Pathogenic Vibrios of public health and aquatic animal health

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Introduction

Classification of Vibrios

Domain - Bacteria
Phylum - Proteo bacteria
Class - Gamma proteobacteria
Order - Vibrionales
Family - Vibrionacea
Genus - *Vibrio*

Vibrios are the diverged group of organisms and mostly had the history of pandemics. They are inhabitants of natural aquatic ecosystem like ocean, River, wells and ponds. They are gram negative facultative anaerobes motile by using single polar flagella. Vibrios do not form spores and capsules. Most of the Vibrios are not fastidious and they tolerate high alkaline pH. Vaibrios can do both Oxidative and fermentative utilization. They are distributed throughout the world with more occurrence in the tropical region. Vibrio occurrence in the temperate regions are more in hotter months. They have the peculiar ability to go to viable but nonculturable state in adverse environmental conditions. Vibrio can be classified in to cholera causing and non-cholera vibrios. Cholera causing vibrios can produce the cholera-toxin and they are *Vibrio cholera* and *Vibrio mimicus.* The important non-cholera pathogenic vibrios are *Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio harveyi* and *Vibrio alginolyticus.*

Vibrios are zoonotic in nature and with the fish they can affect the higher vertebrates also. In aquaculture, any inhabitant in water can affect the production adversely. The sudden onset of diseases, especially by *Vibrio spp*. is becoming a great concern in larval and juvenile penaeids and fishes. Hence, monitoring of aquaculture environments for pathogenic Vibrios is essential to control the spread of Vibrio infections. The members of the genus *Vibrio* are the most important food-borne and aquatic pathogens, which are responsible for illness in humans and cause large-scale mortality in the aquaculture sector. Nowadays, in the international trade of marine fishes, testing of *Vibrio species* has become a criterion of microbiological testing. Even though *Vibrio species* are common inhabitants of the aquatic environment, some species are emerging as pathogens, which can cause up to more than 50 % of deaths of all clinical cases. Major Vibrio sp. viz. *V. harveyi, V. parahaemolyticus, V. alginolyticus, V. anguillarum, V. vulnificus, V. mimicus, and V. splendidus* are usually associated with shrimp diseases. *V. harveyi* is associated with luminescent vibriosis in shrimps e.g., *Litopenaeus vannamei* and *Penaeus monodon* and it is the most important etiological agent for mass mortality in *P. monodon*. The mode of infection in fish mainly consists of penetration of bacterium to the host tissue mainly by the chemotactic activity, followed by deployment of the iron sequestering system and eventually damages the fish through extracellular products i.e., hemolysin and protease.

Traditional method of detection of pathogenic Vibrio species

There are well-established isolation and biochemical confirmation procedures for pathogenic *Vibrio* spp., which were described in ISO and BAM protocol for Vibrios. First stage in traditional detection methods exploits the ability of *Vibrio* species to grow rapidly at relatively high pH values. Media containing sodium chloride and with a pH of about 8.6, such as alkaline saline peptone water (ASPW), are used for enrichment. Typically, a 6-hour preliminary enrichment (at 41.5 ^{o}C for fresh products, or 37 ^{o}C for frozen or salted products) is followed by a second enrichment in ASPW at 41.5 ^{o}C (for *V. cholerae* and *V. parahaemolyticus*) or 37 ^{o}C (for other species) for 18 hours. Preliminary identification based on colony appearance on TCBS agar is traditionally confirmed using classical biochemical tests. The second enrichment culture is inoculated onto thiosulphate citrate bile salts sucrose (TCBS) agar and one other optional selective medium and incubated at 37 ^{o}C for 24 hours. On TCBS agar, *V. mimicus* colonies are green, *V. parahaemolyticus* colonies appear blue-green and *V. harveyi* colonies are green in color. Selective chromogenic agar media specifically designed for the differentiation of pathogenic *Vibrio* species are also available.

***Vibrio cholera* as a human pathogen and aquatic pathogen**

Vibrio cholera is the organism responsible for the disease cholera, an acute illness. The diarrhea cause by cholera is specific with rice water stool. The body will become dehydrated and mortality can occur in hours. This can be cultured with alkaline peptone water enrichment and Thiosulphate citrate bile salt sucrose agar streaking. After 24 h, the TCBS will have yellow round flat colonies of 2-3 mm size. *Vibrio cholerae* has more than 200 serotypes with O antigens. Only serogroup O1 and O139 are found to cause cholera epidemics. The 01 serogroup is divided into two biotypes, Classical and El tor, both of which can cause epidemics. The classical bio-types susceptible to polymixin, VP negative and do not produce hemolysin to lyse heamocytes. Whereas El-tor biotype insusceptible to polymixin, VP positive and produce hemolysin to lyse heamocytes. So far 6 pandemics are caused by Cholera bacteria classical biotype now the cholera occurrences are by 7th pandemic are from Eltor biotype. But this is relatively less fatal and it will survive in human body for more days. Human cholera infection starts with ingestion of the cholera bacterium through food or water. It colonizes the small intestine and produce cholera-toxin in to the host cells. This cause rapid efflux of chloride ions and water to the intestinal lumen. This causes the diarrhea and dehydration.

Vibrio cholera is not causing any apparent cholera disease to fish and shrimp. According to Koch postulate it is not causing any disease. But it can be isolated from aquaculture environment and fish gut. Aquatic environment is the major reservoir of *Vibrio cholerae* before and after the outbreak. Recent evidences support the theory of the fish and water birds can be vectors of cholera outbreak. Most of the Vibrio cholera outbreak are caused by under cooked fish consumption. The Eltor biotype infection in Bengal was brought by Hilsa, which acted as a reservoir.

