MOLECULAR TOOLS FOR IMPROVING SEAFOOD SAFETY

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Summary

Though seafood is generally considered a nutritious and safe food, occasionally, there may be problems due to contamination with pathogenic bacteria and viruses. Rapid detection of contamination and tracing the source of contamination is essential for quality assurance programs in the fish processing industry. Molecular techniques, such as polymerase chain reaction (PCR) and gene probes, are proving to be convenient tools for rapid detection of contamination. Techniques of DNA fingerprinting such as Random Amplification of polymorphic DNA (RAPD) are useful to compare strains isolated from different sources and to trace the source of contamination. Molecular tools are also becoming very useful to detect the presence of antibiotic resistant bacteria in aquaculture systems.

1. Introduction

Fish constitute an important source of protein for the worlds population. While other animal food sources have more saturated fat, fish have a higher proportion of monounsaturated and polyunsaturated fatty acids, and a diet of fish is associated with a lower occurrence of human cardiovascular disease. However, as with any animal food source, there is a risk of acquiring infections through contaminated fish. Compared to other foods, fish is relatively safe, as evidenced by statistics from the United States where only 10 percent of all food-borne illness is attributed to seafood. Nevertheless, the production of fish safe for the consumer is the objective of the fishery industry.

Fish production in the world during 1996 was 94.62 million MT out of which 26.38 million MT were produced by aquaculture. Fish production by aquaculture is a rapidly growing industry. The contribution of aquaculture to the total production increased from 14.4 percent in 1989 to 23 percent in 1995. The cultured shrimp and prawn sub-sector grew at an annual percent rate of 16.8 between 1984 and 1995 and at present, in 2002, aquaculture is considered to be the fastest growing food-production sector.

Fish may occasionally be contaminated with pathogenic microorganisms. Some of these microorganisms may be natural inhabitants of the aquatic environment and others may be derived from post-harvest contamination, occurring during handling and processing. The chances of contamination are greater if fish are cultured in freshwater or coastal waters than in fish harvested in the high seas. There are different methods of aquaculture, ranging from intensive commercial operations to extensive small -scale or subsistence operations, and food safety hazards vary depending on the system management practices and environment. The application of fertilizers to stimulate the production of fish food organisms is a common practice and in some systems, fresh or processed animal manure may be used as fertilizer. Such animal manure may be a source of pathogenic microorganisms. Microbiological hazard associated with fish and fishery products varies with the type of product and depends on whether the product is subjected to a microbicidal process before consumption. Some of the important pathogens, which may be associated with fish and fishery products, are discussed below.

2. Bacterial Pathogens Associated with Seafoods

2.1. *Vibrio* spp

Vibrio spp are common inhabitants of the aquatic environment and are widely distributed all over the world. *Vibrio* species have been isolated from sediments, plankton, mollusks, finfish and shellfish from both tropical and temperate regions. Several studies indicate that the incidence of *Vibrio* species does not correlate with the incidence of fecal coliforms, suggesting that they are autochthonous organisms of the aquatic environment. A number of *Vibrio* species may be pathogenic to man, causing both enteric as well as systemic infections (Table 1). Among these, the cholera causing *Vibrio cholerae* is the most hazardous. More than 150 serotypes of *V.cholerae* are recognized, but only serotypes 01 and 0139 are involved in epidemics of cholera. Other serotypes may occasionally be involved in gastrointestinal infections, but the disease is not as serious as cholera. Studies on the ecology of *V.cholerae* have revealed that even the serotype 01 may be widely distributed and can be isolated from river water in tropical countries as well as in the UK and USA, even though the water is free from fecal coliforms. *V.cholerae* could be a hazard when seafood is eaten without cooking, and it is believed that seafood is significantly involved in the current epidemic of cholera in Latin America.

