Molecular insights into DNA gyrase subunit A of an environmental El Tor variant of V. cholerae O1

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Vibrio cholerae is globally disseminated gram negative zoonotic pathogen and is mainly found in the aquatic flora and fauna where it resides mainly in chitinous aquatic invertebrates. It causes fatal cholera disease in human and mainly occur through the ingestion of contaminated water as well as the consumption of partially cooked or uncooked seafoods. Recently, there have been numerous reports of cholera outbreaks worldwide accounting one lakh death annually with Middle East and Southern Asia contributing to more episodes of endemic cholerae diseases. Pathogenic strain possesses various virulence factors such as genes encoding cholera prophage region (*ctx, ace*, and *zot*) and type IV pilli (*tcp* gene clusters) which are essential for the bacteria to colonize and infect in human host. In addition to this, pandemic strains found to possess various other defence and secretary systems including pandemic islands.

Recently, several outbreak cases were reported due to multidrug resistant *V. cholerae* globally and among these, the reduced susceptibility to ciprofloxacin is major concern. The emergence of the fluroquinolone resistant strains are attributed to the presence of mutation in fluroquinolone resistance determinant element such as *gyrA, gyrB, parC* and *parE* genes. The mutations in bacterial enzymes such as DNA gyrase and DNA topoisomerase IV can change or modify the drug targets or lessen the activity of drug either by reducing its accumulation or deleterious lethal action on cells. The major target for quinolone resistance in gram negative bacteria is DNA gyrase whereas in gram positive bacteria it is *parC* gene. The mutation is due to the amino acid substitution event in quinolone resistance determining region and this region is recognized as DNA binding region of the enzyme with the drug. The mutation in 83rd and 87th position of the amino acid in *gyrA* is considered as the hot spot region where as in parC gene, the hot spot region for mutation is positioned at 85. It was reported that the rate of mutation has a strong positive correlation for quinolone resistance in bacteria. In addition to target-enzyme mechanism of resistance, plasmid mediated resistance termed *qnr* is also common in many gram-negative bacteria. However, in *V.* cholerae the most common mechanism of resistance is enzyme

mediated efflux system where the mutated gyraseA impair the target binding site of the DNA-enzyme complex thereby altering the membrane permeability of the cell.

The research study conducted to monitor the aquatic environment of Cochin, India for the presence of new variants of *V. cholerae* over a period of 05 years (2018-2022) revealed ctx negative O1 positive characteristics in one isolate from coastal water near to a retail seafood market of Thoppumpady fishing harbour, Cochin and the isolate was identified as *V. cholerae* O1 El Tor variant. Antimicrobial susceptibility testing showed that the isolate was resistant to Ampicillin, Chloramphenicol, Cotrimoxozole, Ciprofloxacin and Streptomycin. The isolate possessed several virulence and antimicrobial resistance genes such as *VgrG, mshA, ompT, toxR, ompU, rtxA, als, VasX, makA, hlyA gyrA, dfrA1, strB, parE, sul2, parC, strA*, VC1786ICE9-*floR*, and *catB9* genes. The protein sequence of the gene coding for DNA gyrase A of the isolate was predicted using NCBI BLAST. The protein sequence of the gyrA gene of the isolate showed 99.89% identity to the protein sequences of *gyrA* of *V. cholerae* O1 serotype, ATCC 39315/ El Tor Inaba N16961(Uniprot id Q9KSJ81). The sequence alignment of protein showed mutation in *gyrA* sequence of the isolate at 83rd position (Serine to Isoleucine) (Fig. 1). The SWISS MODEL template search followed by alignment (ProMod3) and Model quality search (QMEAN Scoring Function) resulted 99.89% sequence identity with AlphaFold DB model of C3LLV4_VIBCM (gyrA protein of *V. cholerae* serotype O1 strain M66-2). Active binding site prediction using Biovia Discovery studio Visualizer (Version 21.1.0.20298) revealed the potential drug target of the enzyme (Fig 2). The effect of mutation on the flexibility of the protein needs to be investigated further by employing drug-protein interaction via docking and molecular stimulation and will be undertaken in the future research. It was reported that, global emergence and spread of new variants of *V. cholerae* is mainly attributed due to certain changes in their genetic makeup particularly in genes associated with cholera toxin production and antimicrobial resistance. There are several drug-protein interaction methods such as molecular docking analysis, bio inspired microwell array etc, that were developed to study the interaction between the drug resistant mutation on protein function and also on drug resistance level of the strain. These experimental studies will give better understanding on the underlying mechanism of native and mutated forms of protein on drug and also will give valuable information for the exploration and discovery of new drugs.

