

14. Microbial Safety of Fishery Products

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Introduction

Fish and fishery products constitute an important food component for a large section of world population, more so in developing countries, where fish forms a cheap source of protein. In the last two decades there has been an increase in awareness about the nutritional and health benefits of fish consumption. The low fat content of some fish and the presence of poly unsaturated fatty acids in red meat fishes which are known to reduce the risks of coronary heart diseases, have increased the dietary and health significance of seafood consumption (Din et al., 2004). In USA, seafood consumption has increased from an average of 4.5 kg per person in 1960 to about 7.0 kg per person in 2002 (NOAA, 2003). Total fishery production in 2001 was reported to be 130.2 million tonnes, of which 37.9 (30%) million tonnes was from aquaculture, 90% of which came from Asian countries. On the other hand, seafood is also known to have been responsible for a significant percentage of food borne diseases. With increased fish consumption, there is also an increase in the number of food-borne illness associated with fishery products. In the United States, 10-19% of food-borne illness involved seafood as a vehicle and between 1993 and 1997, 6.8% of the food-borne illnesses involved consumption of fish and shellfish (FAO, 2004). In Japan, where seafood consumption is high and raw fish consumption is popular, 70% of food-borne illness is attributed to seafood. These data highlight the importance of seafood as vehicles for human illness.

However, the true incidence of seafood-borne diseases worldwide is not known, as there is no surveillance system in the developing countries. In addition to being responsible for human health problems, the presence of infectious agents in seafood results in rejection of seafood consignments leading to major economic losses to exporting countries in general, and to seafood processing industries in particular. Maintenance of seafood quality is central to ensuring the safety of seafood. This has been a major area of concern for seafood processing industries and a great challenge to scientists and researchers worldwide. This review focuses on agents

and causes of human illness encountered in seafood and issues related to seafood safety and quality management.

Causes of seafood-borne illness

Seafood often harbour infectious agents that are present naturally in the aquatic environment or introduced through human activities. The illness may be due to the infection caused by the microorganism or due to the intoxication by products of microorganisms. Viruses, bacteria and parasites are the agents of human disease associated with seafoods. The products of microorganism such as bacterial toxins, algal toxins or products of bacterial metabolism such as histamine can cause intoxication when fish or shellfish harbouring these products are consumed. In the case of live finfish, microorganisms may be associated with gill, gut and skin. Filter feeding shellfish like mollusks concentrate different types of microorganisms present in the environment around them. Some of the microorganisms of human health concern may be native to the aquatic environment such as the members of the genus *Vibrio*, but others like *Salmonella*, *E. coli* and many viruses are introduced into the aquatic environment through human activities such as contamination by domestic sewage. The type of microorganism associated with seafood may vary depending upon whether it is fresh or processed. Most of the outbreaks of illnesses occur in countries where seafood is eaten raw or is inadequately cooked. For example, in Japan where seafood is eaten raw, 70% of food borne human illness is seafood associated (Scoging 2003).

Viruses associated with seafoods

Viruses constitute an important cause of seafood borne diseases. The intensity of human illness is known only from developed countries. For example in the USA, it is estimated that 80% of food borne illness are due to enteric viruses (Sair et al., 2002). The major limitation in diagnosing viral infections is the non-availability of techniques or facilities for detecting or identifying viral pathogens. The recent advances in

molecular biology has helped to develop or improve the detection techniques for viruses but these are still not used commonly in developing countries.

Viruses being obligate intracellular parasites cannot multiply in seafood, but molluscan shellfish concentrate these infectious agents in their tissues. This is the reason why molluscan shellfish are associated with most of the well-recorded outbreaks. Nevertheless, very low numbers (1-100) of viruses are known to be capable of causing infections in humans making these organisms all the more important as agents of human illness. The transmission is through fecal-oral route with infected individuals becoming source of viruses to the aquatic environment.

The viruses causing human infections are classified into two groups, viral gastroenteritis and viral hepatitis (Caul, 2000). The illnesses due to viruses are mainly prevalent in countries where eating raw shellfish is a dietary habit. Though there are more than 110 different viruses known to be excreted in human feces, collectively called the "enteric viruses" (Goyal, 1984), hepatitis A virus and Norwalk viruses are most commonly involved in seafood related illnesses followed by rotaviruses and astroviruses which cause infections in children. The most common seafood associated human viruses and bacteria are listed in the Table 1.