Vibrio parahaemolyticus is a halophilic *Vibrio*, which is widely distributed in inshore marine waters throughout the world. This organism is often associated with animals having a chitinous exoskeleton such as shrimps and crabs, though it can also be isolated from molluskan shellfish and free swimming finfish. Outbreaks of gastroenteritis due to *V. parahaemolyticus* are generally due to consumption of raw mollusks (oysters and clams) or cooked crustaceans. Since *V. parahaemolyticus* is extremely sensitive to heat, outbreaks linked to cooked products are due to undercooking or recontamination of the cooked product. Most cases of gastroenteritis due to *V.parahaemolyticus* are caused by strains that produce a thermostable direct hemolysin (TDH) detected on a high salt blood agar medium called wagatsuma agar. Such strains are designated Kanagawa positive. *V. parahaemolyticus* strains associated with the natural environment are predominantly (98%) Kanagawa negative. There are only occasional reports of gastroenteritis associated with Kanagawa negative strains.

Species	Clinical manifestations
Vibrio cholerae 01, 0139	cholera
V. cholerae non-01/139	gastroenteritis
V. parahaemolyticus	gastroenteritis
V. vulnificus	bacteraemia
V. hollisae	gastroenteritis
V. mimicus	gastroenteritis

Table 1. *Vibrio* species pathogenic to man by the oral route.

Vibrio vulnificus differs from other marine pathogenic *Vibrio* species in that it causes bacteremia and septicemia rather than gastroenteritis, though the mode of infection is predominantly through the gastrointestinal tract. It is also an important cause of wound infections in the marine or brackish water environment. The organism has been isolated from coastal water and shellfish all over the world. In temperate waters, incidence is related to water temperature, being detectable when the water temperature rises above 21°C, but in tropical waters, the organism can be found throughout the year. Nearly all cases of food borne *V. vulnificus* infection have resulted from consumption of raw oysters by susceptible individuals - such as those with chronic cirrhosis, hepatitis, thalassimia major, hemochromatosis, and those with a history of alcohol abuse.

2.2. *Salmonella*

Salmonella occurs worldwide, and many foods of animal origin and those subjected to sewage contamination could be vehicles for transmitting these organisms. Though *Salmonella* is generally associated with poultry, the organism may occasionally be isolated from aquaculture systems and therefore fish may also act as vehicles for the transmission of salmonellosis. It is well established that aquatic birds spread *Salmonella* and other pathogens. *Salmonella* species have been reported from eel culture ponds in Japan, catfish culture ponds in the USA, and shrimp culture ponds in Asia. These organisms have been isolated from tropical aquaculture systems that do not use fecal waters or fertilizer, and have also been isolated from the gut of fish such as *Tilapia* and carps. *Salmonella* may also occur in coastal waters subjected to sewage pollution, but generally deep water marine fish do not harbor *Salmonella*. Such fish may become contaminated during post-harvest handling and processing.

2.3. *Listeria monocytogenes*

Though *L. monocytogenes* has been known as a human pathogen for a long time, it was recognized as a food borne pathogen only two decades ago. This organism affects pregnant women, neonates, elderly and immuno-compromised persons. The disease may manifest as abortions, septicemia and meningitis.

Listeria monocytogenes has been isolated from a wide variety of habitats including soil, silage, sewage, food processing environments and raw meat. *L. monocytogenes* has also been isolated from river water, sediments and a variety of fish and fishery products. Unpolluted seawater and ground water are generally free from this organism. In temperate regions, the organism has been isolated from surface water, lakes, and from coastal waters subjected to pollution from industrial, animal or human sources. Nevertheless, very few outbreaks of listeriosis have been linked to fishery products. This organism seems to be a particular problem in the fish smoking industry.

