Chapter 8

Discussion & Conclusion
Primary Proteomic study of unknown pathogenic cagPAI proteins of *H. pylori* help us to unfold their functional details by reference based comparative studies. The software program ProPhyC developed for primary studies of physicochemical parameter of protein; computes frequently required biological physicochemical components only. But it can be extended by adding other protein characterizing components according to need. As expected at the end of proteomic studies the function of almost all the proteins were clear and a membrane protein Cag3 was identified whose function was maintaining the cellular pyrimidine pool and the interacting partners of Cag3 were two other membrane proteins CagC and CagM (Busler *et al.* 2006). Protein interaction was carried out to get rough idea about the assembly and order of assembly of these membrane proteins. The result of interaction studies clarified that CagM initially interacts with CagC-C Dimmer with lowest binding energy followed by Cag3. Along with this it can be said that the interaction between CagC-C dimers are preferable over the CagC monomer interaction to forms stable pilus assembly; as CagC encodes pilus subunit (Olbermann *et al.* 2010). Therefore, we hypothesize that any one of these three easy to access proteins could be targeted to hinder the formation of t4ss. To be specific the formation of CagC-C dimer could be targeted. The formation of this CagC-C dimer could be prevented by competitive inhibition via molecular docking, using either natural or synthetic compound as a competitor compound. The pilus assembly could be hindered and in turn the translocation of onco protein CagA may be prevented. The hypothesis could be primarily confirmed by knocking out CagC gene.

During the proteomic studies different properties and characteristics of proteins are studied using numbers of software and using different database sources. The results of this different database are compiled to characterize each protein, which is a time consuming and massive job while dealing with multiple proteins or proteome of an organism. Therefore considering the organism of interest *H. pylori*, during the present studies “Heli-PyD” A
Comprehensive Database for *Helicobacter pylori* is developed using computer tools like MS-ACCESS, ASP.NET with C# and SQL. This database will bridge up the gap and will provide an integrated database of *H. pylori* having all the information at one platform. Past studies have evident total 18 strains of *H. pylori* from the available 36 strains are contributing to 4 different Gastro intestinal diseases namely, gastric adenocarcinoma, malt lymphoma, peptic ulcer and gastritis (Kawai *et al.* 2011). Therefore, detailed comparative studies are carried out to check possibility of prediction of the type of diseases each strain would cause on bases of their structural annotation. Comparative studies of structural features of different *H. pylori* strains, reported that there are no specific patterns with regards to structural features in strains responsible for particular disease. It was observed that, content of each of the four nucleotides were evenly distributed throughout the genome. Further we can conclude *H. pylori* strains with absence of cagPAI are also likely to cause GI tract disease specifically MALT Lymphoma (Kawai *et al.* 2011; Raymond *et al.* 2010). It should be noted that there are fair chances of getting the pathogenic genes other than so far reported genes, whose virulence is yet undiscovered. Thus, comparative studies of structural genomic features of genomes give important outcomes but, cannot give detailed idea about the kind of disease the strains would cause. Functional analysis of genome or study of gene order and rearrangement of genome may solve the mystery of disease specific genes and give some better target for treatment (Raymond *et al.* 2010).

Further in silico subtractive study was carried out to identification essential proteins of *H. pylori* (Sarangi *et al.* 2009), which could be target for controlling its activity or proliferation. The pipe line described here for subtractive study can be universally used for any organism of interest. The result of subtractive study reported two important candidate targets namely, HP0289, a toxin-like outer membrane protein ([http://www.uniprot.org/uniprot/O25063](http://www.uniprot.org/uniprot/O25063)) of *H. pylori* identified as Vacuolating cytotoxin VacA and MurA, UDP-N-acetylglucosamine1-carboxyvinyltransferase (EC:2.5.1.7); an important
candidate target involved in Peptidoglycan biosynthesis. In current studies the \textit{in silico} attempt of disturbing the function of VacA was not successful. In case of MurA, according to NCBI enzyme commission, MurA catalyzes enolpyruvyl transfer as part of the first step in the biosynthesis of peptidoglycan (PG) of PG layer, a highly dynamic macromolecule that is constantly remodeled to allow cell growth and division. As per our studies MurA is essential to pathogen and non-homologs to human host and could be targeted to control the proliferation of pathogen in host and reduce the growth of stomach disturbances. According to the available literature cysteine 115 of MurA in \textit{E.coli} is the ligand binding site for Terreic acid (C\textsubscript{7}H\textsubscript{6}O\textsubscript{4}) and and Fosfomycin (C\textsubscript{3}H\textsubscript{7}O\textsubscript{4}P) (Han \textit{et al.} 2010). The sequence similarity search in present study reports, cysteine 115 of MurA in \textit{E.coli} corresponds to cysteine 117 of MurA in \textit{H. pylori}. Therefore, docking studies were carried out against \textit{H. pylori} MurA taking Terreic acid and Fosfomycin (C\textsubscript{3}H\textsubscript{7}O\textsubscript{4}P) as ligand and results showed positive inhibition of \textit{H. pylori} MurA. Therefore it is evident that Terreic acid and Fosfomycin can hinder \textit{H. pylori} MurA. Currently the salt of Fosfomycin, Fosfomycin-tromethamine is already available in market having higher absorbance in comparison with Fosfomycin (http://www.drugbank.ca/drugs/DB00828).

Further the results were confirmed by wet-lab taking saliva sample of patient with severe ulcerative colitis and gastric disorder. The results evident that \textit{H. pylori} are sensitive to 1.5gm % of Fosfomycin solution with sterile distilled water and 1.5gm % of Fosfomycin solution with curd-whey. The results also evident that using curd-whey instead of sterile distilled water for making Fosfomycin solution enhances the effect of Fosfomycin against \textit{H. pylori}. From the comparative antibiotic test it is also evident that inhibitory effect of solution of Fosfomycin with curd-whey is better than the present triple medicinal regiment Metronidazole 400mg, Amoxicillin 500mg and Pentraprozoole solution against \textit{H. pylori}. From the antibiotic test, it was apparent that \textit{H. pylori} are sensitive to Amikacin, Ciprofloxacin, Ofloxacin, Norfloxacina and Gentamycin, which can be used as alternative medication for patients who has stop
responding to the current antibiotic regimens. Curd-whey enhanced the antibiotic effect of Fosfomycin against *H. pylori* therefore testing of curd-whey is performed, as Probiotic against *H. pylori* and *H. pylori* were found sensitive to Curd-whey. Curd-whey can be a good option for Probiotic line of treatment and metagenomic study of curd-whey can give better understanding for its anti-*H. pylori* effect (Sachdeva et al. 2014).