Hepatitis A virus (HAV) is a single stranded RNA virus that is classified in the genus Hepatovirus in the picornaviridae family and constitutes the most important group of viral pathogen in seafood. The virus has worldwide distribution and several outbreaks of human illness associated with oyster or clam consumption has been reported from countries such

as Italy, USA, Austria, China and Sweden (Butt *et al.*, 2004a). A major outbreak of hepatitis A in Shanghai, China involved 300,000 cases linked to consumption of shellfish from a sewage polluted area and this constitutes the largest foodborne disease outbreak ever reported. This virus is associated with gastrointestinal tracts of humans, and the infection is transmitted by faecal-oral route. This virus is more resistant to chlorination than other enteric viruses. The infections by this viral group can lead to severe hepatitis with serious liver damage. Jaundice, as the disease is well known, is the clinical symptom followed by headache and vomiting. The incubation period is about 4-6 weeks. Shellfish such oysters and clams accumulate HAV in their tissues and are most commonly associated with infections. Filter feeding shellfish can concentrate the viruses several fold in their tissue and the infectious dose is low (10-100 viral particles). Consequently shellfish harvested from areas negative for coliform counts may transmit the diseases (Butt *et al.*, 2004a). HAV are known to survive for extended periods of time in seawater (Crocini *et al.*, 1999). Infected food handlers can also be important sources of contamination for seafood.

Though laboratory adapted Hepatitis A virus grows in cell culture, wild type strains are difficult to isolate by culture. Reverse transcriptase polymerase chain reaction (RT-PCR) has been used for detection of hepatitis A virus in shellfish. The hepatitis A virus has a highly conserved genome with a single serotype (Lees, 2000). Hence primer design for RT-PCR has been rather straight forward and the targets for amplification have been the capsid protein VP1 (Atmar *et al.*, 1993) or VP1-VP3 protein interphase (Jaykus *et al.*, 1996).

Table 1: Common viral and bacterial agents associated with seafood borne illnesses

Viruses	Bacteria
Hepatitis causing viruses	<i>Vibrio</i> spp
Hepatitis A virus	<i>V. parahaemolyticus</i>
Hepatitis E virus	<i>V. vulnificus</i>
	<i>V. cholerae</i>
Gastroenteritis causing viruses	<i>Clostridium botulinum</i>
Human caliciviruses	<i>Salmonella</i>
Noroviruses	<i>Listeria monocytogenes</i>
Sapporo viruses	<i>Aeromonas hydrophila</i>
Rotavirus	<i>Campylobacter</i> spp
Parvoviruses	<i>Pleisiomonas shigelloides</i>
Sapporoviruses	
Astroviruses	
Coronaviruses	
Human Enteroviruses	
(polioviruses,	
groups A and B coxsackieviruses,	
and echoviruses)	

Hepatitis E Virus

This virus is endemic in many developing countries causing both outbreaks and sporadic cases. Most cases of non-A, non-B hepatitis have been attributed to this virus. This virus shares morphological and biophysical characters of caliciviruses (single stranded RNA viruses showing icosahedral symmetry). However, due to significant genomic differences, hepatitis E virus has not been assigned to any taxonomic group (Lees, 2000). A number of genotypes linked to geographical regions can be differentiated (Tsarevo *et al.*, 1999). The virus causes an acute, self-limiting liver disease, but in contrast to hepatitis A, there could be high mortality rate in pregnant women. Most hepatitis E outbreaks are associated with contaminated drinking water and direct evidence for association with shellfish is lacking (Lees, 2000). However, shellfish would be at risk of contamination and could act as a vector for transmission of the virus.