3. Viruses

Food borne illnesses due to viruses are a serious concern, because data from United States indicate that among all illnesses attributable to food borne transmission, 30 percent are caused by bacteria, 3 percent by parasites, and 67 percent by viruses. Nevertheless, very little is known about food borne viruses because of the difficulties in detecting their presence. Being obligate intracellular parasites, they do not grow on usual culture media, as do bacteria and fungi. Viruses are cultured in tissue culture and chick embryos. Since the food borne viruses do not replicate in foods, they are generally present in very low numbers and therefore extraction and concentration methods are essential for their recovery. The most common source of gastroenteritis causing viruses is shellfish. Being filter feeders, they are known to concentrate viruses. Some of the important viruses that may be transmitted through fish are discussed below.

3.1. Hepatitis A Virus

Hepatitis A virus is distributed worldwide and is transmitted by the fecal-oral route. This virus belongs to the family picornaviridae, as do polio, ECHO and coxasackie viruses, all of which have single-stranded RNA genomes. The virus can survive in feces for one month. Transmission of the hepatitis A virus may occur through contaminated shellfish, such as oysters and clams. Viruses are introduced into rivers and coastal waters through treated or untreated sewage. It has been reported that most sewage treatment processes do not produce a virus-free effluent. The bivalves filter large volumes of water and can retain the viruses, and therefore harbor viruses at higher levels than the surrounding water. Bacterial indicators of fecal contamination such as fecal coliforms have not proven to be reliable with respect to the presence or absence of hepatitis A. Food handlers excreting the virus may also be a source in many cases.

3.2. Norwalk and Norwalk-like Viruses

These viruses are referred as small, round structural viruses (SRSV's) and also as Norwalk agents. They belong to a large heterogeneous family, Caliciviridae.
These viruses cause gastroenteritis. Transmission of these viruses occurs in a way similar to that of hepatitis A. Therefore shellfish could be one of the vehicles of infection, although food handlers may contaminate other types of foods such as salads, sandwiches and bakery products. The virus has been reported to be highly infectious and secondary person-to-person transmission might occur during common source outbreaks. These viruses are more resistant to chlorine than other enteric viruses. Some Norwalk viruses have been reported to be infective even at a residual chlorine level of 5-6ppm.

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Bibliography

Andrew W.H. (1995). Microbiological Methods. In *Official Methods of Analysis of the AOAC International*.(P.Cuniff, ed.) p17.1-17.11, AOAC, Arlington. [Gives detailed protocol for analysis].

Food and Agriculture Organisation. (1995). The use of hazard analysis critical control point (HACCP) principles in food control. *FAO Food and Nutrition Paper* **58**. 53 pp. FAO, Rome. [The concept and applications of the use of HACCP are well described].

Persing D.H., Smith T.F., Tenover F.C. and White T.J. (1993). *Diagnostic Molecular Microbiology - Principles and Applications.* 641 pp American Society for Microbiology, Washington D.C. [Describes the principles of the methods of diagnostic molecular microbiology, and provides details of the protocol].

World Health Organisation. (1995). Application of risk analysis to food standard issues. *Report of joint FAO/WHO Expert Consultant,* 39 pp, WHO, Geneva. [Gives a detailed account of the various applications of risk analysis in food standard issues].

World Health Organisation. (1999). Food Safety issues associated with products from aquaculture. *WHO Technical Report NO*. **883**. 55 pp, WHO, Geneva. [Discusses the various food safety issues for products from aquacultures].

Biographical Sketch

Dr. Iddya Karunasagar took his Masters and Ph.D. degrees in Microbiology from the University of Mysore, India and carried out postdoctoral work at University of Maryland, USA and the University of Wurzburg, Germany. Currently, he works in the University of Agricultural Sciences, College of Fisheries, Mangalore, India as Professor and Head of the Department of Microbiology. He has also worked as Visiting Professor, at the University of Wurzburg, Germany, UNDP Consultant, Department of Fisheries, Govt. of Thailand, Member of Expert Committee, Food and Agriculture Organisation (FAO) and at the World Health Organisation (WHO). His research interests include pathogens associated with seafood, and their detection using molecular methods, bioremediation in aquaculture, marine toxins and seafood safety.