *A.hydrophila*) and gastroenteritis in immunocompromised, aged or infant populations. (Kirov, 2001). Besides gastroenteritis, *A.hydrophila* may causes cholera-like infections (Huss, 1994). This group is considered as controversial gastro intestinal pathogen because definite proof of enteropathogenicity on human volunteer studies or animal model is lacking (Kirov, 2001).

The *Aeromonas* species are natural member of aquatic environments (Huss *et al.* 2003) Hence this group is very commonly found in fish and fish products of marine, brackish water or fresh water origin. *A.hydrophila* is a very resistant microorganism that can survive in food items stored in cold for a period

of time with out losing its pathogenicity (Diesterweg, 1992). Oysters have been implicated in foodborne disease. *Aeromonas* associated diarrhoea has been reported from Bombay, Calcutta, Goa, Vellore, Pondicherry and Chennai in India (Alavandi & Ananthan, 2003).

Some *Aeromonas* spp. are psychrotrophs and some of them are enteropathogenic. Swiss results show 10.9 14.3% positive samples from hot-and coldsmoked fish and 10.5% positive samples from gravled salmon. 61.2% of the identified strains were *Aeromonas hydrophila*, followed by 22.5% *Aeromonas caviae* and 16.3% *Aeromonas sobria* (Gobat & Jemmi, 1993). In Taiwan, of the 66 seafood samples examined, 25.8% were found to be contaminated with *A. hydrophila* (Tsai & Chen, 1996). They showed that very high percentages of the isolates from seafood are able to produce hemolysin (79.2%) and cytotoxin (91.7%). In investigations from Finland (Hanninen *et al.* 1997), *Aeromonas* spp. were identified in 93% samples of fish, in 16% of shrimp and in 100% freshwater samples. Three strains of *A. hydrophila* HG 2 and HG 3 were implicated in two seafood-borne (frozen shrimp) outbreaks. In India, high percentages of the isolates from frozen fish (42%) was identified as *A. hydrophila* (Table 5) compared to that from fresh fish (21%). Psychrotrophic *Aeromonas* strains are able to grow at 4-5°C and to produce toxins in oysters at 5°C (Tsai and Chen, 1996). Combination of chilling, Salting and /or acidification are effective means of preventing growth of *Aeromonas*.

Plesiomonas shigelloides

Plesiomonas shigelloides is an emerging pathogen, mostly associated with water, both fresh water and seawater in warm weather. The organism is predominantly associated with seafood (Kirov, 2001; Huss *et al.* 2003). It has been implicated as being a pathogen in water-associated outbreaks of intestinal and extra-intestinal infections.

P. shigelloides was implicated as the aetiological agent causing diarrhoea after consumption of seafood in Hong Kong (Wong *et al.* 2000), freshwater fish in Zaire (Van Damme & Vanderpitte, 1980), and contaminated raw oysters and shellfish in the United States (Rutala *et al.* 1982; Holmberg *et al.* 1986). In a study on crab (*Scylla serrata*) in India (Anon 2004), the organism was detected in the body meat (Table 5).

Although *P. shigelloides* has been isolated from diarrhoeal patients incriminated in water / food borne outbreaks, negative results of volunteer studies and lack of definite virulence mechanism cast doubt on the enteropathogenicity of the species.

P. shigelloides does not grow at chill temperatures, but they can survive. Therefore, proliferation in foods can be controlled by chill storage or moderate salting/acidification.

Listeria monocytogenes

Listeria monocytogenes has been identified as the cause of listeriosis in humans more recently. Listeriosis is of particular risk and can be lethal for foetuses, pregnant women, neonates and immuno compromised persons. Severe listeriosis can cause meningitis, abortions, septicemia and a number of other maladies, some of which may lead to death. Four sporadic cases of seafood-borne listeriosis (Baker *et al.* 1993) has been reported. Shrimp or shellfish implicated listeriosis outbreaks was reported in the United States, New Zealand and Australia. Several studies indicate that humans can be carriers of *L. monocytogenes*; a prevalence between 5–10% has been indicated (Gledel, 1987).

The reported incidence of *L. monocytogenes* in fish and fishery products (cooked and frozen crabs and shrimp, surimi and smoked fish) from temperate areas of the world varies from very low to 50% (Fuchs & Reilly, 1992; Embarek, 1994). In India, *L. monocytogenes* could not be isolated from fresh and processed seafoods

from Cochin and Mumbai while incidence of 1-3% was reported from Mangalore (Table 5). Like other bacteria *L. monocytogenes* can create a biofilm on stainless steel surfaces (Herald & Zootola, 1988) and food receiving a heat treatment can be recontaminated in the production environment.

