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RESEARCH ARTICLE

A Systematic *in-silico* Analysis of *Helicobacter pylori* Pathogenic Islands for Identification of Novel Drug Target Candidates

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Abstract: ***Background*:** *Helicobacter pylori* is associated with inflammation of different areas, such as the duodenum and stomach, causing gastritis and gastric ulcers leading to lymphoma and cancer. Pathogenic islands are a type of clustered mobile elements ranging from 10-200 Kb contributing to the virulence of the respective pathogen coding for one or more virulence factors. Virulence factors are molecules expressed and secreted by pathogen and are responsible for causing disease in the host. Bacterial genes/virulence factors of the pathogenic islands represent a promising source for identifying novel drug targets.

***Objective*:** The study aimed at identifying novel drug targets from pathogenic islands in *H. pylori*.

***Material & Methods*:** The genome of 23 *H. pylori* strains were screened for pathogenic islands and bacterial genes/virulence factors to identify drug targets. Protein-protein interactions of drug targets were predicted for identifying interacting partners. Further, host-pathogen interactions of interacting partners were predicted to identify important molecules which are closely associated with gastric cancer.

***Results*:** Screening the genome of 23 *H. pylori* strains revealed 642 bacterial genes/virulence factors in 31 pathogenic islands. Further analysis identified 101 genes which were non-homologous to human and essential for the survival of the pathogen, among them 31 are potential drug targets. Protein-protein interactions for 31 drug targets predicted 609 interacting partners. Predicted interacting partners were further subjected to host-pathogen interactions leading to identification of important molecules like TNF receptor associated factor 6, (TRAF6) and MAPKKK7 which are closely associated with gastric cancer.

***Conclusion*:** These provocative studies enabled us to identify important molecules in *H. pylori* and their counter interacting molecules in the host leading to gastric cancer and also a pool of novel drug targets for therapeutic intervention of gastric cancer.

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1. INTRODUCTION

Gastric inflammation, ulcer, and cancer are induced by *H. pylori* infection. *H. pylori* is also responsible for other disorders like skin, oropharynx, endocrine, respiratory, haemopoietic, central nervous system, eye and reproductive system, *etc*. [1, 2]. Bacterial virulence factors are important for the development of gastric carcinoma [3, 4]. Stomach cancer is increased by the presence of the Cag Pathogenicity Island (PAI) of which a Cag gene encodes an immunodominant protein called CagA. This belongs to the type IV secretion system along with it VirB proteins. Zanotti and Cendron [5] revealed a large number of copies of CagA and VirB proteins in the type IV secretion system. Once CagA is injected into the host cell, tyrosine is phosphorylated, which interferes with several cancer pathways [5]. Reproduction in male and females is also affected by *H. pylori* infection [6-8]. These bacterial genes/virulence factors of the pathogenic islands represent a promising source for identifying novel drug targets.

Identification and validation of novel drug targets is a key process for discovery of new compounds. Various methods and approaches are available for discovery and validation of drug targets for infectious diseases [9]. Dutta *et al.* [10] used subtractive genomics for identification of essential genes in *H. pylori* strain HpAG1, Hp26695 and J99. Kiranmayi *et al.* [11] identified essential transporter genes in *H. pylori* using bioinformatics approaches. Neelapu and Pavani [12] identified 17 novel drug targets in *H. pylori* strains HpB38, HpP12, HpG27, HpShi470, HpSJM180 using *in-*

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silico genome and proteome analysis, whereas in a similar type of analysis carried out in the strain HpAG1 29 novel drug targets were identified [13]. Nammi *et al.* [14] used comparative genomics, proteomics *etc*. for 23 *H. pylori* strains to identify 29 novel drug targets. Mandal and Das [15] used *in-silico* approach for identifying drug targets in *H. pylori.* Sarkar *et al.* [16] used metabolic pathway analysis to identify drug targets in *H. pylori*. Cai *et al.* [17] used reverse docking to identify drug targets in *H. pylori*. However, there are no specific reports to date, on screening of pathogenic islands in *H. pylori* to identify drug targets. Therefore, the current paper deals with screening of pathogenic islands to identify novel drug targets in 23 strains of *H. pylori*.

