INTRODUCTION

Gastric cancer is caused by infection of class 1 carcinogen Helicobacter pylori (Zhang, 1994). Treatment for H. pylori infection includes drugs to relieve from pain and acidity, but not for gastritis, peptic ulcers, and gastric cancer. Carcinogenic activity of H. pylori suggests the need for discovery of new drug targets and drugs for prevention of H. pylori. Laboratory techniques and bioinformatics approaches are used to identify drug targets which can influence growth, colonization and virulence of H. pylori (Neelapu et al., 2014). Availability of the complete H. pylori genome sequence of pathogens provides us the platform and opportunity to mine the genome and harness the potential drug targets. Comparative genomic analysis between host and pathogen would provide us with a tremendous amount of information that can be useful in drug target identification (Neelapu et al., 2013). Comparative genomics analysis of host with pathogens revealed potential drug targets in Staphylococcus aureus (Uddinet et al., 2014), H. pylori (Neelapu and Pavani, 2013; Neelapuet al., 2015; Nammi et al., 2016) Listeria monocytogenes (Hossain et al., 2013), Leishmania infantum (Sutharet al., 2009), L. major (Florez et al., 2010), Mycobacterium leprae (Wiwanitkit, 2014), Pseudomonas aeruginosa (Sakharkearet al., 2004), Schistosomamansoni(Caffreyet al., 2009). Metabolic pathway analysis (Sarkaret al., 2012), reverse docking (Caiet al., 2006) and screening for essential genes (Duttaet al., 2006) are used to identify drug targets in H. pylori. However, there are no specific reports to date, on comparing genomes of H. pylori strain Hp26695 with host Homosapiens to identify drug targets in H. pylori. Therefore, comparing genome of host and pathogen may provide novel drug targets for H. pylori strain Hp26695.
Rising antibiotic resistance due to efflux pumps (Rauws and Tytgat, 1990; Graham et al., 1991), potential reinfection of *H. pylori* even after successful eradication therapy (Arora and Czinn, 2005), inhibition of T-cell stimulation by vacuolating cytotoxin (Vac A) (Molinari, 1998; Reyrat, 1999), and the highly inflammatory nature by the constituents of the cell wall suggests vaccination as an alternative for protection against *H. pylori*. For better immunization the half-life of the antimicrobial agents should be long enough to be effective and also penetrate mucosal barrier (Arora and Czinn, 2005). Hence, the use of vaccines with appropriate immunogens may provide immune protection against *H. pylori*. Therefore, mining the genomic sequences via bioinformatics approaches for immunological data would provide suitable vaccine candidates. The objective of the current paper is to screen and identify novel drug targets and vaccine candidates for therapeutic intervention of *H. pylori*.

**MATERIAL AND METHODS**

**Sample**

Complete genome of *H. pylori* strain Hp26695 with the geographical origin of Europe has the following accession number and genome length NC_018939 and 1,667,867 bp respectively (Manolov et al., 2014). In our study, identification of novel drug targets and vaccine candidates for *H. pylori* has been accomplished for the first time in *H. pylori* strain Hp26695. Novel drug targets were screened and identified using an integrated approach of genome, proteome and metabolic pathway analysis followed by primary property analysis of the genes/proteins using computational resources. Novel vaccine candidates were screened and identified by searching for pathogenic factors, followed by non-homology, secondary patterns and subsequent analysis for antigens, non-allergens and epitopes using computational resources.

**Screening and Identification of Drug Targets for *H. pylori***

The following protocol was followed for screening and identification of novel drug targets in *H. pylori* (Figure 1)

**Drug target screening for identification of unique molecules in *H. pylori***

Comparative genome analysis was performed to screen the drug targets for pathogen *H. pylori*. Genonome of *H. pylori* strain Hp26695 was initially annotated and further reconstructed for metabolic pathways using Rapid Annotation Subsystem Technology (RAST) server (Aziz et al., 2008). Comparative genome analysis between pathogen *H. pylori* and host *Homo sapiens sapiens* was performed using RAST to screen unique genes that are only present in pathogen and not present in the host (Table 1). Genes which are unique to *H. pylori* in the above method were filtered and catalogued.

**Drug target screening for confirmation of unique molecules in *H. pylori***

Bacterial genes which are non-homologous to humans are essential for pathogen. To identify the non-homologous molecules in *H. pylori*, homology at the level of sequence and structure of molecules were used as the parameters. BLASTp (Altschul et al., 1990) which is based on principle of homology was used to confirm the uniqueness of the catalogued genes in *H. pylori* by comparing genes against *Homo sapiens sapiens* (Table 1).

**Drug target identification**

A set of computational resources were used to analyse the characteristic features of the genes, to identify the potential drug targets. BTXpred (Saha and Raghava, 2007), SRTpred (Garg and Raghava, 2008), VGIchan (Saha et al., 2007) and VICMpred (Saha and Raghava, 2006) are the potential targets servers (Table 1) to identify the potentiality of the drug targets. Catalogued genes were verified for their potentiality as drug targets using the above list of servers.

**Screening and Identification of Vaccine Candidates for *H. pylori***

The following protocol was followed for screening and identification of vaccine candidates in *H. pylori* (Figure 2).

**Screening of proteome for identification of pathogenic factors in *H. pylori***

The bacterial genome was retrieved and the translated protein sequences of the pathogen are screened for pathogenic factors. Virulence factors, secretory proteins, outer-membrane proteins, bacterial toxins are the pathogenic factors. VirulentPred, EffectiveDB, CELLO, BTXpred (Table 1) are used to screen virulence factors, secretory proteins, outer-membrane proteins and bacterial toxins respectively. Further, the pathogenic factors of the bacteria are screened for non-homologous proteins as per the procedure described above in “Drug target screening for confirmation of unique molecules in *H. pylori*”.