Human caliciviruses

There are single stranded RNA viruses with positive polarity. The viruses show icosahedral symmetry and the exterior surface is made up of a single major protein. Two genera infect humans: Noro viruses previously called Norwalk-like viruses or small round structured viruses and Sapporo viruses. The RNA genome of noroviruses contain three open reading frames. The other genera Sapporo virus has RNA genome with two open reading frames (Butt *et al.*, 2004a). Norovirus was first discovered in 1972, being isolated from a clinical case in Norwalk, Ohio, U.S.A. Presently this virus is the leading cause of non-bacterial gastroenteritis in USA accounting for about 52% of such cases. Norovirus outbreaks have been associated with consumption of oysters, mussels, cockles and clams (Butt *et al.*, 2004a). The wide prevalence of this virus is indicated by the presence of serum antibodies in 70.83% of the population in different parts of the world (Butt *et al.*, 2004a; Koopmans and Deizer, 2004). In contrast to other viral agents causing gastroenteritis such as rotavirus, astroviruses and adenovirus that affect mainly children, norovirus can affect all age groups. The virus is excreted in the feces of infected persons and is able to persist in the environment. Infected individuals may shed the virus for over 1 year and this suggests that food handlers may be source of contamination in foods (Butt *et al.*, 2004a). Sewage contamination of shellfish growing waters is the major reason for seafood borne outbreaks (Berg *et al.*, 2000). The infectious dose of the virus is believed to be < 100 viruses (Berg *et al.*, 2000) and attack rates can be as high as 83% (Kohn *et al.*, 1995) and the detection of

the virus is difficult because it is not culturable. Foodborne outbreak caused by norovirus has been reported from India (Girish *et al.*, 2002).

Detection of norovirus in shellfish can be accomplished using RT-PCR amplifying the viral RNA polymerase gene (Jiang *et al.*, 1992; Atmar *et al.*, 1995; Jaykus *et al.*, 1996). Epidemiological studies have suggested the diversity of Norovirus strains, though recent reports have suggested existence of two broad genogroups (Lees, 2000). Nevertheless, genomic regions with sufficient commonality to permit design of primers reacting with both groups have been documented (Lees, 2000). However, the major problems for virus detection by PCR is the presence of PCR inhibitors in crude shellfish extracts (Lees, 2000). Therefore, a number of attempts to refine virus extraction and nucleic acid extraction have been reported (Lees *et al.*, 1995; Hayliges *et al.*, 1997). Depuration of shellfish is likely to reduce viruses levels and for public health protection, use of nested PCR for sensitive detection of this virus in shellfish has been reported (Green *et al.*, 1998). These primers react with both genogroup I and II and could be useful in shellfish monitoring programme.

Other viruses

Astroviruses (single stranded RNA viruses) have been implicated in food borne outbreaks, but direct epidemiological link with food consumption is lacking (Lees, 2000). Rotaviruses (double stranded RNA viruses) are the commonest cause of diarrhoea in children and have been detected in shellfish, but rotaviruses have not been linked to infectious disease outbreaks following seafood consumption (Lees, 2000). Similarly adenoviruses (double stranded DNA viruses) have been detected in polluted water and shellfish, but no shellfish related outbreaks have been reported, because this virus predominantly affect children, who are not primary consumers of seafood (Lees, 2000). Similarly enteroviruses (single stranded RNA viruses) including polioviruses, coxsackie viruses, echoviruses have been detected in shellfish, but there is no epidemiological evidence linking shellfish to enterovirus disease (Lees, 2000).

Strategies for prevention of seafood borne viral diseases

Fecal contamination of shellfish growing areas is the major route of entry for human pathogenic viruses into shellfish. Thus control of fecal contamination of shellfish growing areas would be a major strategy. Both European Union (EU) and USA have a system of classifying shellfish harvesting areas based on fecal

coliform levels. In EU, fecal indicators are measured in shellfish tissues, while in USA, indicators are measured in shellfish growing waters. Shellfish harvested from areas with < 14 fecal coliforms (geometric mean) per 100 ml water are designated by US FDA as “approved” while in EU samples of shellfish with < 230 *E.coli* or < 300 fecal coliforms per 100 g are classified as category A. Shellfish from these areas can be used for human consumption without further processing. Shellfish harvested from areas with higher fecal coliform or *E.coli* counts are to be used only after depuration, relaying or heat processing. In both EU and enterovirus genome USA, commercial depuration is subject to legal control and purified shellfish are required to comply with end product standard for shellfish sold live. However, human volunteers studies in Australia (Grohmann *et al.*, 1981) and natural outbreak studies in UK and USA (Lees, 2000) suggest that depuration may fail to eliminate enteric viruses from contaminated shellfish and that compliance with *E.coli* or fecal coliform standard does not guarantee absence of viruses. This is further

confirmed by a number of studies that show that viruses are eliminated at much slower rate compared to fecal coliforms (Schwab *et al.*, 1998)

