Seafood Safety - Proceedings of the Symposium on Seafood Safety - Status and Strategies, 28-30 May 2002, Cochin, India,350-358.

Inherent Microbial Hazards Associated with Seafood

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The food borne pathogens that are naturally present in the marine or estuarine environments are members of the family *Vibrionaceae, Aeromonadaceae* and the species *Clostridium botulinum*, particularly *Clostridium botulinum* type E. Among members of the family *Vibrionaceae* that are important as inherent pathogens in seafood, the genera *Vibrio* and *Plessiomonas* are significant. The role of pathogenic vibrios in food-borne gastroenteritis and also other disease manifestations have been thoroughly investigated all over the world. The important species in this group are *Vibrio parahaemolyticus, V. vulnificus* and to a lesser extent *V. hollisae,* and *V. mimicus. Aeromonas hydrophila, A. sobriae* and *Plessiomonas shigelloides*, though not frequently associated with seafood borne infections, are also to be considered as human pathogens of aquatic origin. Unlike the above pathogens, *Clostridium botulinum* is an anaerobic, Gram positive, spore former present in the aquatic systems. It is a well-known pathogen causing severe intoxication and death in humans. Inadequately heat processed and smoked seafood are identified as the common vehicle of this organism. The distribution of these pathogens in seafood of different origins, significance as a pathogen and strategies for their removal and control are highlighted in this paper.

Key words : Microbial hazards, seafood, *Clostridium, Vibrio, Aeromonas, Plessiomonas*

Seafood-borne diseases are divided into three categories, based on the major source of the etiological agent. They are (i) agents naturally present in the environment, (ii) agents derived from pollution and (iii) agents acquired by the food by way of handling, processing and marketing. The pathogens in seafood that are naturally present are members of the family *Vibrionaceae, Aeromonadaceae* and the species *Clostridium botulinum*, particularly type E. Among the members of the family *Vibrionaceae*, genera *Vibrio and Plessiomonas* are important. The important species in the genus *Vibrio* that are considered as inherent pathogens are *Vibrio parahaemolyticus, V. vulnificus* and to a lesser extent *V. hollisae* and *V. mimicus.* The most recognized member of this group, namely *V. cholerae*, is considered as terrestrial organism and hence not included among inherent pathogens of seafood.

Clostridium botulinum

Botulism is the hazard caused by consuming food in which *Clostridium botulinum* has grown and produced toxin leading to severe intoxication. The toxin acts on the peripheral nervous system and may result in paralysis and death. Human botulism is serious but relatively rare. However, apart from the human health risks, an outbreak of disease can lead to massive economic problems to the processors. *Clostridium botulinum,* the etiological agent of botulism, is divided into 7 types, A, B, C (C1, C2) D, E, F and G based on immunological differentiation of the neurotoxin (Sakaguchi, 1979). Based on their proteolytic activity, they are grouped as Group I, II, III and IV. Group I and II are most important with respect to human botulism. Group I includes type A and proteolytic strains of type B and F. Group II contain all type E and non-proteolytic strains of type B and F.

Clostridium botulinum has been widely distributed in nature. It has been isolated from fresh water, brackish water or marine environments and also from the aquatic creatures (Huss, 1980; Smith, 1977; Hauschild, 1989; Dodds, 1993) *C. botulinum* type E is the most prevalent in temperate countries where as types C and D dominate in the tropical areas (Dodds, 1993).

In an exhaustive study on the distribution of *C.botulinum* in Indian waters, fish and fishery products (Lalitha, 1999; Lalitha & Gopakumar, 2000; Lalitha & Surendran, 2002), it was reported that *C. botulinum* types C and D dominate, followed by types A and B. Types C and D are not significant from the point of view of human botulism even though there are some differing reports (Hauschild, 1993). The conspicuous absence of *C. botulinum* type E in these studies further substantiates the previous reports of its absence in the tropical climate where the ambient temperature is always on the higher side.

