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The Study of Microbial Contamination in Seafood and Fish

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Abstract

Fish and seafood are a main source of animal protein in the diet. Because of their health advantages over red meats, the consumption of fish and seafood has increased. Catches can be gathered from seas, rivers and lakes whose water can range from pristine to contaminated. Often contamination is from human and animal sources; thus, fish and seafood can be involved in the transmission of pathogenic microorganisms and toxins. Geographical region, season, and, for fish, whether they are pelagic (surface to mid-water) or demersal (bottom) feeders will influence the numbers and types of microorganisms present on freshly caught seafood.

Seafood-associated infections are caused by a variety of bacteria, viruses, and parasites; this diverse group of pathogens results in a wide variety of clinical syndromes, each with its own epidemiology. Some seafood commodities are inherently riskier than others, owing to many factors, including the nature of the environment from which they come, their mode of feeding, the season during which they are harvested, and how they are prepared and served. Prevention of seafood-associated infections requires an understanding not only of the etiologic agents and seafood commodities associated with illness but also of the mechanisms of contamination that are amenable to control. Defining these problem areas, which relies on surveillance of seafood-associated infections through outbreak and case reporting, can lead to targeted research and help to guide control efforts. Coordinated efforts are necessary to further reduce the risk of seafood-associated illnesses. Continued surveillance will be important to assess the effectiveness of current and future prevention strategies.

Keywords: Contamination; Outbreak; Seafood

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Introduction

Fish are classified as any of the cold-blooded aquatic vertebrates of the super class Pisces typically showing gills, fins and a streamline body. In addition, 'fish' also refers to the flesh of such animals used as food. This super class of vertebrates includes all the bony and cartilaginous finfish, and excludes molluscs and Crustacea. However, some regulatory agencies such as the US Food and Drug Administration (FDA) will include molluscan shellfish, crustaceans, and other forms of aquatic animal as part of their 'fish' definition. In this chapter, "fish" will be used for fresh and seawater finfish. Fish are an important part of a healthy diet since they contain high quality protein, but typically present a low-fat percent when compared to other meats. In addition, most fish contain omega 3-fatty acids and other essential nutrients. Although fish is broadly similar in composition and structure to meat there are a number of distinctive features. Protein content in fish fillet varies typically from 16 - 21%. The lipid content, which can be up to 67%, typically fluctuates between 0.2 - 20%, and is mostly interspersed between the muscle fibres. Fish fillets are a poor source of carbohydrates, offering less

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than 0.5% [1]. Fish fillet composition can vary significantly within the same species due to feed intake, migratory patterns, and spawning season. The lipid fraction is the component showing the greatest variation; it shows a typical season pattern especially in migratory species such as herring or mackerel. Fish can be divided into fatty and lean fish; lean fish are those fish that store most of their fat in the liver, while fatty fish have fat cells distributed along their bodies. Muscle composition and structure of fish also differ from those found in other meat. Fish flesh is dominated by the abundance of white muscle in relatively short segments, giving it its characteristically flaky structure. The connective tissue content of fish is also lower than that found in meat, typically 3 and 15% of total weight, respectively [1].

Seafood is a nutrient-rich part of a healthful diet, and seafood consumption is associated with potential health benefits, including neurologic development during gestation and infancy and reduced risk of heart disease [2,3].

Initial Microflora

Some seafood commodities are inherently riskier than others owing to many factors, including the nature of the environment from which they come, their mode of feeding, the season during which they are harvested, and how they are prepared and served. Fish, mollusks, and crustaceans can acquire pathogens from various sources. All seafood can be susceptible to surface or tissue contamination originating from the marine environment. Bivalve mollusks feed by filtering large volumes of seawater. During this process, they can accumulate and concentrate pathogenic microorganisms that are naturally present in harvest waters, such as vibrios. Contamination of seafood by pathogens with a human reservoir can occur when growing areas are contaminated with human sewage. Outbreaks of seafood-associated illness linked to polluted waters have been caused by calicivirus, hepatitis A virus, and *Salmonella enterica* serotype Typhi [4].

Identified sources of seafood contamination have included overboard sewage discharge into harvest areas, illegal harvesting from sewage-contaminated waters, and sewage runoff from points inland after heavy rains or flooding. Additionally, seafood may become contaminated during handling, processing, or preparation. Contributing factors may include storage and transportation at inappropriate temperatures, contamination by an infected food handler, or cross-contamination through contact with contaminated seafood or seawater. Adequate cooking kills most pathogens; however, unlike other foods, such as meat and poultry, that are usually fully cooked, seafood is often consumed raw or prepared in ways that do not kill organisms [4].

