I. Introduction
Members of the genus *Vibrio* are Gram negative bacteria autochthonous to the aquatic ecosystem (Thompson *et al*., 2004). Among the 98 species currently recognized in the genus *Vibrio* (http://www.vibriobiology.net/), 11 species have been identified as human pathogens (Joseph *et al*., 1982; Janda *et al*., 1988; Shinoda and Miyoshi, 2011.) and several others are potentially pathogenic. The most important human pathogenic vibrios include *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* and their infection follow either direct contact with aquatic environment or indirectly via contaminated food and water. Among them, *Vibrio parahaemolyticus* is considered as an foodborne pathogen, autochthonous to the water and sediments of coastal and estuarine ecosystem. It is responsible for the seafood-borne infections when raw or contaminated seafood is consumed (Daniels *et al*., 2000; Iwamoto *et al*., 2010). This organism was first identified from the cases of seafood-borne gastroenteritis in Japan during 1950’s and credit for the discovery of *V. parahaemolyticus* goes to Tunesaburo Fujino. The organism was first isolated form the source of semi-processed Japanese anchovy, *Engraulis japonicus* “shirasu” during an outbreak of gastroenteritis in Osaka, Japan (Fujino *et al*., 1951; Fujino *et al*., 1953). Similar organism was identified from gastroenteritis outbreaks from India in 1970. Studies undertaken at the Cholera Research Centre, Calcutta during 1970s indicate that about 5-10% of the gastroenteritis cases admitted annually at Infectious Disease Hospital, Calcutta were due to *V. parahaemolyticus* infection. Now this pathogen is a common cause of food-borne illnesses in many Asian countries, including China, Japan and Taiwan, and is recognized as the leading cause of human gastroenteritis associated with seafood consumption around the globe. The global dissemination of this pathogen emphasizes the importance of understanding its many virulence attributes and their effects on the human host.
Many virulence characters are described to play a role in the pathogenicity of *V. parahaemolyticus*. The most important virulence property is the production of thermostable direct hemolysin (TDH) and/or TDH-related hemolysin (TRH) coded by gene *tdh* and *trh* respectively (Nishibuchi and Kaper, 1995). TDH produces well-defined clear haemolysis in a high salt blood agar (Wagatsuma agar) and the resultant action is termed as Kanagawa phenomenon. Kanagawa negative strain isolated for the first time from the patients during gastroenteritis outbreaks in the Republic of Maldives known to contain *trh* gene. (Honda et al., 1987a). It is well known fact that, both *tdh*\(^{+}\) and *trh*\(^{+}\) isolates of *V. parahaemolyticus* were known to associate with human gastroenteritis (Nishibuchi and Kaper, 1995). The genes coding for TDH and TRH share about 70% nucleotide sequence similarity (Nishibuchi et al., 1989). Isolates of *V. parahaemolyticus* from clinical samples are able to produce Kanagawa phenomenon in Wagatsuma agar but only few percentage of environmental or seafood isolates show Kanagawa positivity.

The presence of gene encoding for hemolysins (TDH and/or TRH) has been used as a virulence marker in studying the pathogenicity of this organism. However recently, studies have shown that apart from *tdh* and *trh*, there exists several other putative genes that are associated with pathogenesis (Makino et al., 2003; Ono et al., 2006; Honda, et al., 2008; Kodama et al., 2008; Hiyoshi et al., 2010). Many Gram negative pathogens utilize Type III secretion systems (T3SS) to induce pathogenesis by translocating effector proteins into the cytosol of eukaryotic cells. The T3SS is composed of a needle shaped apparatus comprising protein components and a large number of effector proteins. Upon entering the host cell, the active form of effector protein manipulates the host cell signalling pathways to disrupt host immune response. Genome mapping of a clinical isolate (RIMD2210633) confirmed the presence of two sets of T3SS clusters (Makino et al., 2003). Further based on the divergence
in nucleotide sequences of T3SS2 gene clusters in *V. parahaemolyticus*, two distinctive phylotypes of T3SS2 have been recognized in clinical isolates namely T3SS2α in *tdh*\(^{+}\)ve strains and T3SS2β in *trh*\(^{+}\) strains (Okada *et al.*, 2009). The toxins secreted by these T3SS are alleged to have an apparent responsibility in the pathogenesis of the organism. These virulence factors are responsible for both cytotoxicity and enterotoxicity (Broberg *et al.*, 2011; Karunasagar *et al.*, 2012). Hence, screening for hemolysin genes of *V. parahaemolyticus* alone may underestimate the pathogenic potential of this organism.

The aforesaid virulence attributes have been detected and well studied in the clinical strains of *V. parahaemolyticus* and hair’s breadth of reports in isolates of environmental origin (Noriea *et al.*, 2010). In the previous studies, the prevalence of *V. parahaemolyticus* in seafood harvested along southwest coast of India (Deepanjali *et al.* 2005; Dileep *et al.* 2003; Kumar *et al.* 2011; Parvathi *et al.* 2006; Raghunath *et al.* 2008) has been reported but till date there is no information available regarding the T3SS. This leading to a paucity of data; consequently their virulence potential ruins underestimated in *V. parahaemolyticus* isolated from tropical environments. Hence, this study was principally focused to identify, characterize of T3SS2β genes and to understand their distribution in *trh*\(^{+}\) *V. parahaemolyticus* isolated from seafood. The proposed objectives of this study are as follows;

**Objectives**

1. Identification of T3SS2β genes in *V. parahaemolyticus* isolated from seafood.
2. To clone and express genes encoding T3SS effector proteins of *V. parahaemolyticus*.
3. To develop polyclonal antibody against T3SS effector protein of *V. parahaemolyticus*.
4. To study the effect of *V. parahaemolyticus* on HeLa cells.
5. To study the level of expression of putative virulence genes during infection of HeLa cells.