Cooking shellfish is another alternative for public health protection. Millard *et al.* (1987) demonstrated that hepatitis A virus could be inactivated by more than 4 log₁₀ infectious units by raising the internal temperature of shellfish (cockle) meats to 85-90°C for 1 min. However, gastroenteritis caused by norovirus is the most common illness associated with shellfish consumption and heat inactivation data for this virus would be useful. The major problem in getting this data is that noroviruses can not be cultivated. Studies of Slomka and Appleton (1998) indicate that feline calicivirus is inactivated more readily than hepatitis virus. Though adequate cooking will reduce the risk of human infection with viruses, a study with multi-state outbreak of norovirus gastroenteritis associated with oysters suggested that home or restaurant cooking offered little or no protection (McDonnell *et al.*, 1997).

Table 2: Techniques used for detection of food borne viruses

Viruses	Technique	Comments	References
Hepatitis A virus	RT-PCR amplifying 192 bp genomic region corresponding to VP1-VP3 capsid protein interphase	PCR product confirmed by hybridisation with internal oligonucleotide probe	Jaykus <i>et al.</i> , 1996
	RT-PCR amplifying 248 bp fragment of VP1 capsid protein gene	PCR product confirmed by hybridisation with internal oligonucleotide probe	Atmar <i>et al.</i> , 1993, 1995
Norovirus	RT-PCR amplifying 260 bp fragment of viral polymerase	PCR product confirmed by hybridisation with internal oligonucleotide probe	Jaykus <i>et al.</i> , 1996
	RT-PCR amplifying 470 bp fragment viral polymerase gene	PCR product confirmed by hybridisation with internal oligonucleotide probe	Jiang <i>et al.</i> , 1992 Atmar <i>et al.</i> , 1995
	Nested RT-PCR amplifying the viral RNA polymerase gene	Can detect both genogroup I and II	Green <i>et al.</i> , 1998
Enterovirus	RT-PCR amplifying 197 bp fragment of the highly conserved 5' untranslated region of enterovirus genome	PCR products confirmed by hybridisation with internal oligonucleotide probe	Jaykus <i>et al.</i> , 1996

Bacterial pathogens associated with seafoods

The microflora of fish is a reflection of the flora of the aquatic environment in which fish live. Both pathogenic and non-pathogenic bacteria are present on the surface of fish, gills and intestine. The bacteria that result in the spoilage of fish are numerous, are of concern only with respect to the organoleptic quality of fish and will not be discussed here. The bacteria species that can cause human illness encountered in seafood fall under at least 10 genera which can be classified into three general groups (Reilly and Kaferstein, 1997, Feldhusen, 2000)-bacteria indigenous to the environment in which fish live (marine/estuarine waters) but pathogenic to man. E.g the members of family vibrionaceae such as *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*. The second group comprises of bacteria introduced via fecal contamination, which by far, includes members of the family enterobacteriaceae such as *Salmonella*, *Escherichia coli*, *Shigella* and *Campylobacter* spp. The third group consists of bacteria introduced while processing or storage such as *Listeria monocytogenes*, *Clostridium perfringens* and *Staphylococcus aureus*.

Vibrio cholerae

Vibrio cholerae is a natural inhabitant of fresh and estuarine waters. This species is not homogenous and over 200 serotypes can be recognized. Of these only serotypes O1 and O139 are associated with cholera. The non-O1/O139 are associated with cholera. The non-O1/O139 serotypes are rarely associated with human infections causing mild gastroenteritis or septicaemia (West, 1989; Powell, 1999). The non-O1/O139 diarrhoea is characterised by diarrhea, abdominal cramps, and fever. The disease is self-limiting in most of the cases. On the other hand cholera is characterized by severe diarrhea. There are several outbreaks of cholera linked to seafood (Glass and Black, 1992). There have been several studies on prevalence of *V. cholerae* O1 in shrimp and generally these indicate absence of this serotype (Karunasagar *et al.*, 1990, 2003; Otta *et al.*, 1999). Dalsgaard *et al.* (1995a) isolated *V. cholerae* O1 from 2% aquacultured shrimp in Thailand. Subsequent molecular studies indicated that these are non toxigenic (Dalsgaard *et al.*, 1995b). Choleraogenic *V. cholerae* are characterized by their ability to produce cholera toxin that is encoded by *ctx* gene (Kaper *et al.*, 1995). The *ctx* gene has been sequenced and molecular tests based on *ctx* gene has been used for specific detection of choleraogenic *V. cholerae* in seafoods (Karunasagar *et al.*, 1995; 1997). It is not possible to distinguish O1 from non-O1 by general laboratory techniques. Detection of cholera toxin (CT) by