Processing and its Effects on the Microflora

Wild finfish are usually caught by net, hook and line, or traps, with very little control over the condition of the fish at the time of death or the duration of the killing process. This contrasts greatly with the meat industry, in which the health of each animal can be assessed prior to slaughter, and the killing process is designed to minimise stress. However, in recent decades, aquaculture practices have been expanding worldwide, offering better control of fish health prior to, and during harvest. The length of time that set nets have been in the water or the time trawlers' nets are towed, has an effect on the amount of stress and physical damage that the fish will suffer during capture. Physical damage such as loss of scales, bruising and bursting of the gut will increase the number of sites open for bacterial attack and spread. In addition, cortisone levels increase during prolonged stress and can alter the fillet quality [1]. After capture, the fish may be stored in the vessel for periods ranging from just a few hours to several weeks in melting ice, chilled brine or refrigerated seawater at -2 °C. Inadequate circulation of chilled brines may result in localised anaerobic growth of some microorganisms, and spoilage, with the production of off-odours. Used refrigerated brines can be contaminated with high numbers of psychrotrophic spoilage bacteria, and their re-use will increase the crosscontamination of other fish with such microorganisms. Increasingly, and especially when fish is stored on board for longer periods, freezing facilities (-18 °C) may be used to prevent the catch from deteriorating [5].

Fish may be eviscerated prior to storage at sea - a practice that may have both advantages and disadvantages. The action of intestinal enzymes and activity of the gut bacteria on the flesh around the belly cavity may produce discolouration, digestion and off-flavours in uneviscerated fish. In eviscerated fish, however, the cuttings provide areas of exposed flesh that are open to microbial attack. If evisceration is carried out at sea, care should be taken in removing all the gut contents and washing the carcass thoroughly prior to refrigerating, icing or freezing. The decision to eviscerate the catch at sea will depend greatly on the size of the fish and the duration of storage at sea, with fish such as tuna and cod being more commonly eviscerated than sardines, mackerel or herring [6-8].

Spoilage

Food spoilage can be considered as any change that renders the product unacceptable for human consumption [9]. Spoilage of fish starts upon death due to autoxidation (oxidation of unsaturated lipids), reactions caused by activities of the fish's own enzymes, and metabolic activities of

microorganisms present in the fish. Over time, loss of the fresh characteristics may be simply measured by comparative visual and smell analysis. Loss of freshness and spoilage cannot be separated as processes, but it is a commonly held view that loss of freshness is related to autolytic degradation and spoilage is more microbial in origin [10].

Degradation of whole fresh fish stored in ice generally follows a set pattern, and this pattern is the basis of freshness grading schemes. The eyes turn from convex and clear to concave and opaque, the gills from pink and shiny, with no smell, to brown and slimy with an intense offodour; the skin turns from iridescent to dull and bleached with bacterial slime; and the flesh turns from bright and elastic to dull and soft [12].

When working with fillets, evaluation of taste is unavoidable as the visual pattern of degradation of the eyes and gills is unavailable for analysis. Taste panel evaluation of cooked fish may be used to score or grade colour, texture, smell and taste of cooked fish. Generally, the cooked analysis of fish passes through four phases of spoilage:

i) Delicate sweet, sea-weedy taste, possibly slightly metallic.

ii) Neutral taste, little flavour.

iii) Traces of sour, fruity and/or bitter off-flavours; development of sickly sweet, cabbage-like, ammoniacal, sulphurous and/or rancid smells; texture becomes soft and watery or hard and dry.

iv) Enhancement of the spoilage characteristic of phase iii, spoiled and putrid.

The processes of degradation being analysed by the quality scoring methods above are a complex mix of physical, chemical, biochemical and microbiological actions. These processes are strongly influenced by the physical conditions of storage.

After death, rigor mortis is the first noticeable change in the fish. From being flaccid, the muscles harden as residual adenosine triphosphate (ATP) is reduced and the myosin and actin filaments bind to form actomyosin [13]. After some time, rigor resolves, the muscles relax again and the fish returns to a flaccid state. The pH of the muscle will drop after death depending upon the amount of residual glucose or glycogen that is reduced anaerobically to lactate with the coproduction of ATP; this will generally correlate with the length and severity of rigor. Because fish tend to have relatively little residual glycogen compared with mammals, the pH drop of the muscle is correspondingly less; post-rigor values are typically in the range of pH 5.8 to 6.5 [14].