**Screening of non-homologous proteins for identification of secondary patterns in *H. pylori***

The non-homologous proteins of the bacteria are screened for secondary patterns – helices using Chou Fasman method by CFSSP: Chou and Fasman Secondary Structure Prediction Server (Ashok Kumar, 2013) (Table 1). Proteins with alpha-helices ≥ 3 are selected for further analysis.

**Predicting function of proteome**

Metabolic pathway analysis using RAST (Aziz et al., 2008), BLASTp (Altschul et al., 1990), NCBI Conserved Domain Database (Marchler-Bauer et al., 2004), ProtFun 2.2 Server (Jensen et al., 2002) (Table 1) and literature-search are used for identifying the function of proteins.

**Screening of proteome for antigens**

The proteins fulfilling the above criteria are screened for antigens using Antigenic Emboss Server (Kolaskar and Tongaonkar, 1990) (Table 1). These proteins are catalogued and subjected to further analysis.

**Screening of proteome for non-allergenicity**

The proteins which are antigenic in nature are screened for allergenicity using server Allergen Online (Maria et al., 2006) (Table 1). The non-allergens are shortlisted and catalogued for further analysis.
Table 1: Computational resources used for identification of potential drug targets and suitable vaccine candidates in *Helicobacter pylori*

| S. No | Server Name | Server Function | Reference |
|-------|-------------|-----------------|-----------|
| 1     | RAST        | Rapid Annotation Subsystem Technology Server is used for prediction of unique genes based on metabolic pathways. | Aziz et al., (2008) |
| 2     | BTXpred     | Server is for prediction of bacterial toxins and its function from primary amino acid sequence. | Saha and Raghava, (2007) |
| 3     | SRTpred     | Server classifies protein sequence as secretory or non-secretory proteins. | Garg and Raghava, (2008) |
| 4     | VGIchan     | Server predicts voltage gated ion-channels and classifies them into sodium, potassium, calcium and chloride ion channels from primary amino acid sequences. | Saha et al., (2007) |
| 5     | VICM pred   | Server aids in broad functional classification of bacterial proteins into virulence factors, information molecule, cellular process and metabolism molecule. | Saha and Raghava, (2006) |
| 6     | VirulentPred| VirulentPred predicts virulence proteins using reliable Support Vector Machine (SVM) algorithm. This server has a prediction accuracy of 65%. | Garget et al., (2008) |
| 7     | EffectiveDB | EffectiveDB predicts putative effectors by identifying eukaryotic-like protein domains and by detecting the 2 known types of signal peptides. This server has a prediction accuracy of 80%. | Jehlet et al., (2011) |
| 8     | CELLO-V     | CELLO (subcellular localization predictor) predicts protein present in outer membrane directly from protein sequences. The server uses two-level support vector machine (SVM) system: the first level contains SVM classifiers and the second level SVM classifier function to generate the probability distribution of decisions for possible localizations. This server has a prediction accuracy of 90%. | Yu et al., (2004) |
| 9     | CFSSP Server| Chou & Fasman Secondary Structure Prediction (CFSSP) server predicts protein conformation like helices, beta sheets, random coils based on Chou & Fasman algorithm. This server has a prediction accuracy of 88%. | Ashok Kumar, (2013) |
| 10    | Antigenic   | Antigenic server predicts potentially antigenic sites of a protein sequence. The server uses semi-empirical method consisting physicochemical properties of amino acids and their frequencies of occurrence in experimentally known epitopes. This server has a prediction accuracy of 75%. | Kolaskar and Tongaonkar, (1990) |
| 11    | ABCpred     | ABCpred server predicts B-cell epitope using Recurrent Artificial Neural Network-(ANN-) based algorithm. This server has a prediction accuracy of 65.93%. | Saha and Raghava, (2006) |
| 12    | HLApred     | HLApred server identifies the experimentally proven binders taken from MHCBN database based on quantitative matrices HLA alleles which were obtained from literature. [http://www.imtech.res.in/raghava/hlapred/index.html](http://www.imtech.res.in/raghava/hlapred/index.html) | Saha and Raghava, (2007) |
| 13    | Allergen Online | Cross reactive allergens are predicted using server Allergen Online based on BLOSUM50 scoring matrix algorithm. This Server has a prediction accuracy of 70%. | Maria et al., (2006) |

**Figure 1** Screening and identification of novel drug targets for *H. pylori*

**Screening of non-allergic proteome for identification of antigenic and epitope regions in *H. pylori*:

The proteins fulfilling the above criteria are screened for promising epitopes which include both B cell & T cell epitopes. B-cells epitopes are screened and identified using ABCpred Server (Saha et al., 2006) (Table 1), whereas T-cell epitopes are screened and identified using HLApred (Table 1). Finally, proteins satisfying the above three criteria’s i.e. proteins showing positive predictions for antigenic, B-cell, T-cell activities are short listed as vaccine candidates for vaccine designing.