2. MATERIALS AND METHODS

2.1. Sampling

Genomes of 23 *H. pylori* strains *Hp*F32 [18], *Hp*F30 [18], *Hp*2017 [19], *Hp*2018 [19], *Hp*26695 [20], *Hp*35A [21], *Hp*51 [22], *Hp*52 [23], *Hp*CuZ20 [24], *Hp*F16 [18], *Hp*F57 [18], *Hp*INDIA7 [25], *Hp*SAT464 [26], *Hp*J99 [27], *Hp*B8 [28], *Hp*908 [29], *Hp*83 [30], *Hp*SJM180 [31], *Hp*AG1 [32], *Hp*Shi470 [33], *Hp*G27 [34], *Hp*P12 [35] and *Hp*B38 [36] are sampled based on availability of complete genome, strain history, pathogenicity report of the strains and geographical origin. In our study, identification of novel drug targets for *H. pylori* has been accomplished for the first time for all the 23 *H. pylori* strains by using an integrated approach of genome, proteome and primary property analysis followed by protein-protein interactions of genes/proteins and host-pathogen interactions using computational resources.

2.2. Screening of Pathogenic Islands by *In Silico* Genome Analysis for Drug Targets

Islands viewer [37] is used to identify pathogenic islands and the virulence genes in pathogenic islands for 23 *H. pylori* strains. Islands viewer is an integrated tool with different genomic island prediction methods such as Island Pick, SIGI-HMM and Island Path. These methods identify virulence genes in genomic islands based on three different criteria and methods. Island Pick is used to identify genomic islands and non-genomic islands. SIGI-HMM identifies genomic islands based on sequence composition, GC% and codon usage by implementing Hidden Markov model. Island Path (DIMOB) identifies functionally related mobile genes like transposases, integrases and abnormal sequence composition based on the origin of the genome. Complete genomes of 23 *H. pylori* strains were submitted to the Islands viewer to screen and identify pathogenic islands in the respective genomes and the virulence genes in pathogenic islands. These virulence genes were screened and confirmed for non homology as per the procedure of Neelapu *et al.* [13]. Potential drug targets among the pool of catalogued virulence genes were identified as per the procedure of Neelapu *et al.* [13].

2.3. Prediction of Protein-Protein Interactions

Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) at http://string-db.org/_is used to predict the protein interactions for the 31 drug targets [38]. STRING is a database consisting of the known and predicted protein interactions data for more than 2000 organisms. Protein-protein interactions were performed based on amino acid sequence for each drug targets. Amino acid sequence of each drug target was submitted to the STRING database against organism *H. pylori*.

2.4. Network Analysis

Cytoscape v3.3.0 is a popular bioinformatics tool for biological network visualization and data integration [39]. Cytoscape was used to integrate and visualize the biological network data predicted in STRING. STRING network data consisting of 609 predicted partners for 31 drug targets is imported to Cytoscape. The network data was integrated using option union to predict and visualize the comprehensive network of 609 predicted partners. Network analysis of Network Analyzer of Cytoscape v3.3.0, was used to compute closeness centrality, stress centrality, betweenness centrality, distribution, shortest path length distribution, shared neighbors distribution, node degree distribution, neighbourhood connectivity distribution, node degree distribution, *etc*., along with other simple parameters of the network such as number of nodes, connected components, network diameter, network radius, network centralization, clustering coefficient, number of selfloops, multi-edge node pairs, shortest paths, characteristic path length, average number of neighbors, network density, network heterogeneity. Data integrated in network was visualized using organic of Y files layout.

2.5. Prediction of Host-Pathogen Interactions

Host-pathogen interactions help us to understand the role and mechanism of infection paving path to understand and identify more efficient strategies to cure or prevent infection. Host-pathogen interaction studies were performed in two ways – first option is by using host-pathogen interaction tools and second option is by text mining of the literature. The following tools were employed to predict the host pathogen interactions: Pathogen-Host interaction search tool (PHISTO) [40], Pathosystems Resource integration Center (PATRIC) [41] and Host Pathogen Interaction Database (HPIDB) [42]. In addition, text mining of literature was performed using drug targets and its predicted interacting partners for data host pathogen interactions.

