Summary

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Vibrio parahaemolyticus is a gram-ve, halophilic bacteria ubiquitously present in the marine and brackish water ecosystem causes diseases to the aquatic invertebrate and vertebrate as well as higher vertebrate around the world. To understand the prevalence of this bacterium in India, shrimp samples were collected from three different states (Andhra Pradesh, Gujarat and West Bengal) of India. The identity of the isolated strains was confirmed using biochemical and molecular techniques (16S rRNA gene and *toxR* gene amplification). The *V. parahaemolyticus* strain of 183 was isolated from 300 shrimp samples which clearly indicate the prevalence of *V. parahaemolyticus* in the culture system in India. Most of the *V. parahaemolyticus* isolated from environmental samples are non-pathogenic but they are acquiring virulent genes from the environment by horizontal gene transfer. Therefore, to understand the distribution of these virulent genes among the isolates nine virulent genes (*vcrD1, vp1680, vopD* under T3SS1; *vcrD2, vopD2, vopB2 vopC* under T3SS2 and three hemolysin gene *tlh, tdh, trh*) were selected and screened in all the isolates. All the isolates were carrying the virulent genes under Type III secretion system I. But the virulent genes under Type III secretion system II (TTSS2) was present only in the 3% of the isolates. 3% of the isolates showed the presence of the *tdh* gene and 8% of the isolates showed the presence of *trh* gene which is considered as a major virulent gene in *V. parahaemolyticus.* Furthermore, the pathogenic potential of three hemolysin genes (*tdh, trh* and *tlh*) was also studied and found *tdh* and *trh* positive bacteria strains of *V. parahaemolyticus* hemolyse human RBCs quite faster in comparison to *tlh* positive strain. Both the *tdh* and *trh* positive isolates showed high cytotoxicity in HEK cell line.

The genetic diversity within and between *Vibrio parahaemolyticus* isolated from three different geographical locations was analyzed by using the 16S rRNA gene. The biotype diversity (h) was high for all three populations, however phylogenetic study of *V. parahaemolyticus* revealed an admixture of biotypes. Tajima's D (-2.59470; $p < 0.001$) test of selective neutrality and Fu's *Fs* (-32.422; $p < 0.001$) test were negative and significant, suggesting a sudden population expansion with limited time for population differentiation. This shorter generation time along, random mutations and the mechanisms of genetic recombination possibly helped *V. parahaemolyticus* to evolve very quickly and hence high genetic diversity. Multiple copies, intragenomic heterogenicity and hypervariable regions within 16S rRNA allow greater intraspecies genetic diversity. The discriminatory power of 16S rRNA or single locus is less significance to understand the intraspecies genetic diversity. Therefore, four conserved genes within the bacterial genome have been included to get a better picture of genetic diversity of *V. parahaemolyticus***.** Multilocus sequence typing was used to understand the different genetic population of *V. parahaemolyticus*. Thirty-eight sequence types (STs) were identified which revealed similar genetic pattern.

The extracellular protein was isolated and identified by using MALDI-TOF-MS/MS. Five "moonlighting proteins" viz; RegA protein, WhiA protein, 50S ribosomal protein and RecA proteins along with three metabolic enzymes viz; Acetaldehyde dehydrogenases, Glyceraldehyde-3-phosphate dehydrogenase and Orotidine 5 phosphate decarboxylase were identified in the secretome of *V. parahaemolyticus.* In spite of major metabolic role play by these proteins inside the cell they are also playing a significant

role in pathogenesis like cell adhesion, promote the attachment of bacteria and regulate virulent gene expression when they secreted outside of the cell.

The pathogenicity study of *V. parahaemolyticus* was carried out using *Labeo rohita*. The route of infection and dose of bacteria for the experimental challenge was determined by intraperitoneal and oral administration of *tdh* and *trh* positive strain of *V. parahaemolyticus* in Indian Major Carp, *Labeo rohita* caused 100% mortality at the level of 2.0×10^8 and 1.6×10^8 CFU ml^{-1}, respectively. The histopathological changes like infiltration of blood cells and degenerated hepatic tissue in the liver, degeneration of glomeruli, necrosis of renal tubules and Bowman's capsule in the kidney section, Ragged, irregular shaped villi and necrosis of villus in the intestinal lumen confirm the tissue-specific infection of *V. parahaemolyticus*. Differential gene expression study showed that after experimental challenge the expression of toll-like receptor was increased. Again the expression of proinflammatory cytokines like IL-β, IL-6 and TNFα was also increased in the initial stage of infection. Overexpression of complement factor 3 and Heat shock proteins 70 was also observed after post infection.

Trh is one of the major virulent proteins in *V. parahaemolyticus*. Inhibition of Trh might be reduced the infection. Therefore we are trying to identify the druggability of protein pockets in the Trh protein. But the crystallographic or NMR structure of Trh protein is not available in the Protein Data Bank (PDB). Therefore, 3D model of the Trh protein was generated using a comparative homology modeling approach. This predicted Trh protein model was then validated using Ramachandran plot analysis and ProSA analysis. Additionally, MD simulation, trajectory analysis and average RMSD revealed that the Trh protein was stable throughout the simulation. Furthermore, druggability

probability analyses revealed that theTdh and Trh protein possesses a total of 19 druggable sites out of which pocket 2 is the most significant since it contains eleven amino acids residue which actively participates in the formation of the tetrameric structure which is important for the cytotoxicity of Tdh and Trh. The present study provides an insight into the druggable pockets of Tdh and Trh protein which open new vistas for drug designing and development of medical therapeutics against *V. parahaemolyticus* infections in the near future

Overall the present study provides detail information on the prevalence of *V.parahaemolyticus* in the environmental samples as well as their genetic diversity and distribution of virulent genes in environmental strain and pathogenic strain in India. This information will help to monitor the emergence of new pathogenic strain, development of diagnostics and therapeutics. In addition to that, the prediction of the druggable target site in TRH protein will help in designing a suitable drug to control the infection. Furthermore, pathogenesis and immunogene expression study during infection in model organism *L. rohita* will provide insights on gene regulation and a potential way for therapeutic intervention in humans