Chapter 1

Introduction

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Vibrio parahaemolyticus is a gram -ve, curved rod-shaped, oxidase-positive, non-spore forming, a halophilic bacterium. It belongs to Phylum-Proteobacteria, Class-Gamma Proteobacteria, Order-Vibrionales and Family-Vibrionaceae (Farmer and Janda, 2004). This bacterium is highly mortal in liquid media due to the presence of single polar flagella. It also has numerous lateral flagella which helps bacteria to migrate in semi-solid media and biofilm formation. The bacteria can grow in a wide range of temperature and its optimum growth temperatures lies between 35 °C and 39 °C (Jackson, 1974). The presence of *V. parahaemolyticus* is also identified from the location where the temperature is around 15 °C throughout the year (Johnson et al., 2010). This bacterium can grow in a wide range of salinity from 0.5 % to 10 % but the optimum salinity for growth ranged from 1 % to 3 %. Similarly, it can grow in a broad pH range of 4.8-11.0 but the optimum pH range is from 7.6-8.6 (Jay et al., 2000; Parveen et al., 2013). The doubling time of this organism is less than 20 min under optimal conditions and in certain conditions, the generation time maybe 5 min (Barrow and Miller, 1974; Jackson, 1974). This organism is ubiquitously present in marine and estuarine ecosystems and can be found free-swimming or attached in sediments, zooplankton, fish, eel, crab, clams, oysters, lobsters, scallops, sardines shellfish and squid (Fishbein et. al., 1974; Al-Othrubi et al., 2011; Khouadja et al., 2013; Yano et al., 2014; Lopatek et al., 2015). Under adverse conditions, the *V. parahaemolyticus* can survive under sediment and under favorable conditions, it appears in the water layer (Su et al., 2007) and spread under marine and estuarine environment (Gamble et al., 2007).

In the year 1976, this bacterium was first time reported to be involved in the food born infection of 276 people after consumption of contaminated seafood (sardines) in Japan (Fujino, 1953). Two bacteria were isolated from the stool samples one of them was identified as *Proteus morganii* while another unclassified one was named *Pasteurella parahaemolyticus*. Further analysis showed that only this bacterium is responsible for infection in mice and was able to grow in salt media. Therefore, *Pasteurella parahaemolyticus* was classified as *V. parahaemolyticus* (Broberg et al., 2011). After this, the bacterium was frequently isolated from different countries of Asia associated with the infection due to the consumption of contaminated seafood. The pathogenic strain of *V. parahaemlolyticus* first time emerged in the history as a pandemic strain in 1966 in Calcutta, India. This was found to be associated with a novel strain O3:K6 (Okuda et al., 1997). Since then, it moved to America, Europe, Australia, New Zeeland, Russia and Alaska (Nair et al., 2007). The infection associated with this bacterium was reported from Australia, Bangladesh, Canada, China, France, Germany, Hong Kong, India, Indonesia, England, Italy, Laos, Malaysia, Philippines, Spain, Taiwan, Tanzania, Thailand, Vietnam, and USA (Molenda et al., 1972; Lalitha et al., 1983; Hally et al., 1995; Pan et al., 1997; Nair et al., 2007 and Pal, 2014). *V. parahaemolyticus* is responsible for the gastrointestinal infection. The symptoms include dysentery, diarrhea, abdominal pain, nausea, chills, vomiting, fever, water-like stools and stools with blood (Yeung et al., 2004; Shimohata et al., 2010). Disease outbreaks in the United States associated with *V. parahaemolyticus* were reported since 1969. Every year approximately 2800 cases of gastrointestinal infection due to the consumption of raw oyster were estimated by The Centers for Disease Control and Prevention (CDC), United States.

The whole-genome sequencing of *V. parahaemolyticus* revealed that, it has two circular chromosome, chromosome 1 and chromosome 2. The chromosome 1 and chromosome 2 consist of 3,288,558 bp and 1,877,212 base pair, respectively (Makino et al., 2003). 4,832 genes were identified in the genome of *V. parahaemolyticus*. Phylogenetically *V. parahaemolyticus* is very close to *V. cholerae* and carries almost same sized genome (*V. parahaemolyticus* 3·3 Mb, *V. cholerae* 3·0 Mb). The chromosome 2 of *V. parahaemolyticus* is large in size than *V. cholera. V. parahaemolyticus* has three different pathogenic islands, Type III secretion system I (T3SS1) in chromosome I, Type III secretion system II (T3SS2) in chromosome II and Type VI secretion system which is absent in *V cholera.* The genes present in the type III secretion system encode various effector proteins and these proteins are transported into the cytoplasm of the host cell through a syringe-like apparatus (Park et al., 2004). Type III secretion system (TTSS) also identified within the genome of other diarrhea-causing bacteria like *Shigella* spp., *Salmonella* spp. and *Escherichia coli.* The T3SS1 of *V. parahaemolyticus* is very similar to the secretion system of *Yersinia* whereas T3SS2 is found similar to the secretion system of *Salmonella* and *Shigella* (Troisfontaines and Cornelis, 2005). *V. parahaemolyticus* is a highly diversified species both genetically and serotypically. 13 O serotypes and 71 K serotypes of *V. parahaemolyticus* has been identified worldwide (Iguchi et al., 1995). The diversity within the species is due to the acquisition of virulent genes, mobile genetic elements and genetic islands by recombination which may increase their fitness and their virulence potential (González-Escalona et al., 2008; Yan et al., 2011) in changing the environment (Baker-Austin et al., 2010). The clinical and pandemic strain of *V. parahaemolyticus* (O3:K6) evolve from non-pandemic isolates