C. botulinum is spore-forming bacteria very much resistant to ordinary preservative measures such as low temperature, chemicals like chlorine, radiation etc. Heat sterilization is the only means of its elimination, even though total removal from the system is impossible. The possible routes of contamination of this organism in seafood material are outlined in Table 1. The lethal toxin that is produced is quite stable at freezing temperature and cannot be destroyed by chemicals (Genigeorgis and Rieman 1979). Hence the ultimate safeguard is the very low heat stability of the toxin, which implies that normal household cooking will destroy any pre-formed toxin. Thus the risk is clearly associated with foods that do not require cooking immediately prior to consumption. The best way to control botulism hazard is to prevent intoxication by reducing initial contamination and arresting microbial growth and toxin production by a combination of processing factors.

Table 1. Routes of contamination of seafood with *C. botulinum, V. parahaemolyticus* and *V. vulnificus*

Factors	*C. botulinum*	*V. parahaemolyticus* / *V. vulnificus*
Contamination in the absence of terminal heat process		
Raw product	x	xx
Additives	-	-
Process	-	-
Heat process failure	xxx	-
Post process contamination		
Raw product	x	xx
Equipment	-	-
Cross Contamination	-	xx
Workers	-	-
Cooling water	x	-
Factors permitting growth in processing plant		
Inadequate refrigeration	xx	x
Fermentation	-	-
Anaerobic packaging	xxxxx	-
Growth after the product has left processing plant	x	x

Vibrio parahaemolyticus

Vibrio parahaemolyticus is a Gram negative, halophilic bacterium that occurs naturally in seawater and estuaries and is recognized as important seafood borne bacterial pathogen throughout the world. The organism can cause acute gastroenteritis and on rare occasions, septicemia. Typical symptoms include diarrhoea, abdominal cramps, vomiting and sometimes chill and fever.

V. parahaemolyticus is widely distributed in nature and has been isolated from coastal waters and marine creatures of wide geographical regions

(Karunasagar *et al.*, 1984). It is extremely difficult to completely eliminate it from seafood. The concentration as well as prevalence of *V. parahaemolyticus* varies considerably. Density of *V. parahaemolyticus* in these samples have been found to vary from nil to $10^4.g^{-1}$. But majority of the samples were found to carry *V. parahaemolyticus* counts of less than $100.g^{-1}$.

There are several routes by which *V. parahaemolyticus* may be introduced to the environments. It may be introduced by terrestrial and aquatic birds and animals or through human activities such as "relaying" shellfish. Ship ballast release may be a potential mechanism of introducing this organism (Anon, 2001). It is not an organism of the open sea because it cannot withstand high hydrostatic pressure and cool temperatures of the deep seas (Colwell, 1984). Sewage discharge may indirectly influence the densities of *V. parahaemolyticus* present in shellfish growing areas (Watkins & Cabelli, 1985). Many workers have reported a positive correlation between the density of *V. parhaemolyticus* and warmer climates and moderate salinities (Kaneko & Colwell 1978; Hackney *et al.*, 1980).

Several different virulence markers have been associated with pathogenicity of *V. parhaemolyticus* These include their ability to produce a thermostable direct haemolysin (TDH) and to invade the enterocytes (Akeda *et al.*, 1997) and to produce an enterotoxin (Honda *et al.*, 1976) However, the first two traits are more reliable to distinguish pathogenic from non-pathogenic strains. Factors responsible for the persistence of virulent strains in the environment and impact of environmental factors on these two types needs to be fully elucidated. It is established that isolates from the environment are generally non-virulent compared to clinical isolates. Seafood carries *V. parahaemolyticus* strains that are predominantly Kanagawa negative. For isolates from water, fish or shellfish from Gulf of Mexico only 0.18% strains were Kanagawa positive (Thompson & Vanderzant, 1976) and in Japan less than one percent of the strains were Kanagawa positive.

The infective dose of *V. parahaemolyticus* is not fully established. Based on human challenge studies, Kanagawa positive strains produced infection when the challenge dose was 10^6-10^7, but the Kanagawa negative strains could not produce illness even at the high dose of $2x10^{10}$. Earlier data from USFDA indicated that *V. parahaemolyticus* in shellfish should not exceed a level of 10,000 viable $cells.g^{-1}$. However, in view of the recent outbreaks, they have proposed that fewer than this number could also initiate illness.