After death, the Eh of fish muscle remains relatively high (Gram *et al*,1998). To a varying extent, all marine fish use/have TMAO (Hebard *et al*, 1981), which has been ascribed a number of possible functions as: a trimethylamine (TMA) detoxified waste product, an osmoregulator, an anti-freeze, or simply a waste product present due to bioaccumulation [15]. TMAO permits a high Eh to remain in the muscle tissue, as little endogenous reduction of TMAO occurs. However, bacterial reduction of TMAO to TMA, an intense odour compound, is significant and may even be responsible for the ultimate sensory rejection of fresh cod and other fish with high initial TMAO content [16].

Changes in the resistance of the fish skin after death are used as the basis for tests that employ an electrode measurement of skin resistance (e.g. the Torrymeter, RT Freshmeter or Fishtester). As the fish degrades, the conductance generally increases; thus, measurements of the falling skin resistance may be made and compared with a calibration curve to estimate the time that the fresh fish has been stored in ice, or its remaining shelf life. The process of ATP breakdown is used as an indicator of fish freshness [17]. By measuring the concentrations of the six components, a ratio of concentration of the hypoxanthine (Hx) and inosine (HxR) to total concentration (ATP, ADP, AMP, IMP, HxR and Hx) gives a quantity (K-value, %), which increases from 0% towards 100% with time. During the initial storage, reduction in ATP and increase in hypoxanthine by endogenous enzymes allow this measurement to be used for determining freshness [18]. However, hypoxanthine, which may also be formed by bacteria, is later significantly reduced by bacterial action, so the measurement is effective only during the initial loss of freshness. This measure is highly dependent upon the temperature of storage and may not reflect the rate of loss of quality equally at different temperatures. Lipid oxidation and other oxidative changes lead to oxidative rancidity, colour changes, and are especially important in the spoilage of frozen fish, as microbial spoilage is limited by the low storage temperature [19].

Lipid oxidation can be a result of enzymic action or a cascade reaction initiated by free radicals produced by aerobic respiration and other forms of metal ion reduction Oxidative rancidity is known to reduce the quality of fatty fish in particular [20].

Chemical degradation continues after the initial post mortem phase; however, the importance of microbial action increases with time. Quality indices based upon the products of microbial metabolism do not explain changes in quality until microbial growth produces measurable changes in the fish; therefore, these measurements are usually used to quantify the amount of spoilage, not to describe freshness. Volatile bases are the bestcharacterised chemical indicators of fresh fish spoilage. Evaluation of Total Volatile BaseNitrogen (TVB-N, also termed TVN), or a specific fraction of

the volatile bases, for example the TMA fraction, using Conway diffusion chambers allows determination of changes of mg-N/100 g fish. The Conway method (and variations of it) uses a strong inorganic base to volatilise the bases in the fish sample, and a segregated weak acid to absorb them; the residual acid is then titrated. The variation in post mortem fish pH may likewise influence the amount of bases being liberated to the air and consequently affect the odour characteristics of the fish. Comparing results for different fish species, however, does not show correlation between muscle pH and the amount of volatile bases contained within the fish at rejection. Neither does the change in pH during storage correlate well with the production of TVB-N.

During spoilage, the majority of volatile bases are produced from the soluble non-protein nitrogen of the fish (free amino acids and other low-molecular-weight nitrogenous compounds), as significant proteolysis is observed only during the latest stages of spoilage and after rejection. For some fish species, a correlation can be made between the spoilage of the fresh fish and the production of TVB-N.

The major spoilage odours and flavours of fresh fish are undoubtedly principally microbial in origin, but rejection of whole fresh fish by sensory methods such as the EC grading scheme is based upon non-specific odour detection and physical appearance [21].

Pathogens responsible for seafood Bacteria

Vibrio species

Vibrio organisms are Gram-negative, halophilic bacteria that are widespread and naturally present in marine and estuarine environments. Environmental factors influence their growth, and their numbers are highest when the water is warm. The genus *Vibrio* includes 30 species, of which at least 14 are recognized as pathogenic in humans. *Vibrio* infections are acquired through ingestion of contaminated seafood or through exposure of an open wound to seawater. Clinical features most often associated with *V. parahaemolyticus* infection include watery diarrhea, abdominal cramps, nausea, and vomiting; wound infections and septicemia occur less commonly [22].

Laboratory diagnosis is made by isolation of the organism from clinical specimens, including blood, stool, and wound samples. Because they can be overlooked on standard agar plates, *Vibrio* organisms in stool or wound samples are best identified on selective media, such as thiosulfate-citrate-bile salts-sucrose (TCBS) agar [23].