immunoassays or the *ctx* gene (encoding for CT), are the only methods to determine the pathogenic potential of *V. cholerae*. *V. cholerae* has been reported in prawns and in water samples (Reilly and Twiddy, 1992; Karunasagar *et al.*, 2003). In shrimp farms, non-O1 and non-O139 strains are more frequent (Daalsgaard, 1998; Karunasagar *et al.*, 2003). Studies conducted in Peru during the 1991 outbreaks of Cholera suggests that adoption of HACCP in shrimp processing would reduce the risk of contamination. During the epidemic, *V. cholerae* O1 was detected in all of the five samples of raw seafood collected from street vendors in Lima and Callao but in only 1/1011 samples of seafood intended for export (DePaola *et al.*, 1993). Often, in developing countries, seafood marketed domestically is not handled with adequate care and presence of choleraogenic *V. cholerae* in seafood marketed in Malaysia has been reported recently by Elhadi *et al.* (2004). Thus for prevention of contamination of seafood with choleraogenic *V. cholerae*, it is important to adopt hygienic handling and processing. *V. cholerae* O1 is sensitive to cooking with the D value of 2.65 min at 60°C (ICMSF, 1996). Therefore adequate cooking could reduce the risk of getting cholera through seafood consumption.

Vibrio parahaemolyticus

V. parahaemolyticus is a marine/estuarine bacterium causing gastroenteritis in humans through consumption of seafood. This is the most important species incriminated in human disease associated with seafood consumption in Japan and the US. The abundance or presence of these organisms is influenced by water temperature and salinity in the temperate waters (Kaneko and Colwell, 1975; Kiiyukia *et al.*, 1989; DePaola *et al.*, 2003). Shellfish filter particulate matter from the surrounding water and thereby accumulate microorganisms in their tissues. Raw or undercooked shellfishes such as shrimp, clams, cockles, crab, lobster and scallops have all been implicated in transmission. *V. parahaemolyticus* can be isolated at levels exceeding 10³ /g in oysters (DePaola *et al.*, 2003) and at levels <10²/g in tropical shrimp (Karunasagar *et al.*, 1984). Not all *V. parahaemolyticus* are pathogenic. Pathogenicity is associated with ability to produce a thermostable direct hemolysin (TDH) or a TDH-related hemolysin (TRH) and more than 90% of clinical isolates, but less than 1% environmental isolates produce TDH (Joseph *et al.*, 1982; Honda *et al.*, 1988). Molecular methods such as PCR and oligonucleotide probe hybridization methods have been used for detection of pathogenic *V. parahaemolyticus* in oysters and other seafood (Yamamoto *et al.*, 1991; Tada *et al.*, 1992; Karunasagar *et al.*, 1996; DePaola *et al.*, 2003). Detection of *tdh*⁺ *V. parahaemolyticus* in association with tropical seafood has been reported (Dileep *et al.*, 2003). The

infectious dose of *V. parahaemolyticus* is 10^5 colony-forming units (cfu) or more. The symptoms of infection include abdominal cramps, severe watery diarrhoea and occasionally nausea, vomiting and fever. The infection lasts for a week and is self-limiting though treatment with antibiotics resolves the infection quickly.

V. parahaemolyticus multiplies rapidly at room temperatures and reaches hazardous levels. Cooking seafood to temperatures of about 65°C rapidly inactivates this organism (FAO, 2004). The organism can survive at in frozen foods (-20°C) for two months. The organism is heat sensitive and temperatures above 65°C are lethal. Since *V. parahaemolyticus* are present in seafood in numbers upto 10^4 per gram, prevention of multiplication of this organism in seafood is the first step in preventing the seafood associated hazards by this organism. In USA, seafoods harbouring 10^4 *V. parahaemolyticus* per gram are allowed (CDC, 1998), since the percentage of pathogenic strains among these would be low. However, the presence of pathogenic strains harbouring *tdh* or *trh* to numbers exceeding 100/ g may be considered dangerous for raw consumption.