**RESULTS**

**Genome Wide In silico Analysis for Screening of Drug Targets in *H. pylori***

Genome wide in silico analysis for screening drug targets identified 569 unique genes in *H. pylori* strain *Hp26695* (Table 2). These molecules fall under 24 metabolic categories as shown in Table 2. Proteome analysis followed by gene property analysis of 569 unique genes identified seven potential drug targets in *H. pylori* (Table 3). These molecules fall under five metabolic categories as shown in Table 3.
Proteome Wide Insilico Analysis for Screening of Vaccine Candidates in H. pylori

Screening of 1469 proteins in H. pylori strain Hp26695 identified 643 pathogenic factors. VirulentPred, EffectiveDB, CELLO, BTXpred identified 399, 291, 197, 18 proteins respectively (Table 4). Analysis of 643 pathogenic factors identified 146 non-homologous proteins (Table 4). Screening of 146 non-homologous proteins for secondary structure patterns identified 46 proteins with ≤ 3 helices (Table 4). Analysis of these 46 proteins identified 44 proteins which are antigenic in nature. Further analysis of 44 antigenic proteins identified 29 non-allergenic proteins (Table 4). Analysis of these 29 non-allergenic proteins revealed antigens (99), B-cell (198) and T-cell (419) epitopes shortlisting to 16 proteins with antigenic regions (26) and potential epitopes (52) (Table 5, 6, 7, 8). These 16 immunogenic proteins contribute to immune-response and are well-suited for vaccine designing (Table 8). *Insilico* screening of peptides have helped examining the molecular properties, further *in vivo*-studies would be most helpful in bringing out potentially specific vaccine candidates.

Table 2: Total number of unique genes identified for each metabolic category using RAST in H. pylori strain Hp26695 by comparing genomes of pathogen H. pylori and Host Homo sapiens sapiens

| S. No | Metabolic category                        | Hp26695 |
|-------|------------------------------------------|---------|
| 1     | Amino Acids and Derivatives              | 49      |
| 2     | Carbohydrates                            | 37      |
| 3     | Cell Division and Cell Cycle             | 3       |
| 4     | Cell Wall and Capsule                    | 43      |
| 5     | Clustering-based subsystems              | 82      |
| 6     | Cofactors, Vitamins, Prosthetic Groups,  | 50      |
|       | Pigments                                 |         |
| 7     | DNA Metabolism                           | 31      |
| 8     | Fatty Acids, Lipids, and Isoprenoids     | 29      |
| 9     | Membrane Transport                       | 22      |
| 10    | Miscellaneous                            | 14      |
| 11    | Motility and Chemotaxis                  | 38      |
| 12    | Nucleosides and Nucleotides              | 2       |
| 13    | Phosphorus Metabolism                    | 3       |
| 14    | Potassium metabolism                     | 3       |
| 15    | Protein Metabolism                       | 84      |
| 16    | RNA Metabolism                           | 18      |
| 17    | Regulation and Cell signalling           | 7       |
| 18    | Respiration                              | 28      |
| 19    | Stress Response                          | 15      |
| 20    | Sulfur Metabolism                        | 2       |
| 21    | Virulence, Disease and Defense           | 9       |
| 22    | Iron acquisition and metabolism          | 0       |
|       | Total Number of Genes                    | 569     |

Figure 2: Screening and identification of vaccine candidates for H. pylori
DISCUSSION

Insilico methods helped in identifying novel drug targets and vaccine candidates for *H. pylori*. This was the first report on implementation of insilico subtractive genomics and insilico reverse vaccinology to identify novel drug targets and vaccine candidates respectively for *H. pylori* strain Hp26695.

Drug Targets for *H. pylori*

Subtractive genomics, metabolic pathway analysis, essential gene analysis and reverse docking were earlier used to identify drug targets for *H. pylori*. Cai et al. (2006) used reverse docking to identify a drug target in *H. pylori*. Dutta et al. (2006) identified 40 essential genes as drug targets in *H. pylori*HpAG1 strain. Sarkhar et al. (2012) identified lipopolysaccharide biosynthesis pathway as a source of potential drug targets in *H. pylori* using metabolic pathway analysis. Neelapu and Pavana (2013) identified 17 novel drug targets in Hpb38, HpbP12, HpbG27, HpsHi470, HpsJMI180 strains of *H. pylori* using genomics and proteomics. Neelapu et al. (2015)using genomics and proteomics identified 29 novel drug targets in HpAG1 strain of *H. pylori*. Nammi et al., (2016) using comparative genomics identified 29 novel drug targets. In this present study subtractive genomics was used to identify novel drug targets in addition to the existing drug target’s pool of *H. pylori* strain 26695. Docking to identify a drug target in *H. pylori* (2006) identified 40 essential genes as drug targets in *H. pylori*.

**Table 3** Drug targets identified in the *H. pylori* strain Hp26695 by comparing genomes of pathogen *H. pylori* and *HostHomo sapiens sapiens*

| S. No | Drug Target | Metabolic Category | Gene ID | Validated | Novel |
|------|-------------|--------------------|--------|-----------|-------|
| 1    | Menaquinone via futalosine step 1 | Cofactors, Vitamins, Prosthetic Groups, Pigments | GI:15645397 | + | |
| 2    | Type III restriction-modification system methylation subunit | DNA Metabolism | GI:15645218 | + | |
| 3    | Dipeptide transport system permease protein DppB | Membrane Transport | GI:15644927 | + | |
| 4    | Dipeptide transport system permease protein DppC | Membrane Transport | GI:15644928 | + | |
| 5    | Ferric siderophore transport system, biopolymer transport protein ExbB | Membrane Transport | GI:15646054 | + | |
| 6    | Ribonuclease BN | RNA Metabolism | GI:15646017 | + | |
| 7    | NADH-ubiquinone oxidoreductase chain J | Respiration | GI:15645883 | + | |

1. Experimentally validated drug targets either by genetically or biochemically
2. Novel drug targets identified in this study

**Table 4** Pathogenic factors, non-homology proteins, helices, antigens and non-allergens identified in *H. pylori* strain Hp26695

| Analysis Type | Virulence Factor | Secretory Proteins | Outer Membrane Proteins | Bacterial Toxin | Total Proteins |
|---------------|-----------------|--------------------|------------------------|----------------|---------------|
| Pathogenic factors | 399 | 291 | 197 | 18 | 643 |
| Non-homology to humans | 88 | 71 | 57 | 3 | 146 |
| Proteins with ≤3 helices | 37 | 17 | 4 | 0 | 46 |
| Antigenic proteins | 35 | 16 | 4 | 0 | 44 |
| Non-allergenic proteins | 24 | 9 | 3 | 0 | 29 |

Campylobacter jejuni. Disruption of menaquinone via futalosine pathway had shown inhibition of bacteriostatic growth (Arakawa et al., 2011). Therefore, designing an inhibitor for menaquinone via futalosine step 1 would affect the growth of *H. pylori*.

