COMPARATIVE MODELING AND ACTIVE SITE ANALYSIS OF DRUG TARGETS OF *SALMONELLA TYPHIMURIUM*.

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Abstract

Salmonella typhimurium is an enteric bacteria causing gastroenteritis, a life threating disease in human beings. There are virulence associated factors coded by virulent genes clustered on genome employed in the regulation of its pathogenesis. The emergence of multi-drug resistant *Salmonella typhimurium* emphasize the necessacity for the development of newer antibiotics. The present work was focused on the modeling of 3D structures of virulent proteins OmpR of *Salmonella typhimurium* using modeler and validated. The active site of these models was predicted by Q-site finder and the docking interactions with quinalone antibiotics Ciprofloxacin, Ofloxacin and Apramycin were analysed. Docking results revealed that amino acid residue Arginine is highly conserved in most of the interactions. Thus the results of this study would pave a way for the design of novel antibiotics that targets the virulence mechanism of *Salmonella typhimurium.*

Key words:

Salmonella typhimurium,

Virulent proteins,

Molecular modeling,

Docking,

Antibiotics

Introduction

Salmonella are gram negative enteric bacilli that cause life threatening diseases Salmonellosis in human beings. They are divided into two major groups, namely the typhoidal group causing typhoid fever and the non-typhoidal group causing gastroenteritis (***Helms et al., 2005***). Every year millions of human cases are reported world wide resulting in thousands of death. *Salmonella typhimurium* possess virulence associated factors coded by virulent genes clustered on chromosomes and plasmids responsible for pathogenesis(***Mascaretti,2003***). *Salmonellae* is the most prevalent food borne pathogens that causes enteric fever (typhoid) and gastroenteritis in humans. The important survival strategy of Salmonella is colonization, during all stages of infection.

In earlier times antibiotics namely Chloramphenicol, Ampicillin, Co-trimoxazole, Streptomycin and Tetracycline have been used as therapeutic agents for Salmonellosis. Due to the development of drug resistance, third generation Cephalosporins and Quinalones have gained importance in recent years. Emergence of multi drug resistant *Salmonella* is a significant problem world wide (***Chalker and Lunsford,2002***). Now, multi drug resistant *Salmonella* are frequently encountered and the resistance rate is increasing every year. The day is not far to develop wide spread resistance of *Salmonella* against all the drugs.

Structure of biomolecules can be analysed by molecular modeling technique. It generates *insilico* models for structure based drug design and drug interactions. As the molecular mechanisms contributing *Salmonella* pathogenesis have been determined, it is considered as an ideal organism for the drug development (***Silpak et al,2008; Madhulika,2004 & Cloeckaert and Schwartz,2001***). Hence the opportunity exists to develop novel antibiotics that targets virulence mechanisms in *Salmonella* typhimurium. Computational biology is a new genomic approach for the identification of virulent genes and their drug targets.

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In the present study, the Outer membrane protein regulator of salmonella OmpR was chosen, due to its manifestation as regulating virulence factor. To understand the molecular interaction between OmpR and Ciprofloxacin, Ofloxacin and Apramycin, Homology modeling of OmpR and molecular docking studies with those antibiotics was pursued with the purpose to obtain information on the binding patterns of the AHL, which might through light on the design

of potential inhibitors of the multidrug resistance bacterias which is considered as a most promising clinical problem in modern medicine over the past several decades.

METHODOLOGY:

Target Sequence and Potential Template Search

The sequence of 'vir' genes encoding proteins OmpR of *Salmonella typhimurium* was retrieved from the National Centre for Biotechnology Information (NCBI) (ID: AAL22364). The homologous sequences were searched against PDB using NCBI-BlastP (Basic Local Alignment Search Tool).

Comparative modeling

The 3D model of *Salmonella typhimurium* OmpR was generated using PDB ID: 1ODD_A-chain, as the potential template structures. The best model with the least RMSD value was selected by superimposing the model with their respective templates using SwissPDBViewer (Guex and Peitsch,1997). These models were subjected for energy for further analysis.

Active site prediction

To determine the interactions between antibiotics and OmpR the amino acids in the binding site of the models were predicted,

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Docking studies

The Ciprofloxacin, Ofloxacin and Apramycin antibiotics was docked with the amino acids in the binding site of OmpR using FlexX (Rarey,1996), with the following parameters i) default general docking informations, ii) base placement using triangle matching, iii) scoring of full score contribution and threshold of 0, 30 and no score contribution and threshold of 0, 70.

RESULTS AND DISCUSSION

Salmonella typhimurium a gram negative bacilli is a causative organism of gastroenteritis in human beings. Globally mortality rate in millions of human cases were reported. The pathogenecity of *Salmonella typhimurium* is due to clustered virulent genes on chromosomes and plasmids (Cloeckaert and Schwartz, 2001). This bacterium is continuously emerging as multidrug resistant and the magnitude of the problem is largely unknown in many countries.

Sequence Analysis and Potential Template

The BLASTP analysis of 'vir' gene encoding proteins OmpR from *Salmonella typhimurium*, resulted in the crystal structure of (PDBID: 1ODD_A-chain) as the most homologous sequence with the sequence identity of 50%.

Comparative modeling

The pair wise alignment of OmpR and its respective template obtained from BLASTP was refined using the alignment obtained from ClustalW. The initial models of OmpR, was generated using Modeler9v9 by applying spatial restraints **Figure.1**. Using these initial structures, 50 models of respective proteins were developed randomly. The best models were selected for further analysis based on their structural compatibility.

Model Assessment

The Ramachandran plot of the energy minimized models revealed the stereo chemical properties of generated models **(Figure.2)**. The modeled OmpR exhibited 86.2% of the residues in the most favorable region, 10.8% in the additionally allowed region, 2.1% in the generously allowed region and 1.0% in the disallowed region; which ensures that the generated model were good when compared with its template structures 1ODD_A (**Table1)** .

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Docking Analysis

Maicol Ahumedo *et al.* reported that docking calculations of the acyl homoserine lactones (AHLs) and regulatory proteins LasR and TraR, to understand the complex microenvironment, which may serve as site-specific targets for the development of novel antibiotics. Similarly in the present study the docking analysis with the antibiotics and the developed OmpR model was carried. The docking was carried out by using FlexX with the radius of 6.5 A^0 at the site of docking. The three antibiotics Ciprofloxacin, Ofloxacin and Apramycin molecules were docked in the specified binding regions (**Figure.4)**.

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A keen observation of these interacting residues of the modeled proteins and the antibiotics revealed the most important functional groups of the ligand molecules and the amino acids of the proteins favoring the interactions (**Table 2**). Docking scores implied that all the docking interactions were found to be good.

Conclusion

Multidrug resistance inevitably led to the use of more expensive and often more toxic drugs. *Salmonella* spp continues to evolve resistance mechanism to newer drugs including fourth generation antibiotics. The 3D structures of virulence associated proteins were modeled and their binding interactions with the recent quinalones were evaluated. ***Continues…*** Thus the present work implies that the design of potential drug inhibitors based on these amino acid residues will pave a way to overcome the virulence and pathogenesis in *Salmonella typhimurium*.

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[Arrange in Alphabetical Order]

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Conflict of Interest (if Any)

The Authors can express their conflicts (if any). **Or**

The Authors have no Conflict of interest.

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