***Vibrio parahaemolyticus* as a human pathogen and aquatic pathogen**

The first reported occurrence of *Vibrio parahaemolyticus* is in Japan in 1950, where the under-cooked bacteria affected 272 patients and killed more than 20 people. Until then the *Vibrio parahaemolyticus* was not much considered as a pathogen. *Vibrio parahaemolyticus* is a non-cholera Vibrio, which cause gastro-enteritis. This is a halophilic Vibrio which can live in water of 0.5-8 % salt. The infections are caused by consumption of under-cooked or raw

shellfish. It can cause extra intestinal infections also. It can also cause infection to the cooked product from the uncooked product. The occurrence is there in almost all water bodies with necessary sodium requirement. The major virulence factor are hemolysin (TDH, TRH) and cytolysins. The TDH is the major toxin present in 95 % of the *Vibrio parahaemolyticus* and it can be seen as haemolysin in wagatsuma agar. Thermolablile haemolysin also reported from *Vibrio parahaemolyticus*. This also cause similar result in heme supplemented blood agar. The toxins are having cardio-toxicity, cell toxicity and center toxicity. The toxins are releases as monomers to extra-bacteria space and they become oligomer to make pore in the host cells. This can also spread through open wounds and cause septicaemia. The toxin production is correlated with Urease production in the *Vibrio parahaemolyticus*. The disease propagation in cells needs ammonia, which can be produced by the Urease positive *Vibrio parahaemolyticus*. More than 800 food-borne disease outbreaks were reported in china, out of which, 40 % are from *Vibrio parahaemolyticus* alone.

The *Vibrio parahaemolyticus* is a deadly pathogen for shrimp, which causes early mortality syndrome. It causes hepatopancreatic necrosis and sloughing of intestinal epithelium. The *Vibrio parahaemolyticus* infections have caused major losses in aquaculture industry. Food poisoning due to *Vibrio parahaemolyticus* occurs in warmer months. It is associated with Fish, crab, shrimp, lobsters and oysters. If consumers eat the under cooked seafood contaminated with *Vibrio parahaemolyticus*, the disease occurrence is confirmed. The feces of patients are contaminated with these bacteria and it mostly follows the fecal oral route. It causes fever, chills. Nausea and water like stools. The shock from the toxin sometime gives death.

***Vibrio vulnificus* as human and aquatic pathogen**

Vibrio vulnificus is a halophilic aquatic Vibrio, which has relatively low occurrence compared to *Vibrio cholera* and *Vibrio parahaemolyticus.* It can occur world-wide from temperature ranging from 9-35 °C and salinities ranging from 0.5- 35. It causes diseases such as necrotozing fasciitis, Gastero enteritis and wound infections. This mostly infects person with underlying medical conditions such as liver diseases, immune-compromisation and iron storage disorders. The bacteria possess cytolysins, hemolysin and specialized siderophores (Vulnibactin) as immune factors. This can produce amine putrescene and cadavarine from ornithine and Lysine. They can neutralize the gut acid and can cause gastro-enteritis. *Vibrio vulnificus* produces superoxide dismutase and nullify the peroxide present in the neutrophils. So, the infection can also travel through the neutophils. The bacteria have 3 bio types. Biotype one is arginine negative, ornithine Indole and lysine positive. The biotype two is Indole and ornithine negative. The first biotype is known to cause disease to the human. And second biotype is known to cause fish diseases. Third biotype has the mixed characteristics and its geographical distribution restricted to Israel.

The contamination of *Vibrio vulnificus* will not cause any odour or appearance change. It is present in warm waters and can be accumulated in filter feeding bivalves. The fatality is very high compared to the bio-safety level 3 and 4 pathogens such as plague, anthrax and Ebola. In immuno compromised persons the consumption can cause gastro-enteritis, which if untreated can enter bloodstream and can be fatal. The wound infections could start after the handling of infected fish and seafood, especially shellfish and after the practice of aquatic activities such as swimming. More than 50 % of primary septicaemia due to *Vibrio vulnificus* result in death within the first 72 h of hospitalization. If there is infection diagnosed due to

Vibrio vulnificus, immediate and appropriate antibiotic treatment with surgical intervention is necessary.

Vibrio vulnificus is known to cause Gastroenteritis, primary sepsis, and wound infection. Rare cases of spontaneous bacterial peritonitis, Pneumonia, Endometritis, Meningitis, Septic arthritis, Osteomyelitis, Endophthalmitis and Keratitis to human beings.

An aquatic Vibrio Disease - Early mortality syndrome

The AHPND (Acute Hepatopancreatic Necrosis Disease) is caused by *Vibrio parahaemolyticus, Vibrio punensis, V. harveyi, and shewanella sp* with the disease-causing plasmid pVA1. The plasmid code for the Pir toxin A and Pir toxin B (Photorhabdus luminous insect related). This is one of the reasons behind shrimp aquaculture collapsed in South-Asian countries. It develops quickly, starting approximately 8 days post stocking and severe mortality (up to 100 %) occurs within 20–30 days. The toxins can cause opaqueness, organ liquefaction and death.

Control method for zoonotic Vibrio diseases in aquatic food production sectors

- The handlers should not be immune compromised
- The handlers should wear gloves while handling diseased fishes
- Fish source should be disease free.
- Farm should have bio-security measures
- The affected farm water should be treated with bleaching powder before release
- Water quality parameters should be optimum
- Maintenance of personal hygiene including washing hands before and after handling fish.