Cases of *Vibrio* infections have a marked seasonal distribution; most occur during summer and early fall, corresponding to the period of warmer temperatures. Almost all cases of food-borne *Vibrio* infection are associated with a recent history of seafood consumption, primarily raw oyster consumption. Like other organisms found in water, vibrios can be concentrated in the tissues of filter-feeding bivalve mollusks. Measures to prevent food-borne *Vibrio* infections include consumer education regarding the dangers of eating raw or undercooked shellfish, particularly among persons with medical conditions, such as liver disease, that predispose them to severe illness. Thoroughly cooking shellfish and preventing raw seafood from crosscontaminating other foods are effective measures for consumers to reduce risk.

Regulatory control measures have included monitoring of harvest waters and microbiological sampling of oysters. However, it is important that the presence of vibrios is not associated with fecal contamination. Therefore, monitoring waters for fecal coliform bacteria is not effective as an indicator of the presence of *Vibrio* in harvest environments. Finally, postharvest processing methods, such as high-pressure treatment, irradiation, quick-freezing, and pasteurization, are available to make oysters safer. *Vibrio* infections are reportable to state health departments, and traceback of oysters associated with human *Vibrio* infection is strongly encouraged [24-26].

Salmonella

Salmonellae are Gram-negative bacilli. Approximately 2,500 *Salmonella* serotypes have been identified, causing a variety of clinical syndromes ranging from asymptomatic carriage to invasive disease. *Salmonella* most commonly causes acute gastroenteritis, with symptoms including diarrhea, abdominal cramps, and fever. Other clinical manifestations can include enteric fever, urinary tract infections, bacteremia, and severe focal infections. Isolation of *Salmonella* organisms from cultures of stool, blood, or other clinical samples is diagnostic; isolates are referred to public health laboratories for serotype characterization. *Salmonella* is a leading cause of food-borne illness, causing approximately 1.4 million illnesses annually in the United States [27-29]. Incidence is highest among infants and the elderly, and infections are more likely to occur during summer and early fall. Each *Salmonella* serotype has its individual biology and ecological niche. Many natural reservoirs for the different *Salmonella* serotypes have been identified. Humans are the only known reservoir for *Salmonella* serotype Typhi; many animal hosts (birds, reptiles, and mammals) serve as reservoirs for nontyphoidal serotypes. With improvements in sanitation over the past several decades, *Salmonella* serotype Typhi went from being the leading cause of *Salmonella* infection to becoming relatively uncommon.

However, nontyphoidal *Salmonella* infections have emerged as a public health problem since the 1950s [28-30].

Routes of *Salmonella* transmission include food-borne and waterborne routes, person-to-person contact, and contact with animals, particularly reptiles. Most cases of nontyphoidal salmonellosis are caused by ingestion of contaminated food, and outbreaks have been associated with a wide range of food vehicles in the United States. Seafood-associated outbreaks have been caused by fish, shrimp, oysters, and clams. Studies to determine the prevalence of *Salmonella* spp. in oysters from domestic bays and testing of sampled oysters from domestic seafood samples by the U.S. Food and Drug Administration (FDA) have demonstrated the presence of salmonellae in a variety of fish and shellfish, including seafood intended for consumption without further preparation upon distribution, requiring minimal cooking, and shellfish eaten raw [31].

Fish and shellfish can acquire *Salmonella* from polluted waters. Historically, sewage contamination of shellfish harvest beds led to large shellfish-associated outbreaks of *Salmonella* serotype Typhi infections. Control measures aimed at detecting contamination of harvest waters, such as monitoring fecal coliform counts in these waters, have been effective at reducing the risk of *Salmonella* contamination of seafood occurring before harvest. Additionally, seafood can become contaminated with *Salmonella* during storage and processing. *Salmonella* infection can be prevented by adequate cooking, proper storage and processing after harvest, and avoidance of cross-contamination during seafood handling (FDA,2006).

Shigella species

Shigella species are Gram-negative bacilli. Four species have been identified, and clinical presentations vary by species. Clinical manifestations of *Shigella* infection range from watery, loose stools to more severe symptoms, including fever, abdominal pain, tenesmus, and bloody diarrhea. Complications are rare and include seizures in young children, toxic megacolon, bacteremia, Reiter's syndrome, and hemolytic-uremic syndrome. Diagnosis is made by isolation of *Shigella* from feces or rectal swabs. Cases occur worldwide, in endemic and epidemic forms. Most cases occur among children aged <10 years [30-32].