**Drug targets influencing DNA metabolism of the pathogen**

Type III restriction-modification system methylation subunit of restriction–modification (R-M) systems, is identified as the drug target in *H. pylori*. This drug target influences metabolic pathway DNA metabolism of the pathogen. *H. pylori* are naturally competent and prone to take DNA from the environment (Dorer et al., 2010) and bacteriophages also infect *H. pylori* (Heintschel et al., 1995). Missense and frameshift mutations can accumulate and inactivate genes when bacteriophages or free DNA or plasmids enter into other cells. Evidence is there that sometimes even both endonuclease and methylase genes of R-M systems have to be turned off. However, *H. pylori* in a population have a very good defensive system, where R-M systems protect the genome of *H. pylori* from accumulated mutations when bacteriophages or free DNA or plasmids enter into other cells. Mutant strains lacking this display a pleiotropic phenotype, including increased mutability, hyper recombination, and increased sensitivity to DNA-damaging agents. Therefore, designing an inhibitor for type III restriction-modification system methylation subunit decreases the rate of survival of *H. pylori* due to gross changes occurring in the genetic material.

**Drug targets influencing DNA metabolism of the pathogen**

Dipeptide transport system permease protein DppB, dipeptide transport system permease protein DppC and ferric siderophore transport system, biopolymer transport protein ExbB are identified as drug targets in *H. pylori*. These drug targets influence membrane transport of the pathogen. DipeptideDppABCD and oligopeptideDppABC genes are a class of ABC-type transporter in *H. pylori*. Dipeptide transport system permease protein - DppBC are responsible for transporting dipeptides. Dipeptide and oligopeptide system
| S. No | Protein ID | Protein Name | Antigen No | Antigen Sequence |
|-------|------------|--------------|------------|------------------|
| 1     | NP_206874.1| Lipoprotein signal peptidase | AG1 | KSSLVFMGVFVFLGFVDQAIKYAILEG |
|       |            |              | AG2 | YESLMIDIVLFYNEKGASFLSFLSEGLGLKYLLQILILGFVFILM |
|       |            |              | AG3 | GAVGNVLDRIFPVHGGVDVYHYGDFAPFNAQFADMIDVGVBVLL |
| 2     | NP_206881.1| Membrane Protein | AG4 | ALKSKAFRSQVSQWNLVRLALA |
| 3     | NP_206885.1| Cytochrome c oxidase VI a. | AG5 | IAKKAVKKFLGFLVVVLMLMM |
| 4     | NP_206945.1| CBB3-type cytochrome c oxidase Q | AG6 | LRGFAYAFFFLTFLFYLAYFISM |
|       | NP_206947.1| DUF4006 superfamily | AG7 | YGVLALND |
| 5     |             | Translation Protein | AG8 | GNLLIVVILCVAAPFTLKAIHIIQK |
|       | NP_207071.1| Translation Protein | AG9 | YELVNO |
|       |            |              | AG10 | LHFSHL |
|       | NP_207125.1| Flagellar protein FlaG | AG11 | YESLMIDIVLVFNKGVAFSLLSFLEKILLE |
| 6     | NP_207138.1| Translation Protein | AG12 | AG13 | KSVELEYAOR |
|       | NP_207241.1| Transporter Protein | AG14 | ESSLKI |
| 7     | NP_207255.1| Translation Protein | AG15 | LEKILKCFDAYIKPKLSQNS |
|       | NP_207289.1| putative paralog of Hp aA | AG16 | KTQFFIMA |
| 8     | NP_207332.1| Cag pathogenicity island protein cag15 | AG17 | KTYLFTTLINKYLPSAQSQLPKIS |
| 9     | NP_207346.1| 50S ribosomal protein L31 | AG18 | KLLLVLFR |
| 10    | NP_207357.1| 30S ribosomal protein S21 | AG19 | WMYSSTFISLTKHLFIE |
| 11    | NP_207389.1| putative paralog of HpaA | AG20 | HRYFLF |
| 12    | NP_207461.1| Translation Protein Enzyme | AG21 | EEEGVLYGVGSI |
| 13    | NP_207521.1| Transport and binding protein | AG22 | KHYVLGFLKYP |
| 14    | NP_207749.1| Translation Protein | AG23 | ESSLKI |
| 15    | NP_207852.1| Type I restriction-modification system | AG24 | LEKILKCFDAYIKPKLSQNS |
| 16    | NP_207902.1| Hydrogenase expression/formation | AG25 | KTQFFIMA |
|       | NP_207912.1| protein HypC | AG26 | KTYLFTTLINKYLPSAQSQLPKIS |
| 17    | NP_207918.1| Translation Protein | AG27 | KLLLVLFR |
| 18    | NP_207921.1| Translation Protein | AG28 | WMYSSTFISLTKHLFIE |

