Pathogenic Halophilic *Vibrios* in Seafoods

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A number of *Vibrio* spp. viz. *Vibrio cholerae* (01 and non-01), *V. parahaemolyticus, V. vulnificus, V. fluvialis, V. alginolyticus, V. damsela, V. hollisae, V. mimicus, V. cincinnatiensis* and *V. furnissii* are recognized as human pathogens and most of them are native to the aquatic environment. Among halophilic *Vibrios, V. parahaemolyticus* and *V. vulnificus* are the major pathogens.

Vibrio parahaemolyticus

Ever since *Vibrio parahaemolyticus* was isolated from cases of gastroenteritis in 1950, this *Vibrio* has been recognized as a potential enteropathogen all over the world. *V. parahaemolyticus* is of marine origin and can be found in seawater, sediments, plankton, finfishes and shellfishes of coastal and estuarine environments. It is unable to survive or grow at deep ocean hydrostatic pressure. At first, it was thought to be limited to Japan and the Far East, but during the last 20-25 years it has been isolated from numerous marine and brackishwater sources in many countries throughout the world.

Morphologically, *V. parahaemolyticus* is a Gram-negative rod, exhibiting pleomorphism; slightly curved, straight, coccoid and swollen forms can also be observed. All strains of *V. parahaemolyticus* are motile by means of a single polar flagellum. On agar plates, most cultures appear as smooth, moist, circular, opaque colonies with entire edges.

In India, the occurrence of *V. parahaemolyticus* in fish and aquatic environments has been reported by several workers and the incidence in fresh, marine and brackishwater fish varied from 35 to 55%.

V. parahaemolyticus is a facultatively anaerobic, halophilic bacterium. It can grow in ordinary media containing 1 to 8% sodium chloride, but it grows best in the presence of 2 to 4% NaCl: it fails to grow in the absence of NaCl. It prefers an alkaline pH. The recommended pH for the culture media is 7.4 to 8.6. This organism can grow over a pH range from 4.8 to 11.0, but the optimal pH for growth is between 7.0 and 8.6. *V. parahaemolyticus* does not cause marked organoleptic changes to seafoods, even at infectious concentration.

The organism grows between 10 and 44°C with optimum growth at 35-37°C. The minimum and maximum growth temperatures reported are 5 to 8°C and 42 to 45°C respectively. The generation time under optimum condition is very short, generally in the range of 11 to 13 minutes.

V. parahaemolyticus is very heat sensitive and can be inactivated by mild heat. After heating at 60 or 80°C for 15 minutes, no survivors could be detected in shrimp homogenate inoculated with 500 cells/ml. Only when the cell concentration was increased to $2x10^6$/ml were the survivors observed after 15 minutes at 80°C; no survivors were detected after one minute at 100°C.

The extent of inactivation of *V. parahaemolyticus* due to low temperature, however, is far less than that due to heat. Refrigerated temperatures are more detrimental to *V. parahaemolyticus* than freezing. Studies have shown that this organism can survive in frozen fish substratum only upto one month.

V. parahaemolyticus is very sensitive to drying, and hot smoking may kill it. This organism remains viable for weeks in seawater stored at room temperature but in distilled water it is killed within one minute. It is observed that the time of exposure to tap water required to inactivate 90% of the cells of *V. parahaemolyticus* is 2.5 minutes, probably due to the osmotic destruction of the cells. For this reason, washing of fish and items such as containers, chopping board, knives, dishes etc. with tap water may result in some decrease in the number of viable *V. parahaemolyticus.*

Feeding tests with *V. parahaemolyticus* have indicated that Kanagawa-positive strains produced gastroenteritis in human volunteers, whereas others did not. Majority of the strains isolated from patients with gastroenteritis are Kanagawa-positive. It was reported that 20-25% of the *V. parahaemolyticus* isolated from marine and brackishwater fishes of Mangalore and Cochin were Kanagawa-positive.

In man, *V. parahaemolyticus* usually causes either diarrhoea, occasionally dysentery like or gastroenteritis of sudden onset varying from mild to severe. The mortality rate is less than 10%. The size of the infecting dose necessary to produce clinical symptoms may vary with the strain, but it is usually about 10^6 to 10^9 viable cells. The average incubation period is 12-24 hours, but at times ranges from 2-48 hours depending partly on the food and partly on the condition of the stomach. It is self limiting, generally lasting only a few days, with little evidence of spread of the infection from one person to another. *V. parahaemolyticus* and closely related organisms have also been isolated occasionally from infected skin or tissue lesions on palms of fish handlers.

