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T13: Pathogenic Vibrio Species and Their Importance in Fish Processing and Consumer Safety

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Most research and information on human pathogenic vibrios has for the past years accumulated on *Vibrio cholerae* only , probably due to severity of infection and its impact on public health. Of late, other vibrio species are also gaining attention as human pathogens. Of the 42 species of vibrios so far characterised, twelve are recognized as human pathogens. They are *V.alginolyticus, V.carchariae, V.cholerae ,V.cincinnatiennsis, V.damsela, V.fluvialis, V.furnissii, V.hollisae ,V.metschnikovii, V.mimicus, V.parahaemolyticus* and *V.vulnificus*. The nature of infection of these Vibrios may be intestinal, extra intestinal or both. *V. alginolyticus, V. carchariae, V.cincinnatiennsis, and V.damsela* produce extra intestinal infection only. *V.fluvialis, V.furnissii, and V.hollisae* cause only intestinal infections. Others produce both type of infection

Most of the vibrios are the natural inhabitants of the marine habitat of the tropical and subtropical areas of marine, brackish or fresh water environments where they form a major part of the bacterial flora. There is no evidence to show that land animals or birds act as a vehicle of transmission except in the case of *Vibrio cholerae* which is sometimes harbored by aquatic birds.

V. cholerae

V.cholerae is divided into 2 biotypes, Classic and El Tor, based on certain biochemical properties. *V.cholerae* comes under the genus *Vibrio* belonging to the family *Vibrionaceae*. Each biogroup of *V cholerae* is classified into 2 major groups based on the somatic antigenic profile as O1 and non O1 on the basis of agglutination with antisera directed towards cell wall antigens. *V.cholerae* 'O' group has three known serotypes each pessessing distinct antigenic factors. They are Ogawa, Inaba and Hikojima with 'O' antigenic factors AB, AC and ABC respectively.

We are currently in the 8th pandemic of cholera with sporadic cases occurring worldwide even in developed countries. *Vibrio cholerae* belonging to serogroup O1, alone was considered to be causative agent of epidemic cholera and all non-O1 isolates were classified as non- pathogen. A highly epidemic form of cholera like disease on the Indian sub-continent in 1992 prompted the researchers to unveil a highly virulent strain of *V. cholerae* non-O1, now designated as *V.cholerae* O139 Bengal. This strain show striking similarity in biochemical and physiological traits to the *V.cholerae* O1 biotype ElTor. Genetically *V.cholerae* O 139 shares a common antigen with *O 22* and *O 155* and also with one *Aeromonas* species *A.trota*.

Survival in different processing conditions

In artificially contaminated oysters and clams,V.cholerae O1 survives for more than 3 weeks under refrigerated condition and in milk for 4 weeks . Similarly crab meat heated for less than 10 minutes or steamed for 30 minutes were found to harbor viable cells of *V.cholerae*. It is sensitive to pH below 4.5.

Pathogenicity

V.cholerae O1 and some non O1 strains excrete a potent enterotoxin CT (Cholera Toxin) that is responsible for profuse diarrhoea. CT is composed of 5 subunits (binding) of molecular weight

11,000 each surrounding one A (Active) unit. This is composed of 2 subunits A1 and A2. The A subunit is responsible for the activity and B for binding the toxin molecule to ganglioside GMI of mucosal enterocytes

Occurrence in foods

It is clearly established that both O1 and non O1 including *V.cholerae* O 139 are ubiquitous in the environment and are autochthonous to fresh, brackish and marine waters. Although cholera has customarily been associated with contaminated water, recent epidemiological studies have shown that a variety of foods such as fruits, vegetables, milk, egg, cooked rice and meat have been implicated in *V.cholerae* outbreaks due to O1 serotypes. Only one outbreak is so far reported due to O 139 serotype that was specifically related to food. There is a very close association between cholera infection and consumption of seafoods like oysters and crabmeat

Control and prevention

Water is one of the important route of transmission of this pathogen. A well chlorinated water supply free from sewage contamination is a must for proper control of *V.cholerae*. *V.cholerae* are widely reported in aquatic animals and hence the possibility of contamination is more in sea foods. Initial contamination can be controlled by thorough washing with potable water containing 5ppm chlorine. Since they are strict mesophiles further multiplication can be avoided by refrigerated storage or frozen storage depending on the period of storage. Proper cooking of the sea foods and prevention of recontamination of seafood after cooking can prevent the infection. However the disease will continue in person eating raw seafood, especially the high-risk individuals receiving immunosuppressive drug therapy. The water recreational resorts where vibrios are part of the normal flora should also be under close scutiny.