Table 5: Antigens predicted in the proteome of H. pylori strain Hp26695 using Antigenic server
Table 6: B-cell, T-cell and Consensus epitopes predicted in the proteome of *H. pylori* strain Hp26695

| Antigen No | Antigen Sequence | B-cell Epitopes (Threshold > 0.60) | T-cell Epitopes (Threshold > 0.70) | Consensus Epitopes |
|------------|------------------|----------------------------------|----------------------------------|-------------------|
| AG1        | KSSLVFVGVFVFLVFGVDFQAIVKYAILEG | 1                                |                                  |                   |
| AG2        | YESLMDIVVLVFNKGAFSLSSLFLEGGLKLYQLILLGLFFLM | 1                                | 8                                | 1                 |
| AG3        | GAGVSNVLDRFVHGCGVDDTVYHYGDFAFQSFADVMDVG | 7                                |                                  |                   |
| AG4        | ALKSKAFRVQSWNLVRKLALAE | 1                                | 4                                | 2                 |
| AG5        | IACKAVKIVFFGLVVVMIMI | 5                                |                                  |                   |
| AG6        | LRGFAYAFFIFILFTLFELAYIFSM | 1                                | 4                                | 2                 |
| AG7        | YGYLLALND |                                  |                                  |                   |
| AG8        | GNLLIVVILCVAVFTLKHIAIHQK | 1                                | 6                                | 1                 |
| AG9        | YELVQ   |                                  | 1                                |                   |
| AG10       | LHSHIL  | 1                                |                                  |                   |
| AG11       | DVGHIKINENVLFGVFSLLGW |                                  |                                  |                   |
| AG12       | FLPWSMLELKKILLE |                                  |                                  |                   |
| AG13       | KSVELEYQR |                                  |                                  |                   |
| AG14       | ESLIKI   | 1                                |                                  |                   |
| AG15       | LEKILKKCFAKDAYKFRLSQSNS | 1                                |                                  |                   |
| AG16       | KGNYFVQFQFQFQA    |                                  |                                  |                   |
| AG17       | KTYLFLTILPNLPSAOSQLPKIS | 1                                | 4                                |                   |
| AG18       | KLLYLFER  |                                  |                                  |                   |
| AG19       | WMYSTFISLTHLQFIE | 2                                | 3                                | 3                 |
| AG20       | HRYLF   | 1                                |                                  |                   |
| AG21       | ECVYVGLVGI |                                  |                                  |                   |
| AG22       | KHQYGLIYKPN  | 1                                |                                  |                   |
| AG23       | ILKALEFI | 1                                |                                  |                   |
| AG24       | FEEQFLQSLHLEV | 1                                | 2                                | 1                 |
| AG25       | FIDVLLYDK |                                  |                                  |                   |
| AG26       | LKLEGCEKHCCKKKYAEEKIVFEKGLKLEKSVMPY | 5                                |                                  |                   |
| AG27       | RSQIISLMIK  | 1                                |                                  |                   |
| AG28       | SLAILMPSFLAAPDYK |                                  |                                  |                   |
| AG29       | KFTQIDFLF |                                  |                                  |                   |
| AG30       | IKAIAGGILIGTICAY | 1                                | 3                                | 1                 |
| AG31       | WCQVGIITAAIS | 5                                |                                  |                   |
| AG32       | VQISVSNL | 2                                |                                  |                   |
| AG33       | FLWNKAKSFLSFGFVZIPMIPWLDLNSFLVVFYCVNVFFSIAE | 5                                |                                  |                   |
| AG34       | SDILAHSK |                                  |                                  |                   |
| AG35       | SLIKFVRIYSKMLVALGLSSVILGCAM | 2                                | 3                                | 2                 |
| AG36       | SSEHTPLDFNYPIIHQPAQPNHVIGTLIPRIQVSDNLKPYID | 2                                | 2                                | 2                 |
| AG37       | FDQALINQQT |                                  |                                  |                   |
| AG38       | RGYQVQLR |                                  |                                  |                   |
| AG39       | KFSVLDDLKGWILLE |                                  |                                  |                   |
assimilation systems as a response to iron pathogens have developed highly sophisticated iron acquisition systems; receptor proteins involved in siderophore uptake and transporting iron. These proteins are essential for the growth and survival of H. pylori in stressful conditions, as siderophores provide an iron source in the host’s iron-starved environment.

Mutations in the DppBC transport system of H. pylori have been identified, affecting the growth and survival of H. pylori in limiting iron conditions. These mutations lead to decreased iron transport efficiency, which might be a survival strategy in iron-limited environments. The DppBC system is an example of how bacteria can adapt to iron-limiting conditions by altering their iron acquisition pathways.

The production of siderophores, small nonproteinaceous molecules with extremely high affinity for iron (III), is one of the most successful and widely utilized strategies of iron assimilation by many pathogenic bacteria. Siderophores are produced by bacteria to scavenge iron from the host’s iron-rich environment and transport it into the bacterial cell. The efficient transport of iron is crucial for bacterial survival in the host’s iron-limited environment.

The use of siderophores in iron assimilation is a critical adaptation for H. pylori to survive in the human stomach environment. The successful iron-assimilation strategies in H. pylori have implications for the development of new therapeutic strategies against this pathogen.
surfaces of-utiliz-effective inhibitor to the existing multiple p-establish this fact-mutant and complementation studies in-mechanism that utilizes Ton-Pseudomonas putida-Haemophilus influenzae, Neisseria meningitides-impermeable outer membrane. S-transport of iron or iron-biopolymer transport protein in-membrane-(responsible for the transport of iron or iron-H. pylori-ion of heme-et al.-2003-the otherwise impermeable outer-Ferric transport system Exb B-independent heme. Knockout-H. pylori-et al.-2009). Designing anprotein for the-existence of heme. Knockout-Ribonuclease BN can block the function of protein synthesis-(affect the processing of tRNA's and ultimately synthesis of-identified in-Ribonuclease BN is identified as a drug target in-Drug targets influencing RNA metabolism of the pathogen-Ribonuclease BN is identified as a drug target in-H. pylori. This drug target influences RNA metabolism of the pathogen. Ribonuclease, BN, lacking RNase H and RNase D activity was identified in-E. coli and it is different from other exoribonucleases known till date in-E. coli. RNase BN is a substrate specific with specificity towards C-C-A sequence in-tRNA than other types of tRNA and substrate specificity was proved both in vitro and in vivo. Mutants of these proteins affect the processing of tRNA's and ultimately synthesis of protein (Asha et al., 1983). Hence, an effective inhibitor for Ribonuclease BN can block the function of protein synthesis.

