Quality Issues in Fishery Byproducts

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Fish and fishery products constitute an important component of human diet. Contribution of seafood in the average animal protein consumed worldwide is around 16.6 %. Due to the increasing focus on nutritional significance of fish, the per capita consumption has increased to 18.6 kg in 2010. Seafood has become one of the most internationally traded commodity (109 billion US$ in 2010), the safety concerns related to seafood consumption has also become global in nature.

Quality issues related to seafood sector have been diverse, which include progressive changes associated with loss in freshness quality as well as those introduced by poor post-harvest handling practices. The much discussed about issue is presence of biological hazards, which includes human pathogenic bacteria and their toxins. Fish and fishery products are known vectors for disease causing pathogens like *Vibrio cholerae*, *Salmonella*, V*ibrio parahaemolyticus*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Clostridium botulinum.*

In absence of a foodborne disease surveillance mechanism, it becomes difficult to ascertain the magnitude of disease. In USA alone, there are 48 million cases of food-borne diseases, 128,000 hospitalizations and 3000 deaths, are reported to occur each year (CDC, 2011). WHO estimates of 2005 shows that 1.8 million people die every year due to contaminated food and drinking water. The burden of foodborne disease will continue and it is highly probable that new food-borne pathogens of zoonotic origin and twice likely to cause new and emerging disease will be discovered in the 21st century.

Like other food sectors, seafood industry is not immune to emergence of new pathogens, which are becoming inherently unpredictable. As value-added seafood products incorporating plant based ingredients or other animal meat are not uncommon across countries, seafood mediated transmission of emerging pathogens like *Escherichia coli* O157:H7 and *Campylobacter jejuni* cannot be ignored. Further, it requires a national surveillance network to source-track pathogens and design suitable control measures.

Microbial Safety with respect to seafood

Developed countries like USA and Japan are biggest importers of seafood. In USA, 85% of the seafood consumed domestically is imported from third world countries. As per USFDA, during 1996-2006, seafoods contributed 21.6% of total outbreaks due to foodborne diseases and 11.3% of total foodborne illnesses. A glance on the actual causes of foodborne disease outbreaks in USA reveals that 87% are bacterial origin, 8.5% are viral origin and <1% by parasites. In India there is no database on disease outbreaks related to seafood consumption. The figures of import refusals and rejection of export consignment by importing countries like USA, EU and Japan provide an indirect estimate of the current level of hazards in Indian seafood. For example, during 2010-2011, out of total 65 import refusals of Indian seafood by USFDA, 37 were due to presence of Salmonella. During the same period, there were 2 RASFF (Rapid Alert System for Food and Feed) alert notifications on *Salmonella* and 1 on *Vibrio cholerae* for seafoods exported to European Union.

Phenomenon of emergence of microbial pathogens

Genetic diversity among bacterial species as evidenced by whole genome sequence information provides basis of emergence and re-emergence of pathogens. With this flurry of genetic information, the distinction between pathogen and non-pathogens, virulence factor and colonization factor is getting gradually blurred. As revealed through genome sequence data, biological function of 30-50% of the predicted genes of food borne pathogens is still unknown. Apart from this an array of mechanisms exist which contribute to evolution of pathogenic bacteria such as extensive lateral gene transfer, genetic recombination and duplication, antigenic variation by slipped strand miss-pairing, genome decay or change in fidelity of proof-reading enzymes.

Mechanism like gene duplication although introduces additional genetic element, helps the pathogen to survive extreme environments due to expansion in functional characteristics. Multiple paralogous groups resulting from 260 gene duplications are observed in *Vibrio vulnificus*, which is implicated in foodborne diseases associated with consumption of bivalves like mussel and oysters. Mutations are shown to occur frequently in these duplicated sequences leading to changes in pathogenicity and survival of this emerging pathogen.

Acquisition of genetic elements from other microorganisms through lateral/horizontal transfer remains the most potent source of genetic variation and speciation in pathogenic bacteria. The lateral gene transfer is mediated by mobile genetic elements that include conjugative plasmids, bacteriophages, transposons and pathogenicity islands. These mobile genetic elements contain genes that encode virulence factors. For example, the SpvR, SpvA, SpvB, SpvC and SpvD virulence factors in *Salmonella typhimurium* are carried by the plasmid. Similarly, promiscuous plasmids that move freely between cells are pathogenic determinants in enterotoxigenic *Escherichia coli*.