after acquisition of *toxRS* gene along with other seven pathogenic islands VPaI-1 to VPaI-7 (Hurley et al., 2006) by horizontal gene transfer. Acquisitions of genes from the environments increase the fitness of the bacteria and also increase the diversity of the bacteria.

V. parahaemolyticus is one among the twelve known pathogenic *Vibrio* spp. which causes infection in humans and vibriosis in finfish, shellfish and marine invertebrates (Gopal et al., 2005; Khouadja et al., 2013, Peng et al., 2016). *V parahaemolyticus* is responsible for exophthalmia, ulcers, septicemias and corneal opaqueness in fish (Egidus, 1987) and Acute Hepatopancreatic Necrosis Disease (AHPND) in shrimp (Tran et al., 2013). Shellfish are cultured in brackish or saltwater environment which is also a natural habitat for *V. parahaemolyticus*. Vibriosis is one of the major disease problems in aquaculture caused by *Vibrio* spp. (Chen et al., 2000). Vibriosis is a common bacterial disease in finfish and shellfish which causes great economic loss in aquaculture industries. Aquaculture is presently one of the fastest-growing food sectors in the world and is established itself as a resource to support nutritional security for billions through supplementing cheapest source of animal protein. The world population is predicted to reach nine billion by 2050, leading to a greater demand for global food security. Considering the global fish production, India is the second-largest fish producing country in the world with 8.8 MT total fish production of which, 4.5 MT contributes from culture-based fisheries (FAO, 2011). Shrimp farming remains the largest export-oriented aquaculture production sector and it contributed Rs. 4500 crore to the world's farmed shrimp production (Ayyapan et al., 2011). Giant Tiger Shrimp, *Penaeus monodon*, and the

Pacific White Shrimp, *Litopenaeus vannamei* are the two species dominating the Indian shrimp farming. However, in last two decades due to high intensification and improper management of these shrimp farming resulted in serious disease outbreaks mostly by virus and bacteria. This has led to serious economic losses of the shrimp culture industry in recent years (Walker and Mohan, 2009). Early Mortality Syndrome (EMS) is one of the major problems in shrimp farming caused by *V. parahaemolyticus.* Shrimp post-larvae of 20-30 days after stocking are prone to the EMS / AHPND disease often resulted in 100 % mortality. The intensive mortality in shrimp farms due to this bacterium was first reported from China in 2009 and it was found to spread successively to Vietnam (2010), Malaysia (2011) and Thailand (2012). The loss to the Asian shrimp culture sector was estimated to be USD 1 billion by Global Aquaculture Alliance. The antibiotics resistant in several bacterial genera arises due to excessive use of antimicrobials in human, agriculture, and aquaculture systems (Cabello, 2006; Kang et al., 2016). The presences of antimicrobial resistance *V. parahaemolyticus* isolates in aquaculture ponds and shrimp samples were reported from Thailand (Yano et al., 2014), China (Zhang et al., 2014), Malaysia (Al-Othrubietal., 2011) and Poland which (Lopatek et al., 2015) is a serious concern over global spread of Multi-Drug Resistant strain. The movements of live aquatic animals and animal products accelerated the accidental spread of this bacterium into new populations or different geographical regions.

The most pandemic strain of *V. parahaemolyticus* was first isolated from a case of gastroenteritis in India by Chatterjee et al. (1970) and 10 % of the patients admitted to the Infectious Disease Hospital in Kolkata, India due to gastroenteritis were infected

with *V. parahaemolyticus* (Deb, 1975). New strains belonging to the O3:K6 serovar was found for the first time in Calcutta, India during February 1996. It accounted for 50 % to 80 % of the strains isolated from clinical specimens in 1996 in India from February to August (Wong et al., 2005). Strains of the same serogroup were isolated few months later in neighboring countries like Bangladesh,Vietnam, Indonesia, Laos, Japan, Korea, and Thailand (Nair et al., 2007). It was thought that the pandemic strain of *V. parahaemolyticus* (O3:K6) was evolved in India and later and spread throughout the world, including countries in Asia and the United States. Trade-in lives aquatic animals and their products accelerate the spread of these bacteria globally. The prevalence of the *V. parahaemolyticus* was isolated and identified through molecular diagnosis like PCR and Dot-Blot Hybridization from finfish and shellfish collected from the different parts of India by many workers (Das et al., 2013; Thakur et al., 2003; Jayasree et al., 2006; Subhashini et al., 2011; Mishra et al., 2006). However, the genetic diversity and pathogenicity of *V. parahaemolyticus* were not studied well in India.