| Antigen No | Antigen Sequence | Epitope No | Consensus Epitopes |
|------------|------------------|------------|--------------------|
| AG2        | YESLMIDIVLVFNKGVAFLSSFLGLLEGGLYVKQLLGLLGFILML | EPC5      | LLILGLFIF |
| AG4        | ALKSKAFRVSIQWNALVRKPLLAA | EPC37      | LVRKLLALE |
| AG6        | LRGFAYAFFTFLFTFLYAYIFSM | EPC56      | FLYAVIFSM |
| AG8        | GNNLIVVILCVAVFELKAIHQK | EPC67      | LFLYAIFFS |
| AG19       | WMYSTFSLKTHLQFIE | EPC67      | LFLYAIFFS |
| AG24       | FEEFQLHSLILEV | EPC132      | LKTHLQFIE |
| AG30       | IKAAGLIGIVGTICAY | EPC140      | FQLHSLILE |
| AG35       | SLIFKVKRISKMLVALGGLSSLVILGACAM | EPC185      | LIJGTCYI |
| AG36       | SSEEHTPLDENYPHIHOAPNQHHVGILTRPIQVSDNLPYID | EPC224      | FKKVRIYSK |
| AG41       | LDKSYLYYRLFNISIVIGLVALFSYGAVILVYPILFLFAILKPSFFYTTYTLLVSLSIISKYLLSHA | EPC262      | ILVVPILFL |
| AG46       | HPEYIPKCVTCTSG | EPC263      | ILVVPILFL |
| AG53       | IGLSVALLLGIPVLSFANTLFLGAVFVGGLIAAQI | EPC266      | YVPLISF |
| AG55       | AMAISGLSAGVILSILFIIYIFDYITKPSLIGK | EPC274      | ILSIFIR |
| AG58       | AIPSKVIALKDNVLVTLETLGQRE | EPC300      | VVVLTELG |
| AG59       | GESVYKGDYVLLHGIVYMSK | EPC302      | VHVG |
Drug targets influencing respiration of the pathogen

NADH-ubiquinone oxidoreductase chain J is identified as a drug target in *H. pylori*. This drug target influences metabolic pathway and effect respiration of the pathogen. The NADH ubiquinone oxidoreductase (Complex I), provides the input to the respiratory chain from the NAD-linked dehydrogenases of the citric acid cycle. The complex couples the oxidation of NADH and the reduction of ubiquinone, to the generation of a proton gradient which is then used for ATP synthesis. The complex occurs in the mitochondria of eukaryotes and in the plasma membranes of purple photosynthetic bacteria, and the closely related respiratory bacteria. All inhibitors affect the electron-transfer step from the high-potential iron-sulphur cluster to ubiquinone. Class I inhibitors appear to act directly at the ubiquinone-catalytic site which is related in complex I and glucose dehydrogenase (Friedrich et al., 1994). Inhibitors designed to bind to NADH-ubiquinone oxidoreductase chain J competitively inhibit the protein from functioning which results in chemical asphyxiation of cells.

Vaccine Candidates for *H. pylori*

Constructive screening protocol was implemented to identify suitable vaccine candidates for *H. pylori*. Choosing such a conservative way to face vaccine design inevitably implies missing some pathogen antigens, but still this is a small price to reach a valuable compromise. Forwarding further bioinformatics analyses on selected ones, may prove successful (Sandroet al., 2006). Bioinformatics approach has helped us in shortlisting and in identifying pathogenic factors from the proteome of the pathogen. Screening of pathogen factors for non-homology would shortlist the proteins which have the potential to cross react when vaccine is administered. Usually a protein has high probability of failure to cloning and express in experiment when it is likely to have more helices. Hence, proteins with alpha-helices < 3 are selected for further analysis. (Sandroet al., 2006; Capecchiet al., 2004; Pizza et al., 2000).Screening of proteome for allergenicity would avoid the proteins which can elicit undesirable reaction during vaccination. Further, proteins showing positive predictions for antigenic, B-cell, T-cell activities are characterized as potential immunogenic which are suitable for vaccine candidates.

CONCLUSION

Comparative genomics of *H. pylori* and *Homosapien sapiens* identified seven bacterial genes which are non-homologous to humans and are essential for pathogen. Four genes of the 7 predicted drug targets are already experimentally validated lending credence to our approach. These novel drug targets may have possible therapeutic implications for gastric cancer. Systematic *insilico* analysis approach identified 16 immunogenic proteins which are suitable vaccine candidates for *H. pylori*. Thus, bioinformatics approaches helped in rapid identification of novel drug targets and vaccine candidates for *H. pylori* strain Hp26695.

Acknowledgements

AMCP, DN and NNRR are thankful to the GITAM University, Visakhapatnam, India for providing the facility and support. AMCP, NNRR and DN are thankful to University Grants Commission, New Delhi for the project (UGC Project F.No.42-636/2013 (SR) letter dated 25-03-2013). DN is thankful for the Project Fellowship sponsored by UGC, New Delhi, India. The authors also thankful to Professor IskaBhaskar Reddy and Professor Malla Rama Rao for constant support throughout the research work. We profusely thank Dr ChallaSurekha, GITAM University, Visakhapatnam, India for critical comments and reviewing of the manuscript.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990): Basic local alignment search tool. *J. Mol. Biol.*, 215(3): 403-410.