According to the Centre for Disease Control, USA, the dose response under conditions of normal population exposure is different from that of feeding trials and possible reasons for this include food matrix or immunological effect of pre-exposure of the organism (Anon, 2001). Based on the simulation studies, an 'at harvest' guidance level of 10^5, 10^4, 10^3 and $10^2.g^{-1}$ could reduce the onset of illness by 2, 15, 50 and 90%, respectively (Levine *et al.*, 1981).

A comprehensive study on the *Vibrio parahaemolyticus* isolates from food poisoning outbreaks from 1992 to 1997 revealed the presence of 5 'O' and 20 'K' as the common serotypes associated with food poisoning (Wong *et al.*, 2000). Of these, the new serotype *V. parahaemolyticus* 03:K6 that has been isolated for the first time in the year 1996 is anticipated to be the cause of major food poisoning outbreaks in the future years. This clone emerged abruptly in Calcutta, India in the year 1996 and was detected in Bangladesh in 1997-98. It has been reported from other Asian countries like Taiwan, Laos, Japan, Thailand, Korea and also US (Okuda *et al.*, 2000; Vuddhakul *et al.*, 2000; Daniel *et al.*, 2000). Due to high infection frequency and capability to spread globally, this organism need to be intensively monitored internationally.

Three basic factors found to be associated with the consumer risk of this pathogen are (i) the level of pathogenic *V. parahaemolyticus* in seafood at harvest, (ii) effect of post harvest handling and processing, and (iii) the ability of the organism to multiply to an infective dose. A schematic diagram showing the factors responsible for the illness is given (Fig. 1).

Vibrio vulnificus

The *Vibrio vulnificus* called the "terror of the deep" is one of the most invasive species ever described (Oliver, 1989). It is an important member of the so called 'lactose positive' vibrios. It produces two types of clinical manifestations, septicemia and gastroenteritis. Food poisoning occurs after eating contaminated raw or undercooked shellfish. The organism produces primary and sometimes secondary septicemia and may be a cause of death in immuno-compromised individuals and it seldom infects normal healthy individuals (Oliver 1989). Illness is usually associated with certain risk factors such as liver and gastric diseases, malignancy and chronic renal insufficiency.

V. vulnificus is wide-spread in the marine and brackish water environment. In U.S, infection via the intestinal tract is most often associated with consumption of raw oysters. Kelly and Dinuzzo (1985) demonstrated

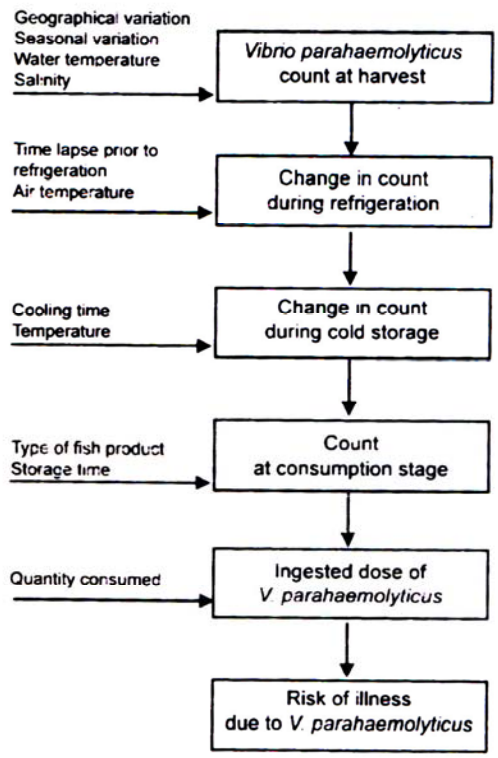

Fig. 1 Risk assessment of *Vibrio parahaemolyticus*

that high numbers of *V. vulnificus* in oyster was probably due to filter feeding nature of the animal. The presence of *V. vulnificus* in water and shellfish is seasonal, being most prevalent when water temperature is above 20°C.