3. RESULTS

3.1. Potential Drug Targets for *H. pylori*

Island Pick, SIGI-HMM and Island Path methods of Islands viewer predicted 31 pathogenic islands with genes/virulence factors for 22 *H. pylori* strains (Table **1**). No pathogenic islands were detected in *H. pylori* strain HpSAT464. The features of the pathogenic islands predicted are mentioned in Table **2**. Nearly 642 genes/virulence factors associated with pathogenic islands were identified in 23 *H. pylori* strains Table **2**. Of them 282 were known with known functions and rest 361 were hypothetical proteins (Table **2**). Analysis of 642 bacterial genes identified 101 genes which are non-homologous to humans and are essential for pathogen. Gene property analysis of 101 genes identified 31 potential drug targets (Table **3**).

Literature screening based on the keywords identified that all the drug targets are experimentally validated. Sixteen of the 31 predicted drug targets are critical for the survival of

Table 1. Pathogenic islands identified in 23 *H. pylori* strains.

S. No	Strain Name	No of Genomic Islands	Method	Start Position	End Position	Size
1	Hp57	1	IslandPath-DIMOB	284,185	324,544	40,359
2	Hp12	1	IslandPath-DIMOB	484,957	489,788	4,831
3	HpB8	1	IslandPath-DIMOB	448,052	533,220	85,168
4	Hp India 7	3	IslandPath-DIMOB	749,965	797,920	47,955
—	—	—	IslandPath-DIMOB	1,217,751	1,246,359	28,608
—	—	—	IslandPath-DIMOB	1,616,790	1,626,196	9,406
5	Hp51	1	IslandPath-DIMOB	992,739	1,036,302	43,563
6	HpF32	1	IslandPath-DIMOB	1,051,313	1,085,082	33,769
7	HpF16	2	IslandPath-DIMOB	470,749	493,591	22,842
—	—	—	IslandPath-DIMOB	832,543	872,347	39,804
8	HpF30	1	IslandPath-DIMOB	828,728	868,864	40,136
9	Hp35A	1	IslandPath-DIMOB	1,032,831	1,072,644	39,813
10	HpB38	1	IslandPath-DIMOB	1,509,346	1,522,857	13,511
11	Hp2018	1	IslandPath-DIMOB	982,216	1,004,243	22,027
12	Hp908	1	IslandPath-DIMOB	973,392	990,660	17,268
13	Hp2017	2	IslandPath-DIMOB	497,212	532,400	35,188
—	—	—	IslandPath-DIMOB	974,279	996,463	22,184
14	HpAG1	1	IslandPath-DIMOB	512,700	550,135	37,435
15	HpSJM180	1	IslandPath-DIMOB	1,372,341	1,416,207	43,866
16	HpJ99	2	IslandPath-DIMOB	908,817	912,007	3,190
—	—	—	IslandPath-DIMOB	1,010,607	1,061,274	50,667
17	HpShi470	1	IslandPath-DIMOB	874,701	915,726	41,025
18	HpG27	1	IslandPath-DIMOB	1,045,375	1,082,440	37,065
19	HpCuz20	3	IslandPick	205,446	215,461	10,015
—	—	—	IslandPath-DIMOB	226,353	260,258	33,905
—	—	—	IslandPath-DIMOB	562,530	600,842	38,312
20	Hp26695	2	IslandPath-DIMOB	449,710	479,634	29,924
—	—	—	IslandPath-DIMOB	1,042,255	1,070,401	28,146
21	Hp83	2	IslandPath-DIMOB	73,583	107,449	33,866
—	—	—	IslandPath-DIMOB	852,516	892,293	39,777
22	Hp52	1	IslandPick	654,123	662,394	8,271

H. pylori. Analysis showed that GTPase, transposase, conjugal transfer protein, cag island DNA transfer protein cag 5, cag pathogenicity island protein Cag 3, cag pathogenicity island protein CagC, type IV secretion system protein virB8, type IV secretion system protein VirB4, type IV secretion system protein VirB9, cag pathogenicity island protein M, cag pathogenicity island protein W/9, relaxase, competence protein comB9-like competence protein, ATPase, Holliday junction resolvase, type II adenine specific DNA methyltransferase might be the critical drug targets for survival of *Helicobacter* species.