Arakawa, C., Kuratsu, M., Furihata, K., Hiratsuka, T., Itoh, N., Seto, H. and Dairi, T. (2011): Diversity of the early step of the futalosine pathway. *Antimicrob. Agents Chemother.*, 55(2): 913-916.

Arora, S. and Czinn, S.J. (2005): Vaccination as a method of preventing *Helicobacter pylori*associated gastric cancer.*Cancer Epidemiol. Biomarkers Prev.*, 14: 1890-1891.

Asa, P.K., Blouin, R.T., Zanimewski, R. and Deutscher, M.P. (1983): Ribonuclease BN: Identification and partial characterization of a new tRNA processing enzyme. *Proc. Natl. Acad. Sci. USA*, 80: 3301-3304.

Ashok, K.T. (2013): CFSSP: Chou and Fasman secondary structure prediction server. *Wide Spect.*, 1(9): 15-19.

Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T., Edwards, R.A., Fasman, G., Gerdes, S., Lass, E.M., Kubal, M., Meyer, F., Olsen, G.J., Olson, R., Osterman, A.L., Overbeek, R.A., McNeil, L.K., Paarmann, D., Paczian, T., Parrello, B., Pusch, G.D., Reich, C., Stevens, R., Vassieva, O., Vonstein, V., Wilke, A. and Zagnaio, O. (2008): The RAST server: rapid annotations using subsystems technology. *BMC Genomics*, 9: 75.

Caffrey, C.R., Rohwer, A., Oellien, F., Marhöfer, R.J., Braschi, S., Oliveira, G., McKerrow, J.H. and Selzer, P.M. (2009): A comparative chemogenomics strategy to predict potential drug targets in the metazoan pathogen. *Schistosomamansoni.*PLoS ONE, 4(2): e4413.

Cai, J., Han, C., Hu, T., Zhang, J., Wu, D., Fang, W., Liu, Y., Ding, J., Chen, K., Yue, J., Shen, X. and Jiang, H.
(2006): Peptide deformylase is a potential target for anti-
*Helicobacter pylori* drugs: reverse docking, enzymatic assay, and x-ray crystallography validation. *Protein Sci.*, 15(9): 2071-2081.

Capecchi, B., Serruto, D., Adu-Bobie, J., Rappuoli, R. and Pizza, M. (2004): The genome revolution in vaccine research. *Curr. Issues Mol. Biol.*, 1(6): 17-28.

Chou, P.Y. and Fasman, G.D. (1978): Empirical predictions of protein conformation. *Annu. Rev. Biochem.*, 47: 251-276.

Dorer, M.S., Fero, J. and Salama, N.R. (2010): DNA damage triggers genetic exchange in *Helicobacter pylori*. *PLoS Pathog.*, 6(7): e1001026.

Dutta, A., Singh, S.K., Ghosh, P., Mukherjee, R., Mitter, S. and Bandyopadhyay, D. (2006): *in silico* identification of potential therapeutic targets in the human pathogen *Helicobacter pylori*. *In Silico Biol.*, 6(2): 43-47.

Florez, A.F., Park, D., Bhak, J., Kim, B.C., Kuchinsky, A., Morris, J.H., Espinosa, J. and Muskus, C. (2010): Protein network prediction and topological analysis in *Leishmania major* as a tool for drug target selection. *BMC Bioinformatics*, 11(1): 484.

Friedrich, T., Heck, P., Leif, H., Ohnishi, T., Forche, E., Kunze, B., Jansen, R., Trowitzsch-Kienast, W., Höfle, G., Reichenbach, H. and Weiss, H. (1994): Two binding sites of inhibitors in NADH: ubiquinone oxidoreductase (complex I). *Eur. J. Biochem.*, 219(1-2): 691-698.

Garg, A. and Gupta, D. (2008): VirulentPred: a SVM based prediction method for virulent proteins in bacterial pathogens. *BMC Bioinformatics*, 9(1): 62.

Garg, A. and Raghava, G.P.S. (2008): A machine learning based method for the prediction of secretory proteins using amino acid composition, their order and similarity-search. *In Silico Biol.*, 8(2): 129-140.

Graham, D.Y., Lew, G.M., Evans, D.G., Evans, D.J. and Klein, P.D. (1991): Effect of triple therapy (antibiotics plus bismuth) on duodenal ulcer healing. A randomized controlled trial. *Ann. Intern. Med.*, 115(4): 266-269.

Heintschel V.H.E., Nalik, H.P. and Schmid, E.N. (1997): Vacuoles induced by *Helicobacter pylori* toxin contain both late endosomal and lysosomal markers. *J. Biol. Chem.*, 272: 25339-25344.

Nammi, D., Srinath—Tirumula-Peddinti, R.C.P.K. and Neelapu, N.R.R. (2016): Identification of drug targets in *Helicobacter pylori* by *in silico* analysis: possible therapeutic implications for gastric cancer. *Curr. Cancer Drug Targets*, 16(1): 79-98.

Neelapu N.R., Nammi, D., Pasupuleti, A.C., Surekha, C. (2014): *Helicobacter pylori* induced gastric inflammation, ulcer, and cancer: A pathogenesis perspective. *Interdiscip. J. Microinflamm.*, 1(2): 113.

Neelapu, N.R.R. and Pavan, T. (2013): Identification of novel drug targets in *Hp* B38, *Hp* P12, *Hp* G27, *Hp* S110 strains of *Helicobacter pylori*: An *in silico* approach for therapeutic intervention. *Curr. Drug Targets*, 14(5): 601-611.

Neelapu, N.R.R., Mutha, V.R.N. and Akula, S. (2015): Identification of potential drug targets in *Helicobacter pylori* strain *Hp* AG1 by *in silico* genome analysis. *Infect. Disorders Drug Targets*, 15(2): 106-117.