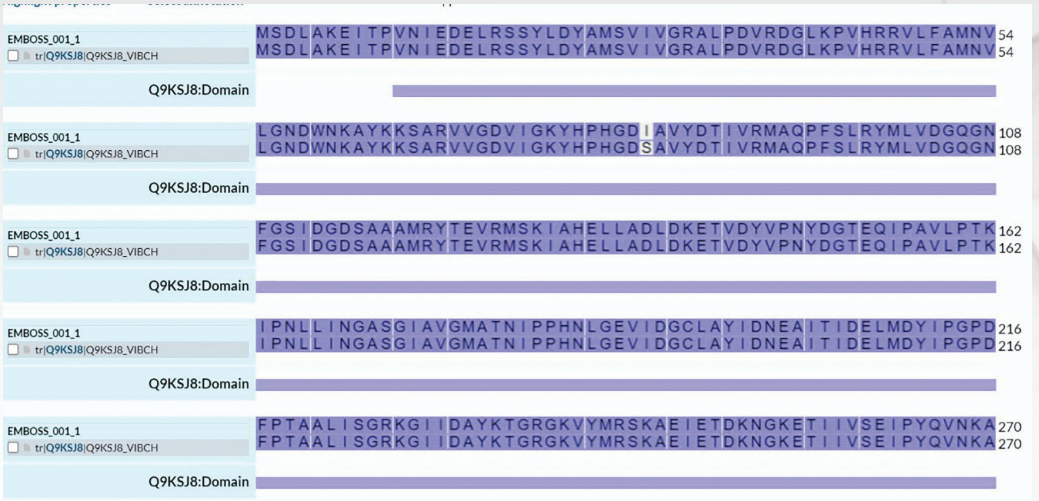

Fig. 1. Sequence alignment of gyrA protein sequence (EMBOSS 001_1- environmental El Tor variant V. cholerae O1; Q9KSJ8:Domain- gyrA protein sequence of V. cholerae O1 serotype O1 strain ATCC 39315/El Tor Inaba N16961(Uniprot id Q9KSJ81).

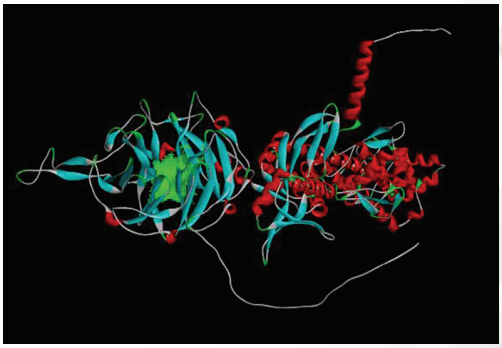

Fig. 2. Predicted 3D structure of gyrA enzyme of environmental El Tor variant V. cholerae O1. The active binding site shown in green grid box with coordinates (X=-45.0889, Y= -7.579, Z= 36.3016)

References

George A.J., 2005. Mechanisms of Resistance to Quinolones, Clinical Infectious Diseases, Volume 41, Pages S120–S126, https://doi.org/10.1086/428052

Rauta, P.R., Ashe, S., Nayak, D. and Nayak, B., 2016. In silico identification of outer membrane protein (Omp) and subunit vaccine design against pathogenic Vibrio cholerae. Computational biology and chemistry, 65, pp.61-68.