L. monocytogenes is halo- and psychrotolerant and can grow well in refrigerated foods. Hazards from *L. monocytogenes* can be prevented by thoroughly cooking seafood and by preventing cross-contamination once the seafood is cooked. Since the infective dose of *L. monocytogenes* is thought to be small, time/temperature abuse of food products may not be necessary to result in illness.

Clostridium perfringens

Clostridium perfringens is an anaerobic enteric pathogen, associated with general environment soil, water, foods and intestines of animals and humans (Labbe, 1989). The organism has been implicated as the etiological agent in many food poisoning outbreaks. It is widely distributed in aquatic environments, intestines of animals and humans all over the world. In terms of number of cases of bacterial foodborne illness reported each year, *C. perfringens* generally ranks second in Canada and the United Kingdom and third in the United States (Huss, 1994., Mc Clane, 2001). Fish are not commonly involved in food-poisoning outbreaks due to this organism. However, both raw and processed seafoods were implicated in *C. perfringens* food poisoning outbreaks in United States and Japan (Taniguti, 1971., Caico, 1998).

This organism was isolated from 2-3% of the seafood samples from San Francisco and Seattle retail markets in USA (Abeyta, 1983). It has been detected in high percentage (68-88%) in fish from Japan (Taniguti, 1971) and India (Table 5).

Food poisoning caused by *C. perfringens* may occur when foods such as meat, fish or poultry are cooked and held without maintaining adequate heat or refrigeration before serving. Control measures emphasize proper food preparation and storage techniques, especially temperature control.

Bacillus cereus

Bacillus cereus is an aerobic pathogen, present in general environment such as soil, dust and water. The organism causes two types of illness – diarrhoeal and vomiting (emetic) types. *B. cereus* associated foodborne illness accounted for 2% of outbreaks with confirmed aetiology that were reported to the Centers for Disease Control and Prevention of the US FDA during 1973–1987 (Bean & Griffin, 1990). In eight *B. cereus* food poisoning outbreaks reported in USA, rice and shellfish were involved.

Contamination of fresh and processed fish by *B. cereus* has been reported in India (Table 5). Measures to reduce or eliminate the threat of food poisoning by *B. cereus* include avoid holding cooked foods at room temperature, cool foods below 45°F (7.2°C) within 4 hours of preparation and hold/store hot foods above 140°F (60°C) until served.

Pathogens indigenous to the human/ animal reservoir

***Salmonella* spp.**

Salmonella are among the most important causes of gastrointestinal disease globally. *Salmonella* are distributed in the gut of man and animals and polluted environments. *Salmonella* food infection causes nausea, vomiting, abdominal cramps and fever. The infective dose in healthy people varies according to serovars, foods involved and susceptibility of the individuals (Huss *et al.* 2003). Outbreaks of *Salmonella* food infection have been associated with raw oysters, salmon, tuna salad, shrimp cocktail, stuffed sole and gefilte fish.

Surveys in Japan, southeast Asia and US has shown *Salmonella* species in eel culture ponds (21%), in cultured prawn (16%) and in freshwater cultured (5%) catfish (Saheki *et al.* 1989, Reilly & Twiddy 1992; Wyatt *et al.* 1979). Aquatic birds have been suggested as one possible means of spreading this organism from area to area (Huss *et al.* 2003).

In UK, *S. typhi* was detected in more than 1.6% of shellfish sampled from open harvesting waters (Rippey, 1994; Anon, 1991; Lipp & Rose, 1997). *Salmonella* was isolated from 6-17% fresh fish/ shellfish samples from Cochin and Coimbatore markets and 9-15% frozen fish samples from cold storages at Cochin and fish processing units in Mumbai as given in table 5. In a study on different seafood products processed in six industries (three European Union approved (EUA) and three EU non-approved (EUN)) in India, 16.7% of cuttle fish, 28.5% of the shrimp and 40% of the squid from EUN industries were found positive to *Salmonella*. However, no positive samples of *Salmonella* spp. were found in the EUA plants (IAEA, 2005).

The predominant serovar in Europe was *S. enteritidis*. In the US, non-typhoidal salmonellae have been associated with fish and shellfish, *S. paratyphi* and *S. enteritidis* with shrimp and bivalves. *S. typhi* was the most common bacterium associated with shellfish vectored disease. *S. weltevreden* was identified as the principal serotype found in ponds, and to a lesser extent *S. anatum* (11%) *S. wandsworth* (8%) and *S. potsdam* (8%). In India, *S. typhi* was the most predominant among *Salmonella* isolates and *S. paratyphi* B biovar Java heavily contaminated samples of marine prawns in Calcutta markets (Boonmar *et al.* 1998). Reilly and Twiddy (1992) have reported that strains isolated from most human cases of salmonellosis appear to be different from those found in farmed products, leading to the conclusion that these products constitute a very low risk to public health.