Apart from this, several other pathogenic vibrio species like *V. hollisea, V. furnissii, V. alginolyticus* and *V. cincinatiensis* have been isolated, but either their frequency of occurrence is rare or their pathology is poorly understood. Like other Gram negative pathogens, members of the genus *Vibrio* are sensitive to many of the methods used to process food such as heat, acid, radiation, sub-freezing temperatures and chemicals (Oliver, 1989) However, low temperature storage cannot be relied upon as a method for total elimination of vibrios.

***Aeromonas* species**

The genus *Aeromonas* consists of a group of ecologically diverse microorganisms that are naturally present in aquatic systems and is an opportunistic pathogen for humans. Based on DNA–DNA hybridization studies and multilocus enzyme electrophoresis analysis, 12 genospecies have been identified of which 10 have taxonomic standing in literature. Of these, *A. hydrophila* and *A. sobriae* are inherently pathogenic and are included in HG II (Janda & Kokka, 1991). *A. hydrophila* has been consistently recovered from water and aquatic creatures and is present in marine environment also (Stelma, 1989).

Diseases caused by *A. hydrophila* are broadly divided into 2 types - intestinal and extra-intestinal (Stelma, 1989). Disease outbreak due to *A. hydrophila* are mostly sporadic in nature and hence its role in major food poisoning outbreaks are to be ruled out. An outbreak in US in which *A. hydrophila* has been implicated as causative organism has been reported (Abeyta *et al.,* 1981). The outbreak was due to the consumption of raw oyster. Even though *A. hydrophila* is isolated from stool specimens of patients, it was not yet possible to produce the symptoms in human volunteer studies. Probably other virulence factors are also thought to be involved in the initiation of the disease (Morgan & Wood, 1988).

Plessiomonas shigelloides

Plessiomonas shigelloides, formerly classified as *Aeromonas shigelloides*, has been implicated as the cause of human gastroenteritis for more than 40 years. Symptoms of the infection include diarrhoea (94%), abdominal pain (74%), nausea (74%), chills (49%) and fever (37%) (Miller & Koburger, 1985). *Plessiomonas shigelloides* is widespread in aquatic systems, being mostly associated with fresh water, but may also be seen in brackish water and seawater. It is more common in warmer months. The minimum growth temperature of the organism is 8°C., but a few strains could grow at 0°C as well. The organism is sensitive to pH 4.0 or below and a salt level 5.5% or above (Koburger, 1989).

Inherent pathogens and seafood safety

Like any other food commodity, seafood has an in-built microbial flora that might cause potential safety problems. All seafood, excepting those eaten raw, have a good safety record. An insight into previous data shows that virus and sewage associated bacteria are by far the greatest hazard and bacteria of truly marine origin accounted for only a minor fraction of illness. However, emergence of newer pathogens like *V. vulnificus* indicates that people in the high risk categories should be more cautious with seafood than healthy individuals. Some of the control measures that can be adopted are listed below:

i. Continued and coordinated efforts for water quality management, disease surveillance, consumer education in seafood harvesting, processing and marketing

ii. Based on surveillance data, potential areas in which associated pathogens causing largest number of seafood associated food poisoning should be identified for each region and their prevention measures have to be concentrated.

iii. Venues at which seafood are most frequently consumed in reported outbreaks such as commercial establishments, catering centres, restaurants, etc., have to be pinpointed

iv. Long term eradication procedures based on GMP have to be imposed in such establishments and its success monitored periodically

v. Food poisoning outbreaks that are seasonal can be controlled by seasonal marketing regulations and targeted education of high risk groups.

Since these inherent microbial hazards are naturally present in the system, complete eradication is impossible. But reducing the initial density and preventing further multiplication and growth are the best possible means for their control. This emphasizes the need for selection of most ideal storage temperature, avoiding temperature abuse and developing new techniques for reducing initial cell density of these pathogens.

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