Vibrio vulnificus

V. vulnificus a human pathogen capable of causing wound infections and septicaemia especially in immunocompromised individuals and in persons with underlying debilitations such as chronic liver disease. The infections often can be fatal. In US, 50% of seafood-associated deaths are due to *V. vulnificus*. There are two biotypes of which biotype 1 is human pathogenic while biotype 2 is pathogenic to eel. The general symptoms include fever, chills and nausea. People with severe alcoholism (or liver disfunction), in particular, are at risk. Infections generally occur due to exposure of wounds to seawater or by eating contaminated oysters (Oliver, 1989; Hlady and Klontz, 1996). *V. vulnificus* is widely distributed in temperate and tropical waters and numbers exceeding 10^3 /g can be found during certain periods in a year (Kelly, 1982; Karunasagar *et al.*, 1987, 1990). Unlike in temperate waters where the abundance of this organism has been correlated with the temperature of water (Kasper and Tamplin, 1993; Motes *et al.*, 1998), the abundance of this organism in tropical waters is influenced more by salinity than temperature (Parvathi *et al.*, 2004a). The organism can be enumerated by colony hybridization using probe binding to hemolysin gene and when present in low numbers can be detected by a combination of selective enrichment PCR approach (Parvathi *et al.*, 2004a, 2004b). *V. vulnificus* multiplies rapidly at temperatures 20 to 35°C in seafood reaching high numbers quickly. Rapid chilling of freshly harvested seafood will prevent

multiplication in seafood as the organism grows poorly at temperatures below 15°C (ICMSF, 1996). The number rapidly decreases in frozen storage.

Salmonella spp

Salmonella is one of the most important food-borne pathogens, being responsible for about half the reported cases and outbreaks of food borne diseases in US (Butt *et al.*, 2004a). There are several instances of rejection of shrimp from South East Asia due to contamination with *Salmonella*. It is generally believed that like *E. coli*, *Salmonella* are not present in the natural aquatic environment but are derived as a result of sewage contamination. However, unlike *E. coli*, *Salmonella* is associated with number of non-human hosts eg. reptiles (Winfield and Groisman, 2003). *Salmonella* has been reported to survive and persist in the aquatic environment. This bacterium has been detected in environmental soil samples collected from both agricultural and recreational areas (Winfield and Groisman, 2003). It has been reported that *Salmonella* can survive and even multiply in this ecosystem in contrast to *E. coli* that has a half life of 3 days in soil (Thomason *et al.*, 1975; Bagosian *et al.*, 1996; Winfield and Groisman, 2003). *Salmonella* has been detected in the gut of tilapia and carp (Iyer *et al.*, 1989; Ogbondeminu, 1993). In Japan, *Salmonella* could be detected in 21% of eel culture ponds (Saheki *et al.*, 1989) and in US, 5% of cultured catfish have been found to harbour *Salmonella* (Wyatt *et al.*, 1979). *Salmonella* has been detected in 16% of tropical prawns and in 22.1% of water and mud samples (Reilly and Twiddy, 1992). Using PCR, higher occurrence could be documented in tropical seafood (Kumar *et al.*, 2003). *S. weltevreden* is the main serotype present in seafoods. The presence of multiple drug resistant *Salmonella* can be very hazardous as the infection cannot be treated with normal drugs. Further, there are chances of transfer of drug resistance genes to other bacteria of the same or other families of bacteria. However, the presence of serotypes most commonly associated with clinical cases of salmonellosis are rarely encountered in seafood and therefore, the risk of *Salmonella* infection from seafood consumption needs to be ascertained. The high rate of *Salmonella* isolation from tropical seafood needs further investigation to determine if some strains constitute indigenous and thus non-pathogenic strains of *Salmonella* in seafood.