The infective dose of *Salmonella* is relatively high for healthy individuals and very low for the elderly or medically compromised individuals. Time/temperature abuse has been identified as contributing factor in many of the outbreaks. But illness could result even without time/temperature abuse in the sensitive group. Hazards from *Salmonella* can be prevented by holding chilled seafood below 4.4°C, preventing post-cooking cross contamination. People who are carriers or suffering from salmonellosis should not be allowed to work in food processing operations to prevent cross-contamination.

Shigella spp.

Shigella produces an illness called Shigellosis, which causes mild diarrhea, fever, abdominal cramps and severe fluid loss. Its presence in the environment is associated with fecal contamination. *Shigella* is transferred to seafood through sewage pollution of the coastal environment or by contamination after harvest. *S. dysenteriae* causes epidemic shigellosis. Seafood (shrimp-cocktail, tuna salads) has been the cause of a number of outbreaks of shigellosis.

In the US, *Shigella* was implicated in 111 shellfish related cases and four outbreaks from 1898 to 1990 (Rippey, 1994). FDA reported seven cases of *Shigella* infections associated with seafood per year and 200 estimated cases per year (FDA, 1994; Lipp & Rose, 1997). In India, this organism was isolated from 4% of the 185 seafood samples (Table 6). *Shigella* could not be detected in any of the processed fish samples analysed in Cochin, India (Varma & Iyer, 1987; Sanjeev & Mukundan, 2003). *Shigella* may be an important potential disease agent as it has a low infectious dose and long survival time in clams and oysters (Anon, 1991).

Hazards from *Shigella* can be prevented by eliminating human waste contamination of water supplies and by improved personal hygiene for people who are ill or are carriers of *Shigella* and work in food operations.

Pathogenic *Escherichia coli*

Most of the *E. coli* strains are harmless commensals. However, some strains are capable of causing disease either intestinal or extra-intestinal. *Escherichia coli* is a normal inhabitant of the gastrointestinal tract of man and animals. Commensal enteric *E. coli* may be the natural reservoir of pathogenic strains (Falkow, 1996). *E. coli* food infection causes abdominal cramping, water or bloody diarrhea, fever, nausea, and vomiting. Most infections appear to be related to contamination of water or handling of food under unhygienic conditions. Strains responsible for intestinal diseases are thought to cause much of their pathology in the small intestine. Pathogenic *Escherichia coli* is a group of bacteria, including enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), diffuse-adhering *E. coli* (DAEC), enteroaggressive *E. coli* (EAggEC), and enterohemorrhagic *E. coli* (EHEC/VTEC).

Human gastrointestinal illness caused by EPEC, EIEC and ETEC has been recognized for several decades. The DAEC and EAggEC cause various variants of diarrhoea. *E. coli* O157:H7 is the most common EHEC serotype. Shiga toxin-producing *E. coli* (STEC) O157: H7 infection, which can cause haemolytic uremic syndrome and death, is a global public health concern (Tarr, 1995). Pathogenic strains of *E. coli* are transferred to seafood through cross contamination or through dirty water. Where animal manures, particularly bovine, are used as pond fertilizers, there is a risk that pathogenic strains of *E. coli* may be present in the pond water (Reilly, 1998). In 1997, German investigations of seafood samples showed no contamination with *E. coli* O157:H7. In India, Kumar *et al.* (2001) reported occurrence of shiga-toxigenic *E. coli* (STEC) in fresh seafood marketed in Mangalore (Table 6). Thampuran *et al.* (2005) did not find this organism in 280 raw unprocessed and 134 frozen fish samples

collected respectively from retail markets and cold storages in Cochin.

Hazards from *E. coli* can be prevented by heating seafood sufficiently to kill the bacteria, holding chilled seafoods below 4.4° C, preventing post cooking cross contamination, and prohibiting people who are ill from working in food operations. The infective dose of *E. coli* is dependent upon the particular strain from only a few organisms to millions. For this reason, time/temperature abuse of food products may or may not be necessary to result in illness.