Outbreaks of this *Vibrio* infection occur only in the summer season in Japan where it is one of the most important causative agents of food poisoning. It was considered a local problem until recently, but it has now been detected in almost all countries.

The natural reservoir of *V. parahaemolyticus* seems to be the sea, estuary and the animals harvested from there. Preventing contamination of raw materials, therefore, would be almost impossible. To safeguard seafoods, efforts should be directed to prevent contamination of the finished products, especially those foods that are to be consumed without further cooking. Seafood processors must eliminate the time and temperature abuse as far as possible. This is essential in controlling the organism which can multiply rapidly.

V. parahaemolyticus is sensitive to heat, disinfectant, low temperature, low pH and tap water. However, none of these treatments except heat, would inactivate *V. parahaemolyticus* to a safe level. If people choose

to eat raw or under-cooked foods, they expose themselves, whether knowingly or unknowingly, to certain unnecessary risks. The most important means of controlling infection in human beings lies in simple hygienic measures to prevent multiplication of *Vibrio* in seafoods and to prevent cross contamination of cooked foods from raw seafoods. Refrigeration or freezing is the most important method for preventing multiplication of *V. parahaemolyticus* in seafood. If foods are heated to 100°C shortly before consumption, this kind of food poisoning would not occur.

Vibrio vulnificus

Vibrio vulnificus is an emerging pathogen, phenotypically similar to *Vibrio parahaemolyticus* and has been identified as an etiological agent for three syndromes - primary septicaemia, skin infection and acute diarrhoea. Infection is known to occur by two portals of entry. Ingestion of raw seafood may result in primary septicaemia. Septicaemia generally leads to secondary cutaneous lesions and necrotic ulcers of the extremities; approximately 60% of the known cases result in fatality. The majority of the victims have some underlying chronic disease, typically involving the liver. A second portal involves wound infections resulting from exposure of skin lesions to *V. vulnificus* in seawater and/or shellfish. Infection is associated with the consumption of raw seafood, particularly oysters, when this bacterium becomes concentrated through filter feeding. Consequently the occurrence of this bacterium in aquatic environments is a significant concern of the shell-fish industry and public health agencies.

This organism is part of the normal microflora of estuarine and coastal waters and occurs in high numbers in molluscan shellfish. *V. vulnificus* is transmitted to humans as a result of consumption of raw or insufficiently cooked seafoods, particularly bivalves.

V. vulnificus is a Gram-negative, halophilic, oxidase-positive, lactose-positive, motile and rod-shaped bacterium. The number of *V. vulnificus* cells which must be ingested to produce primary septicaemia or

gastroenteritis in humans is unknown. All *V. vulnificus* strains, both environmental or clinical, produce a haemolysin that affects human erythrocytes in contrast to *V. parahaemolyticus* in which mainly clinical isolates are haemolytic.

The incidence of *V. vulnificus* in water samples and bivalves of some countries has been reported as 25% and its load has been reported to be 10^6/100 g in Gulf Coast oysters in summer. Studies have shown that there is strong correlation between temperature, salinity and presence of *V. vulnificus* in water and oyster.

Raw oysters eaten directly from the shells are more frequently associated with *V. vulnificus* infection than raw oysters taken from commercially packed containers. This suggests that something in the processing reduces or eliminates *V. vulnificus* from the oyster. Ten minutes heat treatment at 50°C has been reported to be adequate to reduce *V. vulnificus* to a non-detectable level. Studies conducted on the survival of *V. vulnificus* in oyster homogenate held at 4°C indicated a rapid and dramatic decrease in viability not attributed to either cold shock or the oyster homogenate alone but to a combination of the two. Fish or shell-fish kept in ice apparently are not a likely source of *V. vulnificus* infection. Effect of salting and drying on this organism is not known. *V. vulnificus* is closely associated with oyster tissues and is not removed completely by controlled purification method such as UV light assisted depuration.

No effective means currently exist for elimination of this health hazard in oyster intended for raw consumption. Therefore it is safer to avoid consumption of raw seafoods.