Escherichia coli

Traditionally, *E. coli* has been used as an indicator organism for the presence of other pathogenic bacteria in water and food. However, this perspective has changed with the identification of the ability of *E. coli* to cause human illness. Of late, more than 200 serotypes of *E. coli* are categorised under at least 5

pathogenic types- Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli*(ETEC), Enterohemorrhagic *E. coli* or Shiga toxin-producing *E. coli* (EHEC or STEC), Enteroinvasive *E. coli* (EIEC) and Enteroadherent/ aggregative *E. coli* (EAEC/EaggEC). Unlike *V. cholerae*, *Shigella dysenteriae* or *L. monocytogenes*, which are one-disease organisms, *E. coli* can cause a variety of different types of diseases which include diarrhea, dysentery, hemolytic uremic syndrome, bladder and kidney infections, septicemia, pneumonia and meningitis. Though *E. coli* contamination of tropical seafood is quite common (Kumar et al., 2004), the distribution of different pathogenic types in seafood is poorly studied except for a recent document on the presence of Shiga toxin-producing *E. coli* (Kumar et al., 2001) in Indian seafood. In general, different strains are associated with different diseases and the food processing industry is mainly interested in pathogenic *E. coli* strains that may be infective through the gastrointestinal route. Most strains of *E. coli* are not pathogenic and a food quality control laboratory should be able to differentiate such strains from pathogenic ones. 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 (Kumar et al., 2004). The use of animal manures can result in the contamination by certain pathogenic types such as Shiga toxin-producing *E. coli* for which bovine reservoirs have been identified. Therefore, it is important that all stages of fish production, handling and processing are monitored for *E. coli* contamination.

Listeria monocytogenes

The genus *Listeria* comprises of six species and these comprise *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. ivanovii* and *L. grayi* of which *L. monocytogenes* is an important food borne pathogen. The infection due to *L. monocytogenes* occurs in aged people, pregnant women and infants. The bacterium causes infection of the central nervous system and the severe septicemia which can be fatal in susceptible

population especially in older people and newborn babies. The infection of the pregnant women causes stillbirth, abortion or infections of the new born. The latter can result in fatal meningitis of the newborn. The organism is widely distributed in nature and is associated with soil, decaying plant material and abiotic surfaces. *L. monocytogenes* has been isolated from variety of seafood both fresh and processed, such as frozen seafood, smoked fish, surumi, etc. (Rocourt et al., 2000). The organism can grow at refrigeration temperature and can survive in the food processing environments. *Listeria* species can attach to and grow on a wide range of materials used in seafood processing plants. This ability of *Listeria* to colonise the food processing environments makes it a constant source of contamination. A zero tolerance level has been prescribed in the US for ready to eat products. Presence of *L. monocytogenes* in tropical seafood is rare compared to seafood from temperate environments (Embarek, 1994). Dhanashree *et al.* (2003) isolated *L. monocytogenes* from 0.95% of seafood samples (n=210) analysed. Experience from the salmon smoking industry in certain ready to use foods is unattainable and unrealistic requirement that would restrict food production and consumption without having a positive impact on public health (Feldhusen, 2000). *Listeria monocytogenes* can be readily eliminated from seafood products by applying heat (Doyle et al., 2001). In the case of smoked products, care must be taken to see that the products are not post process contaminated.

Human parasitic worms in seafood

Parasites are involved in substantial numbers of seafood related illness especially in countries where seafood is eaten raw (FAO, 2004). More than 40 million people are affected worldwide every year by trematode infections (WHO, 1995). Parasite worms are commonly associated with seafood and in certain countries of Eastern Asia some parasites are endemic. The parasites of concern belong to different groups, nematodes, cestodes and trematodes. Table 3 summarises important food borne parasites of concern.

Table 3: Parasites associated with seafood borne diseases

<i>Round worms (Nematodes)</i>	<i>Flukes (Trematodes)</i>	<i>Tape worms (Cestodes)</i>
<i>Anisakis</i> spp.	<i>Clonorchis</i> spp.	<i>Diphyllobothrium</i> spp.
<i>Gnathostoma</i> spp.	<i>Opisthorchis</i> spp.	
<i>Capillaria philippensis</i>	<i>Heterophyes</i> spp.	
<i>Angiostrongylus</i> spp.	<i>Paragonimus</i> spp.	
<i>Metagonimus yokagawai</i>		

The parasites have complex life cycles with a definite host (man or animals) and a primary intermediate host (generally snails) and a secondary intermediate host (generally fish or crustaceans). The sexual maturity and the release of eggs take place in man or animals (the definite host) while the larval stages are reached and completed in intermediate hosts. The larval stages in second intermediate host (fish or crustaceans) infect humans when fish or shellfish harbouring them are consumed raw. The larval stages in second intermediate host are known as cercariae or metacercariae. The parasite infections are most prevalent in eastern and Southeast Asian countries such as Japan, Korea, Thailand and also to some extent in European countries and the US.