Vibrio parahaemolyticus

The first reported evidence of food poisoning due to *Vibrio parahaemolyticus* was from Osaka in Japan in the year 1950. This organism is facultative halophile inhabiting marine or other saline environments .

Characteristics

V. *parahaemolyticus* shares the common features of the members of the genus V*ibrio*. The pathogenic strains can be differentiated from non-pathogens based on the haemolytic reaction in a special medium called Wagatsuma agar. The thermostable extra cellular hemolysin responsible for this difference is designated as Kanagawa phenomenon to distinguish from other haemolytic factors present in Vibrio species.

Antigenic factors

V. parahaemolyticus synthesizes 3 major antigens., A thermostable somatic O antigen, a thermolabile capsular K antigen and a flagellar antigen H. Based on this an antigenic scheme has been developed by Sakasaki in 1986 for serotyping *V. parahaemolyticus.* While most of the clinical isolates show confirmity to this scheme, the environmental isolates appear untypable due to heterologous cross reactions

Growth

The organism is a true mesophile showing preferential growth at 37°C. While the minimum temperature reported is 5°C it can grow upto 42°C under laboratory condition. Optimum pH is 7.5 to 8.5 though they resist pH range from 5 to 11. They show a definite Na^+ requirement and need at least 0.5% salt to initiate growth. Growth in a_w of upto 0.937 has been noted.

Survival

V.parahaemolyticus is a relatively fragile organism. It is sensitive to drying and heat. Despite the general agreement that the organism exhibits poor cold resistance, there has much controversy on the long- term storage characteristics at lowered temperatures. They are also sensitive to irradiation.

Disease

Human illness that frequently occur is a mild and self limited gastro-enteritis with a duration of 2-3 days. A more severe form with dysenteric type illness have also been reported. Information accumulated over the past two decades clearly establish that hemolysins are the cardinal factors for diseases.

Food poisoning outbreaks

Seafoods are the main vehicle of this type of infection and mainly happen in people eating raw sea foods. Hence this type of gastroenteritis due to *V. parahaemolyticus* is restricted to countries where raw seafoods are a delicacy. A typical annual cycle is indicated for this organism which is well connected with the disease outbreaks. Thus the disease is more prevalent in summer months when the *V. parahaemolyticus* number also increases.

V.vulnificus

Vibrio vulnificus is an emerging pathogen. Considering the invasive nature and lethality of the infection *Vibrio vulnificus* possibly stands out as an important member of the "non-cholerae group" of vibrios. Even though reports on the infection due to this pathogen started to accumulate from 1970, full characterization of *V.vulnificus* was done only in 1976.

This organism is responsible for three types of clinical manifestations in human, namely primary septecemia leading to secondary lesions, gastroenteritis and wound infections. Discovery of this organism came about not as a result of its ability to produce fatal food borne infection, but by its ability to cause wound infection.

Growth and survival

Regarding the growth and survival characteristics, *V. vulnificus* resemble *V. parahaemolyticus*. Due to its obligately halophilic nature, *V. vulnificus* is constantly associated with aquatic animals and the environs where the salinity and water temperature are on the higher side. Most of the reports pertains to the isolation of *V. vulnificus* from coastal waters and aquatic animals especially oyster. Eating raw seafood like oyster and crab was found to be the cause. Infection by *V. vulnificus* in 85% of the cases indicated onset of primary septicemia. The organism failed to be cultured from stool specimen of patients suffering from the disease though some symptoms associated with enteric infection such as abdominal pain and diarrhoea were noted in some cases. In the case of wound infections, the typical sources are bite from a marine animal, laceration of the skin from rock surface, cuts during cleaning of shellfish or from the utensils or exposure of any infected area to marine waters.

Control measures

The organism is part of the natural flora of estuarine waters and its creatures and hence a complete eradication seems difficult. Like other vibrios, it is sensitive to low as well as high temperatures and this property can be exploited for preventing multiplication or for total eradication. Eating raw sea foods should be strictly avoided by individuals, especially those persons with an underlying history of other infection. Also preventing wound infection is more difficult as it occur in healthy individuals too. Cross contamination of the foods specially sea foods can be avoided by following the GMP .

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