Humans are the primary reservoir of *Shigella*. Transmission occurs through direct or indirect contact with feces of infected persons. *Shigella* infection is highly communicable, because ingestion of as few as 10 viable organisms is sufficient for infection to occur. The low infectious dose contributes to the potential for large outbreaks. Outbreaks have been associated with person-to-person transmission in crowded or unhygienic environments and with ingestion of contaminated food and water. Foods can become contaminated during handling or preparation by an infected food handler. With seafood, contamination can occur if seafood is harvested from sewage-contaminated water, as occurred in an outbreak caused by consumption of raw oysters harvested from waters where sewage was dumped overboard from the oyster harvest boat [33-34]. *Shigella* organisms can survive outside the host, but they are killed readily by cooking. Control strategies to prevent shigellosis associated with seafood have included monitoring of harvest water for fecal coliforms, prohibition of harvesting from sewage-contaminated areas, enforced control of dumping sewage overboard, and guidelines for seafood handling in restaurants.

Clostridium botulinum

Clostridium botulinum is a spore-forming, anaerobic, Gram-positive bacillus that is widespread in the environment. The bacterium produces a potent neurotoxin under anaerobic, low-acid conditions. Seven types of botulism toxin have been identified; toxin types A, B, and E cause most human illnesses. Food-borne botulism is caused by the ingestion of food contaminated with preformed toxin produced by spores of *C. botulinum*. Botulism is characterized by an acute, symmetric, descending flaccid paralysis. Early signs and symptoms of botulism often include cranial nerve palsies, with diplopia, ptosis, slurred speech, and difficulty swallowing progressing to descending weakness and paralysis. Symptoms can progress to cause paralysis of the respiratory muscles, requiring ventilatory support. Cases of botulism are rare but serious; an estimated 60 food-borne cases occur each year in the United States. Most cases are sporadic, but food-borne botulism outbreaks are reported each year [33].

Food-borne botulism cases are most often associated with home-canned foods. Other food vehicles identified in outbreak investigations have included fermented or salted seafood, potatoes baked in aluminum foil, garlic in oil, onions held under butter, and homemade salsa.

Most seafood-associated cases are caused by toxin type E, which is associated most commonly with eating traditional Alaska Native foods, such as fermented salmon heads, salmon eggs, and blubber and skin from marine animals (muktuk). *C. botulinum* type E spores are commonly found in fish and aquatic animals, and implicated seafood has been fermented under anaerobic conditions that favor the germination of *C. botulinum*. Measures to prevent seafood-borne botulism have included educational efforts to promote proper methods to

ferment foods and to boil fermented foods before consumption, especially if they were stored in tightly sealed plastic or glass containers (CDC, 2006).

Discussion

Our research identified specific seafood vehicles frequently associated with illness. This information can help to guide future prevention efforts. Seafood-associated outbreaks of infection were most often attributed to consumption of molluscan shellfish, particularly raw oysters, and most often caused *Vibrio* illnesses and norovirus infections. Over the study period, both the absolute number of outbreaks and the proportion of outbreaks caused by molluscan shellfish increased. Botulism cases associated with fish were also reported frequently, although these outbreaks were reported almost exclusively from Alaska. Etiologies and implicated seafood commodities changed over time during the study period. The cause of these changes is likely multifactorial: enhanced food-borne outbreak surveillance began in the late 1990s; laboratory diagnostic capacities have improved, particularly for norovirus but also for other pathogens, resulting in better detection of outbreaks; control efforts, especially in shellfish sanitation, have evolved; and changes in environmental factors may favor the occurrence of some pathogens.

Our understanding of foods and pathogens responsible for illness is largely derived from information gained from outbreak investigations. However, many outbreaks likely go unrecognized and uninvestigated. Moreover, the Food-Borne Disease Outbreak Surveillance System is a passive system that relies on voluntary reporting, which may lead to further underestimation of the actual number of outbreaks and illnesses that occur. Outbreak reporting may not be uniform across states, which may be due in part to whether states have dedicated food-borne disease epidemiologists. Also, outbreaks comprise only a small proportion of all cases of food-borne illness. No information is available on seafood-borne transmission in sporadic cases of infection other than *Vibrio* illnesses. Enhanced surveillance for food-borne outbreaks began in 1998. As a result, an abrupt increase in reported outbreaks occurred, which should not be interpreted as a true increase in the number of outbreaks. Also, improved laboratory methods and surveillance increased the ability to detect and investigate outbreaks during the study period. For example, subtype-based laboratory surveillance has improved the ability to detect outbreaks of infections associated with seafood items that are widely distributed, as in multistate outbreaks, and with pathogens with long incubation periods, such as hepatitis A virus. Other laboratory methods that evolved during the past decade include testing for the presence of norovirus by PCR and increased use of appropriate selective media to detect *Vibrio* organisms in stool.

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