Round worms

Anisakis simplex and *Pseudoterranova decipiens* are the most important species of anisakid worms infecting humans worldwide while the rest are restricted to some geographical regions only. These worms are widely distributed in tropical and temperate fish. Large outbreaks have been reported from Japan and Korea. When the fish harbouring the larval stage of *Anisakis* are eaten; the young worms can penetrate the gut epithelial cells into the peritoneum. The symptoms include sudden onset of abdominal pain, nausea and vomiting (Sakanari et al., 1989). Anisakid worms are also a major problem in Europe and the US. The infective larval stage is found in the intestine and the muscle of the fish. Therefore rapid chilling following catch and degutting will substantially reduce the worm burden (FAO, 2004). The fish intended to be eaten raw must be frozen at -20°C for at least 24 h. Salting or marinating will destroy the worms.

Tapeworms

Among tapeworms transmitted through seafood, *Diphyllobothrium* is the most important group, *D. latum* and *D. dendriticum* being the most common species infecting humans. *D. latum* can grow to a length of more than 10 m and competes for vitamin B12 in human host causing pernicious anaemia (Butt et al., 2004b). Cases have been reported from US and Japan. The worms are found in the small intestines of fish. The symptoms of infections include abdominal pain, vomiting and diarrhoea. However, the patient may, in several cases, remain asymptomatic for many years.

Trematodes

The trematode worms are most important and the largest group of parasites infecting humans. It has been estimated that About 40 million people are infected annually with these parasites (WHO, 1995). The infections occur mainly in countries where the parasites are endemic and present in large numbers in fish harvesting areas. The parasites occur in the skin of infected fish or shellfish (molluscs or crabs), as metacercariae. The trematode worms are classified based on their organ tropism as liver flukes (*Clonorchis sinensis*, and *Opisthorchis viverrini*, *O. felinus*), lung flukes (*Paragonimus* sp.) and blood flukes (*Heterophyes* and *Metagonimus* spp.) The liver flukes grow and reproduce in the liver and the eggs are passed out in the faeces of infected individual. The growth of worms in liver causes obstruction of bile ducts which can lead to secondary infections by bacteria. Hepatitis and liver carcinoma have been linked to infection of liver by the liver flukes. The trematode, *Clonorchis sinensis*, known as the Chinese liver fluke, is highly prevalent in China, Korea, Taiwan, Vietnam, and Japan while *O. viverrini* is common in South East Asian countries such as Thailand and Laos (FAO, 2004).

The most common human lung flukes *Paragonimus westermani* and *P. skrjabini* are endemic in countries such as Thailand, Japan, China and Korea. Snails are the first intermediate host while crustaceans such as freshwater crabs serve as second intermediate host. The infections are acquired by eating crabs raw and the larvae enter into the body cavity after passing through intestinal epithelium and get lodged into the pulmonary cavity, where they grow into adult worms in the lungs. Man and a number of domestic and wild animals serve as final host for these worms and the adult worms reproduce and release eggs which find their way out into the outside environment through the sputum of the affected individual. Acute abdominal pain and diarrhoea are initial symptoms but the prolonged persistence of the worms can cause lung disease.

The intestinal flukes of importance commonly encountered in seafood are *Metagonimus yokagawai* and *Heterophyes heterophyes*, though more than 50 species are known to infect human. Though both the worms have snails as first intermediate host in their life cycle, they differ in their final host. For *Metagonimus* sp., freshwater fish are the second intermediate host while for *Heterophyes* sp., brackish water fish serve as the second intermediate host. Clinical symptoms of infection by intestinal flukes include abdominal pain, weight loss, fever and diarrhoea. Occasionally, the mature worms can become invasive crossing the epithelium and become systemic causing heart

damage. Like all other worms, intestinal flukes can be destroyed easily by cooking. However, the traditional habits of eating raw fish make control of infections difficult. The use of human or animal faeces for manuring fishponds maintain constant source of infection. Though most of the infections can be treated effectively infections certain worms can be fatal depending on the worm burden. Chronic infections such as those seen in the case of liver or lung flukes can lead to serious complications.

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