3.2. Protein-protein Interactions

STRING's reliable algorithms predicted protein-protein interactions for *H. pylori* in three modes-confidence view,

Table 2. Proteins and drug targets identified in pathogenic islands for 23 *H. pylori* strains.

S. No	Strain Name	No. of Pathogenic Islands	No. of Proteins	No. of Hypothetical Proteins	No. of Potential Drug Targets	No. of Drug Targets
1	Hp51	1	9	12	5	3
2	HpF32	1	12	25	11	2
3	HpF16	2	11	15	10	2
4	HpF30	1	28	2	7	1
5	Hp35A	1	32	4	9	3
6	HpB38	1	13	4	3	2
7	Hp2018	1	11	12	2	1
8	Hp908	1	6	14	0	0
9	Hp2017	2	28	5	3	2
10	HpHPAG1	1	31	0	10	1
11	HpSJM180	1	9	24	6	3
12	HpJ99	2	2	4	0	0
13	HpG27	1	9	16	6	5
14	HpShi470	1	8	27	4	2
15	HpcuZ20	3	4	7	0	1
16	Hp83	2	13	34	8	2
17	Hp26695	2	9	18	2	0
18	HpIndia7	3	10	27	7	1
19	HpB8	1	21	65	5	3
20	Hp12	1	2	0	0	0
21	HpF57	1	11	24	3	2
22	Hp52	1	4	4	0	0
23	HpSAT464	0	0	0	0	0
Total		31	282	361	101	36

Table 3. Drug targets identified in the 23 *H. pylori* strains.

S. No	Drug Target	Metabolic Categories	Gene ID	Strain Name
1	GTPase	Cellular process	GI:387782588	Hp51
2	DNA transfer protein	Cellular process	GI:387782591	Hp51
3	Transposase	Cellular process	GI:385224738	Hp83
4	Putative IS606 transposase	Cellular process	GI:385223561	Hp2017
5	Conjugal transfer protein	Cellular process	GI:317181586	Hp57
6	Bacteriophage-related integrase	Cellular process	GI:384892219	HpcuZ20

(Table 3) contd….

S. No	Drug Target	Metabolic Categories	Gene ID	Strain Name
7	Cag island DNA transfer proteinCag C	Cellular process	GI:208434927	HpG27
8	Cag pathogenicity island protein 5	Cellular process	GI:384896154	Hp35A
9	Cag pathogenicity island protein 3	Cellular process	GI:385223565	Hp2017
10	Integrase/recombinase XercD family protein	Cellular process	GI:308185118	HpSJM180
		Cellular process	GI:188527674	HpShi470
11	Type IV secretion system protein virB8	Virulence factors	GI:298355174	HpB8
12	Type IV secretion system protein VirB4	Virulence factors	GI:298355233	HpB8
13	Type IV secretion system protein VirB9	Virulence factors	GI:317009420	HpIndia7
14	Periplasmic competence protein-like protein	Virulence factors	GI:308185123	HpSJM180
		Virulence factors	GI:208434936	HpG27
15	Poly E-rich protein	Information and storage	GI:385216250	HpF32
16	Cag pathogenicity island protein M	Metabolism molecule	GI:384896163	Hp35A
17	Cag pathogenicity island protein 9	Metabolism molecule	GI:108562930	HpHPAG1
		Metabolism molecule	GI:3848449915	HpF30
18	Cag pathogenicity island protein W	Metabolism molecule	GI:384896173	Hp35A
19	Hac prophage II protein	Metabolism molecule	GI:254780055	HpB38
20	Hac prophage II integrase	Metabolism molecule	GI:254780053	HpB38
21	Mechanosensitive channel	Metabolism molecule	GI:385231894	Hp2018
22	Relaxase	Metabolism molecule	GI:308185146	HpSJM180
23	Putative chromosome partitioning protein	Metabolism molecule	GI:298355190	HpB8
24	VirB7	Metabolism molecule	GI:385224749	Hp83
25	Competence protein	Metabolism molecule	GI:208434918	HpG27
26	ComB9-like competence protein	Metabolism molecule	GI:208434919	HpG27
27	ATPase	Metabolism molecule	GI:387782603	Hp51
28	Outer membrane protein HorC	Metabolism molecule	GI:385216248	HpF32
29	PARA protein	Metabolism molecule	GI:208434932	HpG27
		Metabolism molecule	GI:188527681	HpShi470
		Metabolism molecule	GI:317181592	Hp57
30	Holliday junction resolvase	Metabolism molecule	GI:385217246	HpF16
31	Type II adenine specific DNA methyltransferase	Metabolism molecule	GI:385217221	HpF16

