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Contamination by Pathogenic Bacteria during Handling and Processing of Seafoods

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Pathogenic microorganisms gaining access to the seafood during handling and processing are becoming major hazards. The most important pathogens which gain entry into the fish during handling, transportation and processing are *Salmonella* spp., *Vibrio cholerae*, *Staphylococcus aureus* and *Listeria monocytogenes*. In addition, Enteropathogenic *Escherichia coli*, *Clostridium perfringens* and *Bacillus cereus* may also contaminate the seafood during handling and processing.

Key words : Pathogenic bacteria, seafood handling, seafood processing, *Salmonella* spp., *Vibrio cholerae*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Clostridium perfringens* and *Bacillus cereus*

Since fish is harvested from natural water bodies including farms, it harbours a number of microorganisms found in the environment from where it is caught. In addition to these inherent microorganisms, the fish can get contaminated with other microorganisms during handling, transportation and processing, right from the point of catch to the end product. These microorganisms include both pathogenic and non-pathogenic bacteria. The pathogenic microorganisms can be hazardous to the health of the consumer. The most important pathogens which gain entry into the fish during handling, transportation and processing are Salmonella, *Vibrio cholerae*, *Staphylococcus aureus* and *Listeria monocytogenes*. In addition, enteropathogenic *Escherichia coli*, *Clostridium perfringens* and *Bacillus cereus* may also gain entry to the fish.

In this review, the commonly encountered pathogens, which contaminate the seafood during handling and processing are discussed in brief.

Salmonella

Salmonella is a type of bacteria which causes typhoid fever and many other infections of intestinal origin in man. Typhoid fever is caused by the *Salmonella typhi*. But the illness generally called "salmonellosis" which is common in all parts of the world is caused by other *Salmonella* serotypes. Nearly 2300 serotypes of *Salmonella* are known to exist based on 67 somatic antigens (O-antigen) and many flagellar antigens (H-antigens).

Salmonella is a Gram negative rod-shaped bacteria mostly motile with the exception of *Salmonella pullorum* and *S. gallinarum*. From the epidemiological point of view Salmonella are classified into three main groups. The first group referred as the typhoid group comprises *S. typhi* and *S. paratyphi,* which infect only man and spread directly or indirectly through food and water from person to person. The second group includes those *Salmonella* which are host adapted like *S. gallinarum* in poultry, *S. dublin* in cattle and *S. choleraesuis* in swine. Some of them are pathogenic to man as well. The third group usually referred as "gastroenteric group" is the principal agent of salmonellosis, the food poisoning caused by *Salmonella*, which includes the majority of other *Salmonella* serovars with no particular host preference.

Salmonella occurs widely in animals, water, soil, insects, factory surfaces, animal faeces, raw meats and raw seafoods. The incidence of salmonellosis has been increasing over the past many years, and cases in which fish or shellfish was the vehicle has been around 5% in the United States (Heinitz *et al.*, 2000). Outbreaks due to *Salmonella* have been linked to the consumption of chilled boiled salmon, smoked halibut, cooked cockles, fish & chip, etc. in the US (Francis *et al,* 1989). In UK, 3.2% incidences of *Salmonella* was reported in 156 smoked fish samples (Heinitz & Johnson, 1998), 8 to 35% incidence in bivalves in France (Monfort *et al.*,1994). A survey of 55 seafood samples in Malaysia, indicated a 25% incidence of *Salmonella* in raw prawns (Arumugaswamy *et al.*, 1995). In a detailed study on the incidence of *Salmonella* in fish and seafood by the USFDA, in which 2734 samples comprising of imported cooked crab, smoked fish, dried/salted fish, and other processed items, an incidence of 2.6% of *Salmonella* has been reported (Heinitz *et al.,* 2000). They have also found that *Salmonella* have been detected

in 8.5% of raw imported crustaceans, consisting of prawns (4.3%) and lobsters (8.7%).

