Abstract

The present study was conducted to understand the prevalence of *Vibrio parahaemolyticus,* its genetic diversity and pathogenicity in environmental isolates. Shrimp samples were collected from three major shrimp producing states *viz.* West Bengal, Andhra Pradesh and Gujarat in India from February 2014 to June 2017. A total of 183 *V. parahaemolyticus* were isolated and identified using *toxR* and 16S rRNA gene. The genetic diversity of *V. parahaemolyticus* isolated from three different geographical locations was analyzed by using 16S rRNA gene. Though, the biotype diversity (h) was high for all three populations; however, phylogeny of *V. parahaemolyticus* revealed an admixture of biotypes. For further confirmation, Multilocus sequence typing was used to understand the different genetic populations of *V. parahaemolyticus*. Thirty-eight sequence types (STs) were identified using different housekeeping genes which revealed similar genetic patterns. All the isolates showed the presence of *vcrD1, vp1680* and *vopD1* virulent genes under T3SS1 whereas only 3 % of isolates showed the presence of all the virulent genes (*vcrD2*, *vopD2*, *vopB2* and *vopC*) under T3SS2. The study revealed that 8 % of the isolates had virulent *trh* gene and 3 % of the isolates had *tdh* gene. None of the isolates contained both genes *tdh* and *trh*. The pathogenicity study with *tdh* and *trh* positive strains revealed that, the *tdh,* and *trh* positive isolates were resistant to β-lactam antibiotics and was able to lyse more than 95 % human RBCs. Both *tdh* and *trh* positive isolates showed high cytotoxicity in HEK cell line. 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 of *L. rohita* were observed after experimental challenge. The changes like degeneration of glomeruli, necrosis of renal tubules and Bowman's capsule were observed in the kidney section. Ragged, irregular shaped villi and necrosis of villus were observed in the intestinal lumen. Gene expression profiling of immune-related genes revealed that IL-β, IL-6, TNF-α,C3a, Hsp 70 and Toll-like Receptor were upregulated in response to challenge with *V. parahaemolyticus.* Proteomics analysis revealed that, five "moonlighting proteins" *viz*. RegA protein, WhiA protein, 50S ribosomal protein and *Rec*A proteins along with three metabolic enzymes, *viz*. acetaldehyde dehydrogenases, glyceraldehyde-3-phosphate dehydrogenase and orotidine 5 phosphate decarboxylase were identified which plays an important role in cell adhesion, cell multiplication and regulate virulent gene expression. The *In-silico* analysis of Tdh and Trh protein revealed compactness, dynamics, and residual fluctuations of the generated models. The druggability probability analyses revealed that Trh protein possesses a total of 19 druggable sites out of which pocket 2 is most significant since it contains eleven amino acid residues (E138, Y140, C151, F158, C161, K162, S163, and Q164) which actively participate in the formation of tetrameric structure. Overall the present analysis will help in monitoring the emergence of new pathogen and also help in development of new druggable molecule and a potential way for therapeutic intervention to treat the infection caused by *V. parahaemolyticus*.