evidence view and action view. Twenty six of the 31 drug targets demonstrated interactions with other proteins, whereas no partners were predicted for rest five of the drug targets (Supplementary Table **1**). The evidence view presents information from different sources such as neighbourhood, coexpression, text mining, homology and gene fusion. The evidence view for the 23 drug targets is presented in Fig. **1**. Action view presents interacting information regarding activation, inhibition, binding, phenotype, catalysis, post translation modification and expression whereas confidence view presents score between interaction partners. STRING predicted 609 interacting partners for the 23 drug targets in three modes (Fig. **1**; Supplementary Table **1**).

3.3. Network Analysis

Network analysis on twenty three networks were predicted in STRING when exported and merged in cytoscape. Data visualization and analysis on the merged network demonstrated different protein hubs in the merged network (Figs. **2**-**6**). Protein-protein interactions in bird eye view with 361 nodes and 3146 edges of 609 interacting partners were revealed by cytoscape (Fig. **2A**). Very important interactions with specific proteins responsible for gastric cancer were visualized in protein hubs. Protein-protein interactions of drug target C694_01330 (Type II adenine specific DNA methyl tranferase can be visualized in Fig. (**2B**) by cytoscape. Protein-protein interactions of hub (pz33sb) with IS606A transposase and IS605A transposase were as visualized in Fig. (**3**). Protein-protein interactions of drug target ruvC (Holiday junction resolvase) with DNA repair system is visualized in Fig. (**4**). Blocking the drug target/hub of the pathogen with a molecule would lack DNA repairing mechanism affecting survival of the organism.

Protein-protein interactions of drug targets Cag 5, Vir B, Vir B8, pz19b (mechanosenstive ion channel protein), ftsz/obg/GTPase, Com9 (DNA transformation competence protein), pz23sb (PARA protein), Vir B4 with Che V (chemotaxis proteins), adhesion proteins like BabA, and toxins like Vac A are visualized in Fig. **5**. Interfering with these drug targets which are related to organism movement, adhesion of the organism with the host, and transfer of toxins to host would result in retardation of the growth. Protein-protein interactions of drug targets Cag E, Vir B11, ISO606B transposase with Ure A (urease sububnit aplha) are visualized in Fig. **6**. Urease is an important enzyme for neutralizing the acid in the host and lacking this enzyme would be fatal for pathogen.

3.4. Host-Pathogen Interactions

Tools PHISTO, PATRIC and HPIDB predicted host pathogen interactions. PHISTO predicted five interactions between host and pathogen proteins Table **4**; (Fig. **7A**). Tyrosinase-protein phosphatase non-receptor type II, NCK-interacting protein with SH3 domain, serine/threonine protein kinase MARK2, mitogen-activated protein kinase kinase kinase 7 (MAPKKK7) and TNF receptor associated factor 6 are the proteins from host involved in interactions Table **4**; (Fig. **7A**). Cytotoxin associated immunodominant antigen (with protein ID's P80200, P55980, B5Z6S0, Q9ZLT1) and vacuolating cytotoxin A (Q8RNUI) are the proteins from pathogen involved in interactions Table **4**; (Fig. **7A**). Tyrosinase-protein phosphatase non-receptor type II (Q06124), serine/threonine protein kinase MARK2 (Q9NZQ3), mitogen-activated protein kinase kinase kinase 7 (MAPKKK7) (O43318), TNF receptor associated factor 6 (Q9Y4K3) of host interacted with cytotoxin associated immunodominant antigen with protein ID's P80200, P55980, B5Z6S0, Q9ZLT1 of pathogen respectively Table **4**; (Fig. **7A**). PHISTO also predicted NCK-interacting protein with SH3 domain (Q9NZQ3) of host interacting with vacuolating cytotoxin A (Q8RNUI) of pathogen Table **4**; (Fig. **7A**).