The incidence of *Salmonella* in Indian seafood is also disturbing. Bhaskar *et al.* (1995) reported isolation of *Salmonella* from all the 18 cultured shrimp samples from Southern India. Hatha & Lakshmanaperumalsamy (1997) have recorded high incidence of *Salmonella* in fish (14.25%) and crustaceans (17.4%), collected from Coimbatore fish markets. On the other hand, Prasad & Rao (1995) found only 1% of the 500 prawn samples from East Coast of India, to be contaminated with *Salmonella*. Reilly & Twiddy (1992) have found that 16% of the prawn from 131 brackish water farms in south-east Asia harboured *Salmonella*. The study by Heinitz *et al.*, (2000) involving 2734 samples, imported to the US from different parts of the world indicated that incidence of *Salmonella* was the highest (32%) in products from Vietnam, followed by Indonesia, Philippines and India (10-20%). The incidence was 8% in imports from Thailand and Mexico, 5% from China and Taiwan and only 1.6% from Japan. The most frequently isolated *Salmonella* serovars were *S. welteverden, S. seftenberg, S. lexington, S. paratyphi* and *S. enteritidis.*

Studies by Varma *et al.* (1985) showed that only 1.46% of frozen peeled and deveined shrimp and 1.86% of frozen cuttlefish, processed for export in seafood processing establishments in Cochin (India) were contaminated with *Salmonella*, while Lakshmanan *et al.* (1993) have reported an incidence of *Salmonella* in 3.2% of processed squid and cuttlefish from Cochin. Iyer & Varma (1990) have found that the main sources of contamination of fish by *Salmonella* are the polluted waters (from which fish is caught), process water, ice used, primary processing centres and droppings from lizards, rodents and rarely from the workers.

The USFDA/EU has declared *Salmonella* as "Zero tolerant" in seafood and their standards stipulates that *Salmonella* should be "Nil" in 25 g of the test sample.

Vibrio cholerae

Vibrio cholerae is an aerobic Gram netgative, non-spore forming curved or short rod motile with a single polar flagellum. It's optimum temperature of growth is 37°C, with a very short generation time and

capable of growth at alkaline pH (8.5 – 9.2). This bacterium is responsible for Asiatic or epidemic cholerae. The only known natural reservoir is man. It is present in water contaminated with faeces, in marine coastal waters, fish and shellfish.

Vibrio cholerae produces two antigens *viz.*, 'O' (somatic) and H (flagellar) antigens. O-antigen is heat stable, while H-antigen is heat labile. Strains which agglutinate in antisera raised against 'O' – antigens are called Sero group – O1 and those that do not agglutinate are called Sero group non-O1 or non-agglutinating group (NAG). The Sero group O1 are further subdivided based on the presence of 3 major 'O' group antigens, A, B and C. Of these, antigen 'A' is O1 specific. It occurs in combination with either antigen B or C, or both, giving rise to three O1 serotypes. The *V. cholerae* with 'AB' antigens is called 'Ogawa' serotype, the 'BC' combination the Inaba serotype and 'ABC' combination – the Hikojima serotype (rare).

The non-O1 (NAG) *V. cholerae* strains are morphologically and biochemically similar to O1 group, but have been generally considered as non-pathogenic. They are reported to produce mild cholera like diarrhoea. But in 1992, the world was alarmed by a severe outbreak of cholera in the Bay of Bengal region, caused by a non-O1 strain of *V. cholerae*, now serotyped as O139. It produces a toxin, identical to *V. Cholerae* O1. It is more virulent than O1 and not comparable to any of the 138 serotypes known so far, but behaves like non-O1.

Vibrio cholerae grows at temperature range of 18 to 42°C, but optimally around 37°C. Food that is most frequently implicated in cholera out break are seafoods, both molluscan shellfish and crustaceans. Since *V. cholerae* possess the enzyme chitinase, it can remain viable for long time on the exoskeleton of crustaceans.

Water is one of the important route of transmission of *V. cholerae*. A well chlorinated water supply free from sewage contamination is a must for proper control of *V. cholerae*. Since *V. cholerae* is widely distributed in aquatic animals, possibility of contamination in seafood is there, particularly when the seafood is caught from water bodies contaminated with sewage. Initial contamination can be controlled by thorough washing in water containing 5 ppm chlorine.

Another source of contamination is the unhygienic food handlers and processing workers. Strict personal hygiene of food handlers/processing workers can control such chances of contamination.

Staphylococcus aureus

Staphylococci are Gram positive spherical bacteria that occur in microscopic clusters resembling grapes. The two common species of staphylococci which are important to man are *Staphylococcus aureus* (forming yellow pigmented colonies on agar media) and *S. epidermidis* (white colonies). *S. aureus* colonises mainly the nasal passage, although they have been isolated from most other surface anatomical locales of man. *S. epidermidis* generally inhabits the skin.