Bacteriophages are mostly implicated in lateral gene transfer in foodborne pathogens. The recent emergence of antibiotic resistant strains of Salmonella i.e. *Salmonella typhimurium* DT 104 has been attributed to *Salmonella* genomic island I (SGI-I), containing phage- and plasmid-related genes, and five antibiotic resistance genes to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline. Similarly, the prophage CTXϕ that encodes for cholera toxin genes (ctxA and ctxB) plays a significant role in the life cycle of *Vibrio cholerae*. These toxin genes are acquired by *V. cholerae* after getting infected with the phage CTXϕ with the entire phage genome getting integrated into the *V. cholerae* genome. The emergence of toxigenic *E. coli* O157:H7 from its nontoxigenic, less virulent ancestor, *E. coli* O55:H7 has also been ascribed to bacteriophage that codes for Shiga toxins 1 and 2 (Stx1 and Stx2).

Another class mobile genetic element involved in horizontal gene transfer is "Pathogenicity Island", which are linked blocks of genes (5-100 kb) that impart virulence to the recipient organism. They are generally associated with t-RNA gene loci and have a G+C content different from that of the host bacterial genome. Many type III and type IV secretion systems from foodborne pathogens such as *E. coli* and Salmonellae and Yersinia are products of the pathogenicity islands. In *S. typhimurium* two type III secretion systems (Spi-1 and Spi-2) play an important role in their invasion and colonization. Pathogenicity islands can be both insertion and deletion in different lineages, indicating series of gene acquisition and erosion during the emergence.

Slipped strand mispairing (SSM) is a mutation process that takes place during DNA replication. The DNA strands gets denatured and displaced, resulting in mispairing of the complementary bases. Changes in length of these tracts within or immediately upstream of genes causes alteration in translation and synthesis of proteins. This results in phenotypic variation of a species such as variation in antigenic characteristics of a pathogen which is used to elude immune system of the host. Identification of such genes with variation in repeat sequences helps in investigation of host adaptation and pathogenesis of foodborne pathogen.

Another mechanism of emergence of foodborne pathogens is progressive purging of unnecessary genes from the genome, called as genome decay. Downsizing of the genome content takes place as these genes no longer provides selective advantage to the organism. In *Salmonella typhi* which is only restricted to human host, many non-functional genes called as pseudogenes are observed in comparison to Salmonella Typhimurium. The accumulation of pseudogenes in *Salmonella typhi* is indicative of host-adaptation or genetic drift associated with population pressure following adaptation. Positive selection for gene loss i.e. pathoadaptation also helps in improving fitness of the pathogen and makes it more virulent. In Shigella, loss of cadA gene (which encodes for lysine decarboxylase enzyme) makes it more virulent as cadaverine (product of lysine decarboxylase activity) inhibits the plasmid-encoded virulence factors.

Due to defective DNA replication and mismatch repair system, sometimes mutator strains are more predominant in foodborne pathogens like Salmonella and *Escherichia coli*. These mutator strains have altered response in host microcosm and lead to emergence of new pathogenic groups.

Threat perceptions

Vibrios

Among the different species of vibrios, *Vibrio cholerae, V. parahaemolyticus and V. vulnificus* contribute to most cases of foodborne illness. Other species of vibrios emerging as pathogen due to consumption of seafood include *V. alginolyticus, V. mimicus, V. damsel, V. hollisae, V. cholerae* non-O1 non-O139 and *V. fluvialis*. Highest case fatality rate is observed in *Vibrio vulnificus*. There are already seven pandemics caused due to *Vibrio cholerae*. The pandemic strain of *Vibrio parahaemolyticus* O3:K6 emerged in Kolkata and rapidly spread throughout

Asian continent. The pandemic O3:K6 strain carries the tdh gene but not the trh genes and do not produce urease.