HPIDB visualized interactions between 54 proteins from different pathogens with three human proteins (Table **4**; Fig. **7B**). Proteins 22, 1, 20, 11 were predicted for bacteria, fungi, virus, animal respectively. Among these pool 22 bacterial proteins two proteins from *H. pylori* were interacting with three human proteins (Table **4**). HPIDB predicted three interactions between host and pathogen (Table **4**; Fig. **7B**). Tyrosinase-protein phosphatase non-receptor type II (Q06124), mitogen-activated protein kinase kinase kinase 7 (MAPKKK7) (O43318) and NCK-interacting protein with SH3 domain (Q9NZQ3) are the proteins from host involved in interactions with cytotoxin associated immunodominant antigen A (with protein ID's P80200; B5Z6S0) of pathogen Table **4**; (Fig. **7B**).

PATRIC predicted five interactions between host and pathogen Table **4**; (Fig. **7C**, **D**). Tyrosinase-protein phosphatase non-receptor type II, mitogen-activated protein kinase kinase kinase 7 (MAPKKK7), serine/threonine protein kinase MARK2 and TNF receptor associated factor 6 are the proteins from host involved in interactions Table **4**; (Fig. **7C**, **D**). Cytotoxin associated immunodominant antigen (with protein ID B5Z6S0) and Cytotoxin associated immunodominant antigen A (with protein ID P80200) are the proteins from pathogen involved in interactions Table **4**; (Fig. **7C**, **D**). Serine/threonine protein kinase MARK2 (Q9NZQ3), mitogen-activated protein kinase kinase kinase 7 (MAPKKK7) (O43318), TNF receptor associated factor 6 (Q9Y4K3) and NCK-interacting protein with SH3 domain (Q9NZQ3) of host interacted with cytotoxin associated immunodominant antigen A (P80200) of pathogen respectively Table **4**; (Fig. **7C**, **D**). PATRIC also predicted tyrosinase-protein phosphatase non-receptor type II (Q06124) of host interacting with cytotoxin associated immunodominant antigen (B5Z6S0) of pathogen respectively Table **4**; (Fig. **7C**, **D**). In addition text mining of literature showed that eight proteins of *H. pylori* are interacting with 14 human proteins Table **5**.

4. DISCUSSION

Discovery, identification and validation of drug targets have been a debate from long time. Recent advances on discovery and validation of drug targets for infectious diseases focused on disease understanding and mechanism. Previously subtractive genomics [11-13] was implemented by our group to identify novel drug targets for 23 *H. pylori* strains. Different methods like essential gene identification [10, 11], metabolic pathway analysis [16] and reverse docking [17] were implemented by other groups to identify novel drug targets for *H. pylori*. Though, these methods were successful in identifying novel drug targets for *H. pylori* we foresee