S. aureus is facultatively anaerobic, that grows by aerobic respiration or by fermentation, yielding mainly lactic acid. It is haemolytic on blood agar. It is capable of growth at 15-45°C and can tolerate upto 15% NaCl. Nearly all strains of *S. aureus* produce the enzyme coagulase and it is a potential pathogen. *S. epidermidis* are coagulase negative, non-haemolytic and non-pathogenic.

Some strains of *S. aureus* are capable of producing a highly heat stable protein toxin, called Staphylococcus enterotoxin. Ingestion of the toxin causes the staphylococcus food poisoning. The onset of the symptoms is usually rapid and sometimes acute, depending mainly on the amount of the toxin in food ingested. Infective toxin dose is less than 1.0 microgram in food, and this level is reached only when the *S. aureus* population exceed 100,000 per gram.

The food get contaminated with *S. aureus* from man, and animals, which are primary reservoirs. Fish and shellfish from marine and unpolluted waters do not contain *S. aureus*, but contamination takes place during handling.

Listeria monocytogenes

Listeria is a Gram positive non-spore forming short rod, facultatively anaerobic/micro aerophilic, catalase positive and oxidase negative bacterium, with tumbling motility at temperatures below 30°C. The genus *Listeria* has seven species of which *L. monocytogenes* is the significant pathogen, for both

human and animals. *L. monocytogenes* is an ideal candidate as a food borne pathogen due to its widespread occurrence, tolerance to extreme conditions of environments. The organism can grow at 4°C. *Listeria monocytogenes* can be found almost everywhere, including soil, waste water and vegetation. After eating the contaminated food, incubation periods are in the range of 1-8 weeks. *Listeria* is a threat to public health, particularly among immunocompromised people, pregnant woman and newborns. Manifestations of listeriosis include septicemia, meningitis and gastro-intestinal symptoms. The infective dose of *L. monocytogenes* is fewer than 1000 cells. Implicated foods include raw and smoked fish as well.

The USFDA has imposed a zero tolerance level for this organism in food. Now both USFDA and the EU countries insist that no *Listeria* (not *L. monocytogenes* alone) should be present in seafood imported to those countries.

Clostridium perfringens

Clostridium perfringens is an anaerobic Gram positive spore forming rod. It is widely distributed in the environment, and frequently present in the intestines of human and animals. Spores of *C. perfringens* persist in soil and sediments. It causes a food borne illness called perfringens food poisoning characterised by intense abdominal cramps and diarrhoea, beginning in less than 24 h after ingestion of foods containing large numbers of *C. perfringens* (about 10^8 cells) capable of food poisoning toxin production.

Both raw and processed seafoods were implicated in *C. perfringens* food poisoning outbreaks in the United States and Japan (Taniguti, 1971; Caico, 1998). Contamination of fish/shellfish by *C. perfringens* has been reported by Taniguti (1971; 1978), Madden, *et al.* (1986), Easterbrook & West (1987), Saito (1990), Lalitha *et al.* (1990).

Usually, the *C. perfringens* spores reach the food/fishery products through water, contaminated with faecal/sewage matter. The keys to minimise health risks associated with the presence of *C. perfringens* are prompt and proper refrigeration during storage and distribution, strict adherence to good manufacturing practices and sanitation, and reheating of leftovers of sensitive foods before consumption.

Enteropathogenic *Escherichia coli*

Escherichia coli is a Gram negative facultatively anaerobic motile short rod. It is a member of the Enterobacteriaceae, present in plenty in the large intestines (colon) of man. *E. coli* in general is non-pathogenic, but is a very good indicator of contamination with faecal material, either direct or indirect.

E. coli is classified into more than 170 serotypes. Some of these serotypes are pathogenic like the *E. coli* 0157:H7 which is called enterohaemorrhagic *E. coli* (EHEC). It is responsible for the food borne illness called haemorrhagic colitis. *E. coli* 0157:H7 mostly live in the intestines of cattle and poultry. It reaches food/fishery products through contaminated water or handlers or from fish caught from polluted waters.

Pathogenic bacteria like *Shigella, Campylobacter* and *Bacillus cerues* also can cause food poisoning, but contamination of seafood by these pathogens are very rare. The key to reducing the contamination of seafood by these pathogenic bacteria, is strict adherence to good manufacturing practices (GMP). Sanitation and hygiene are the corner stones of GMP.

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