Listeria monocytogenes

As *Listeria monocytogenes* can survive high salt concentration (up to 20% w/v), low water activity (aw: 0.91), broad pH range (4.3-9.8) and temperature (0.5-45oC) (Lado and Yousef, 2007), this pathogen is reported from different spectrum of fishery products. Compared to other food processing environments, prevalence of this pathogen in fish processing environments is relatively low. Seafoods are implicated in outbreaks of Listeriosis in North America, Europe and New Zealand in crustaceans, smoked fishes and ready to eat products. Although the prevalence of Listeriosis is reported to be below 0.7 per 100000 populations, the fatality rate in outbreaks is quite high

In India prevalence rate of *Listeria monocytogenes* in seafood has been reported as 0-17%. Although earlier reports till 1992 denied presence of this pathogen in Indian fish and fishery products, there has been a surge of reports citing the incidence of this pathogen in fresh finfish and shellfish samples in Goa, Mangalore and Mysore

Salmonella

Salmonella continues to be the leading cause of seafood related disease outbreaks throughout the world (EFSA, 2010; CSPI, 2009). The threat posed by Salmonella is compounded by growing consumption of seafood, preference for minimally processed and RTE products, expansion of international trade and growing aquaculture contribution to the fish trade (Amagliani et al., 2012). The prevalence of Salmonella is very high in tropical seafood compared to temperate waters. In India Kumar et al (2008) reported 30.5% prevalence in fish, 29% in shrimps and 34.1% in clam species. The serovars of Salmonella reported from Indian seafood are *S. worthington, S. weltevreden, S. typhimurium, S. enteritidis, S. bareilly, S. gallinarum, S. rissen, S. derby and S. infantis.*

Foodborne viruses

As viruses do not grow on food and have rapid spread other pathogens, there is no systematic surveillance for foodborne viral disease. Noroviruses which are now recognized as one of the most common causes of gastro-enteritis are transmitted through seafood. Noroviruses and hepatitis A are mostly detected in bivalve molluscan shellfish such as mussels, oysters and clams and as the RASFF portal indicates there are large number of rejections of imported seafood in EU.

Foodborne parasitic zoonotic agents in seafood

Changes in dietary practices including food preferences and eating patterns have influenced emergence of parasitic organisms in fishery products. Parasites like *Anisakis simplex* and *Pseudoterranova decipens* transmit due to ingestion of raw fish in European countries, USA and Japan, where there is growing demand for raw or lightly cooked fish. Consumption of various raw preparations of fish such as Sushi, Sashimi, marinated anchovies, proliferation of these parasites of public health importance. Similarly, consumption of raw freshwater fish is linked to trematode (*Clonorchis sinensis*) linked infections in China, S. Korea and other Asian countries, cestode (*Diphyllobothrium* spp.) in northern Europe. Seafood trade across continents in recent years have also influenced emergence of hitherto unreported parasites. Transmission of lung fluke (*Paragonimus* spp.) from Vietnam to EU countries through export of fishery products has brought to the forefront the significance of this parasite. The zoonotic transmission of major emerging protozoan parasites like Cryptosporidium and Giardia are linked to bivalve molluscs, which are eaten raw in many parts of the world.

Surveillance and Monitoring

It is essential to conduct periodic surveillance and monitoring of human pathogenic bacteria in seafood to prevent outbreak and spread of the disease. The traditional methods like serotyping and phage typing although useful do not provide information on source of the hazard. The modern genotypic tools like pulsed field gel electrophoresis (PFGE) or multi locus sequence typing (MLST) are useful in source-tracking of pathogens, retrospective population studies and determining clonality of strains. For simultaneous determination of precursors of putative virulence determinants such as pathogenicity islands, pathogenicity loci, antibiotic resistance genes, transposons, plasmids and phages and their spread across different species, specially designed microarray would be quite useful.

Quality Issues of Fisheries Byproducts

Seafood industry generates a large amount of byproducts which comprises of several bioactive substances, such as proteins, enzymes, fatty acids, and biopolymers. Seafood by-products are mostly used for human health purposes with wide potential for biotechnological, nutritional, pharmaceutical, and biomedical applications. Considering wide use of these byproducts as human food, the safety aspects cannot be ignored. Microbial pathogens and their toxins as well as chemical contaminants can pose severe health hazard if present in these by-products.