Neelapu, N.R.R., Nammi, D., Pasupuleti, A.C.M and Challa, S. (2016): Targets against *Helicobacter pylori* and other tumor-producing bacteria. In: Villa T.G. and Vinas M. (eds) New Weapons to Control Bacterial Growth, Springer International Publishing, pp. 239-279.

Neelapu, N.R.R., Srimath—Tirumula-Peddinti, R.C.P.K., Deepthi, N. and Pasupuleti, A.C.M (2013): New strategies and paradigm for drug target discovery: a special focus on infectious diseases tuberculosis, malaria, leishmaniasis, trypanosomiasis and gastritis. *Infect. Disorders Drug Targets*, 13(5): 352-364.

Pizza, M., Scarlato, V., Massignani, V., Giuliani, M.M., Arico, B., Comanducci, M., Jennings, G.T., Baldi, L., Bartolini, E., Capecchi, B., Galeotti, C.L., Luzzi, E., Manetti, R., Marchetti, E., Mora, M., Nuti, S., Ratti, G., Santini, L., Savino, S., Scarselli, M., Storni, E., Zuo, P.,...
Broeker, M., Hundt, E., Knapp, B., Blair, E., Mason, T., Tettelin, H., Hood, D.W., Jeffries, A.C., Saunders, N.J., Granoff, D.M., Venter, J.C., Moxon, E.R., Grandi, G. and Rappuoli, R. (2000): Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing. *Science*, 287(5459): 1816-1820.

Rauws, E.A. and Tytgat, G.N. (1990): Cure of duodenal ulcer associated with eradication of *Helicobacter pylori*. *Lancet*, 335(8700): 1233–1235.

Reyrat, J.M., Pelicic, V., Papini, E., Montecucco, C., Rappuoli, R. and Telford, J.L. (1999): Towards deciphering the *Helicobacter pylori* cytotoxin. *Mol. Microbiol.*, 34: 197-204.

Saha, S. and Raghava G.P.S. (2006): Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. *Proteins*, 65(1): 40-48.

Saha, S. and Raghava, G.P.S. (2006): VICMpred: SVM-based method for the prediction of functional proteins of gram-negative bacteria using amino acid patterns and composition. *Geno. Prot. Bioinfo.*, 4(1): 42-47.

Saha, S. and Raghava, G.P.S. (2007): Prediction of bacterial proteins. *In Silico Biol.*, 7(5): 0028.

Saha, S., Zack, J., Singh, B. and Raghava, G.P.S. (2007): VGIchan: Prediction and classification of voltage-gated ion channels. *Geno. Prot. Bioinfo.*, 4(4): 253-258.

Sakharkar, K.R., Sakharkar, M.K. and Chow, V.T.K. (2004): A novel genomics approach for the identification of drug targets in parasites, with special reference to *Pseudomonas aeruginosa*. *In Silico Biol.*, 4(3): 355-360.

Sandro, V., Filippo, B. and Francesco, F. (2006): Nerve: new enhanced reverse vaccine environment. *BMC Biotechnol.*, 6: 35.

Sarangi, A.N., Aggarwal, R., Rahman, Q. and Trivedi, N. (2009): Subtractive genomics approach for *insilicoidentification and characterization of novel drug targets in Neisseria meningitidis* serogroup B. *J. Comput. Sci. Syst. Biol.*, 2(5): 255-258.

Sarkar, M., Maganti, L., Ghoshal, N. and Dutta C. (2012): *Insilico* quest for putative drug targets in *Helicobacter pylori*HpAG1: Molecular modeling of candidate enzymes from lipopolysaccharide biosynthesis pathway. *J. Mol. Model.*, 8(5): 1855-1866.

Suthar, N., Goyal, A. and Dubey, V.K. (2009): Identification of potential drug targets of *Leishmania infantum* by *insilico* genome analysis. *Lett. Drug Des.Discover.*, 6(8): 620-622.

Uddin, R. and Saeed, K. (2014): Identification and characterization of potential drug targets by subtractive genome analyses of methicillin resistant *Staphylococcus aureus*. *Comput. Biol. Chem.*, 63(4): 48-55.

Weinberg, M.V. and Maier, R.J. (2007): Peptide transport in *Helicobacter pylori*: roles of Dpp and Opp systems and evidence for additional peptide transporters. *J. Bacteriol.*, 189(9): 3392-3402.

Wiwanitkit, V. (2005): Analysis of *Mycobacterium leprae* genome: *Insilico* search for drug targets. *Southeast Asian J. Trop. Med. Public Health*, 36: 225-227.

Ye, S., Von Delft, F., Brooun, A., Knuth, M.W., Swanson, R.V. and McRee, D.E. (2003): The crystal structure of shikimate dehydrogenase (AroE) reveals a unique NADPH binding mode. *J. Bacteriol.*, 185(14): 4144-4151.

Yu, C.S., Lin, C.J. and Hwang, J.K. (2004): Predicting subcellular localization of proteins for Gram-negative bacteria by support vector machines based on n-peptide compositions. *Protein Sci. J.*, 13: 1402-1406.

Zhang, Z.W. (1994): Schistosomes. liver flukes and *Helicobacter pylori*. IARC working group on the evaluation of carcinogenic risks to humans. Lyon, 7-14 June 1994. IARC Monogr.Eval.Carcinog. Risks Hum., 61:1-241.

---

**How to cite this article:**

Amita Martin Corolina et al.2017, Screening And Identification of Drug Targets And Vaccine Candidates For *Helicobacter Pylori* Strain Hp26695. *Int J Recent Sci Res.*, 8(4), pp.16384-16395.DOI: http://dx.doi.org/10.24327/ijrsrc.2017.0804.0140

*****