Yersinia enterocolitica

Yersinia enterocolitica causes gastrointestinal disease yersiniosis characterized by appendicitis-like symptoms, including fever and abdominal pain, accompanied by diarrhoea or vomiting. Outbreaks have been associated with oysters and fish. Strains of *Y. enterocolitica* have been identified in fish and shellfish in both wild and aquaculture settings (Anon., 1991; Nedoluha and Westhoff, 1995). In India, *Yersinia enterocolitica* was detected in fresh and processed seafood samples (Table 6) analysed from retail markets situated in Mumbai (Kamat .A and Bongirwar.D 2001). Nambiar and Surendran (2003) could not detect the organism in any of the fresh seafood samples from retail markets in Cochin.

Yersinia is a psychrotrophic bacterium. Hazards from *Y. enterocolitica* can be Prevented by: heating seafood sufficiently to kill the bacteria, holding chilled seafoods below 4°C, preventing post-cooking cross-contamination, salting and acidification of food.

Campylobacter spp.

Campylobacter jejuni, an emerging foodborne pathogen, is the leading cause of diarrhea worldwide and the second most common cause in the United States. Campylobacteriosis is the name of the illness

caused by *C. jejuni* which is also often known as campylobacter enteritis or gastroenteritis. The organism has also been associated with other types of infection, including bacteremic and central nervous system infections in humans. *C. jejuni* inhabits the intestinal tracts of wild and domestic animals. Birds, especially farmed poultry, are the major *Campylobacter* reservoir. An estimated four million *C. jejuni* infections occur each year in the US. The organism is transmitted through contaminated foods (including raw clams, mussels and oysters), person-to-person contact and contaminated water.

In Ireland thermophilic *Campylobacter* spp. were found in 42% of 380 shellfish. (Arumugaswamy & Proudford 1987). *Campylobacter* was detected in 14% of the Oyster flesh and an outbreak has been ascribed to raw clams in the US (Huss *et al.* 2003). In India, occurrence of *Campylobacter* in seafoods is not reported.

Hazards from *C. jejuni* can be controlled by thoroughly cooking seafood and by proper food-handling practices. Since the infective dose of *C. jejuni* appears to be small (Nachamkin, 1997), time/temperature abuse of food products is not necessary to result in this illness.

Staphylococcus aureus

Staphylococcus aureus food poisoning is caused by the ingestion of food that contains one or more *S. aureus* enterotoxins. The disease caused by *S. aureus* is an intoxication characterized by nausea, vomiting, abdominal cramping, watery or bloody diarrhea, and fever. The natural habitat of *S. aureus* is warm-blooded animals including humans. *S. aureus* does not appear as a part of the natural microflora of newly caught fish. However, enterotoxigenic *S. aureus* strains are transferred from food handlers.

S. aureus has been isolated at levels of 2-10% in fish and bivalves but much more commonly in cooked, handled crustaceans

where as much as 24-52% of samples may be positive (Huss *et al.* 2003). *S. aureus* has been reported at levels of 2-10% in fresh seafoods (Table 6). Sanjeev and Iyer (1988) isolated *S. aureus* from palms and throats of workers from fish processing factories. Food poisoning may occur if the product is handled carelessly during processing, resulting in high multiplication ($>1 \times 10^5$ cfu g⁻¹) (Varnam & Evans, 1991; Vishwanath *et al.*, 1998). In a study on different seafood products processed in six industries (three European Union approved (EUA) and three EU non-approved (EUN)) in India, 14.3 % of shrimp and 40 % of the squid samples of EUN plants in India were positive to *S. aureus* (IAEA, 2005).

S. aureus elaborates an enterotoxin on improperly stored seafood, especially if the fish is garnished with cream sauces or mayonnaise (Saavedra-Delgado & Metcalfe, 1993). Growth and toxin formation may easily be prevented by proper chilling of products. Hazards from *S. aureus* can be prevented by: minimizing time/temperature abuse of seafood, especially after cooking, and requiring that food handlers engage in proper hygiene.

Conclusion

In par with rapid strides in food preservation techniques, the safety of the consumer is gradually diminishing. Although seafood was regarded as comparatively safe among other foods, recent surveys indicate that seafood poisoning outbreaks are also steadily increasing all over the world. Apparent absence of illness or pathogen does not guarantee safety of the consumer. Food is a dynamic system and microbial growth in food is a complex process governed by environmental, physiological and genetic factors. Hence relative risks for each type of food or process vary considerably and now it is possible to assess the risk in quantitative data providing critical insight into food - microbe interaction for eliciting better strategies for

human health and consumer safety. Reducing the number of seafood-related outbreak requires continued and coordinated efforts by several agencies involving water quality, disease surveillance, consumer education, sea food harvesting, processing and marketing.

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