Risk assessment of both chemical and microbial hazards in seafood by-products has not been attempted in most parts of the world. In absence of risk assessment data, regulatory agencies are finding it difficult to impose any safety standard.

As manufacture of most of the by-products involve heavy chemical extraction and downstream processing, presence of microbial hazards is mostly due to post-process contamination. On the other hand, many chemical hazards may get concentrated during extraction and may pose severe safety concerns.

Quality issues with feed material obtained from processing of fish or other marine animals

Animal feed (both for terrestrial and aquatic animals) prepared from fish based ingredients are used in large quantity throughout the world. Out of the total fish production in the world, 14 percent (21.7 million tonnes) is destined to non-food uses, of which 75 percent (16.3 million tonnes) is reduced to fishmeal and fish oil (SOFIA, 2014). The average fishmeal production during 2011-13 is reported to be 5.189 million tonnes. Around 73% of global fishmeal production is being used in aquaculture sector alone. The quality of fishmeal and fish oil ultimately influences the aquacultured fish; hence safety standards are also applied to these commodities.

Quality issues with Fish Oil

In recent days there are quality concerns on fish oils, especially omega 3 supplements meant for human consumption. Excess amount of Polychlorinated Biphenyls (PCBs), dioxins, mercury, mislabelling, lower or higher claimed amount of EPA/DHA are considered some of the emerging quality concerns in fish oil.

Limit of undesirable substances in feeding stuff prepared from fish/aquatic products (Directive 2002/32/EC)

Undesirable substance	Product	Maximum content in mg/ kg (ppm) relative to a feeding stuff with a moisture content of 12 %
Arsenic	Feeding stuffs obtained from the processing of fish or other marine animals	15
Lead	Feeding stuffs obtained from the processing of fish or other marine animals	10
Fluorine	Feeding stuffs of animal origin with the exception of marine crustaceans such as marine krill	500
	marine crustaceans such as marine krill	3000
	calcareaous marine algae	1000
Mercury	Feeding stuffs produced by the processing of fish or other marine animals	0.5
Nitrite	Fishmeal	60 (expressed as sodium nitrite)
Cadmium	Feed materials of animal origin	2
Aflatoxin	complete feeding stuff	0.01
Dieldrin	Fish Feed	0.02
Camphechlor (toxaphene) — sum of indicator	Fish, other aquatic animals, their products and by-products with the exception of fish oil	0.02

congeners CHB 26, 50 and 62	Fish oil	0.2
	Feeding stuffs for fish	0.05
Chlordane	All feeding stuff	0.02
DDT (sum of DDT-, TDE- and DDE isomers, expressed as DDT)	All feeding stuff	0.05
Endosulfan	complete feeding stuffs for fish	0.005
Endrin	All feeding stuff	0.01
Heptachlor	All feeding stuff	0.01
HCB	All feeding stuff	0.01
HCH Alpha isomer Beta isomer gamma isomer	All feeding stuff	 0.02 0.01 0.2

Limit of Dioxin and PCBs in Fish oil as per EC regulation 1259/2011 amending Regulation (EC) No 1881/2006

Food stuffs	**Maximum level**		
	Sum of dioxins (WHO-PCDD/F-TEQ)	Sum of dioxins and dioxin-like PCBS (WHO-PCDD/F-PCB-TEQ)	Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6)
Marine oils (fish body oil, fish liver oil and oils of other marine organisms intended for human consumption)	1.75 pg/g fat	6.0 pg/g fat	200 ng/g fat
Fish liver and derived products thereof with the exception of marine oils		20.0 pg/g wet weight	200 ng/g wet weight

Presence of ethoxyquin in crustaceans has been a major cause concern for export of seafood to Japan. Ethoxyquin is commonly used as antioxidant in fishmeal production to prevent rancidity. Japan has amended its requirement from earlier 0.01 ppm to 0.2 ppm (parts per million) in crustaceans, including the farmed shrimp. The SPS notification issued by European Union (G/SPS/N/EU/61/Add.1) in July 2014 has classified Ethoxyquin as pesticide and a limit of 0.01 ppm has been fixed for aquatic products. This is bound to create lots of problems for export of Indian seafood consignments to European Union.