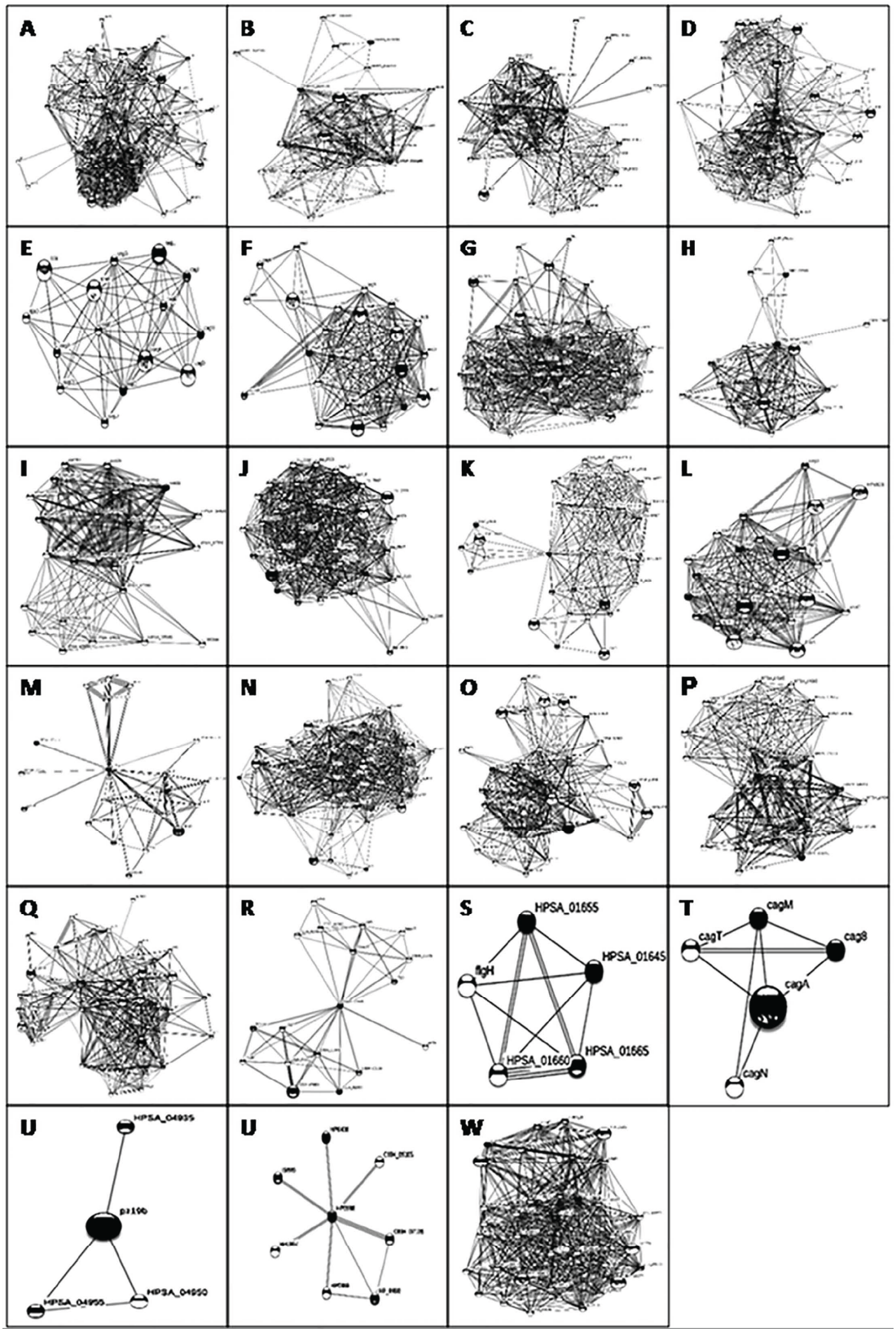

Fig. (1). Evidence view of protein-protein interactions as predicted in STRING for 23 drug targets identified in *H. pylori* **A**). GTPase protein, **B**). ATPase protein, **C**). conjugal transfer protein **D**). Cag 5 protein **E**). Cag C protein, **F**). Cag 3 protein, **G**). integrase/recombinase (XERCD family) protein, **H**).Vir B4 protein, **I**). Vir B9 protein, **J**). Periplasmic competence protein, **K**). Poly E rich protein, **L**). Cag 9 protein, **M**). PARA protein, **N**). Relaxase protein, **O**). Competence like protein, **P**). Com B9 like protein, **Q**). Holiday junction resolvase protein, **R**). Type II adenine methyl transferase protein, **S**). Outer membrane HorC protein, **T**). Cag M protein, **U**). Mechanosensitive channel protein, **V**). Transposase protein, **W**). Type IV secretion system VirB8 protein.

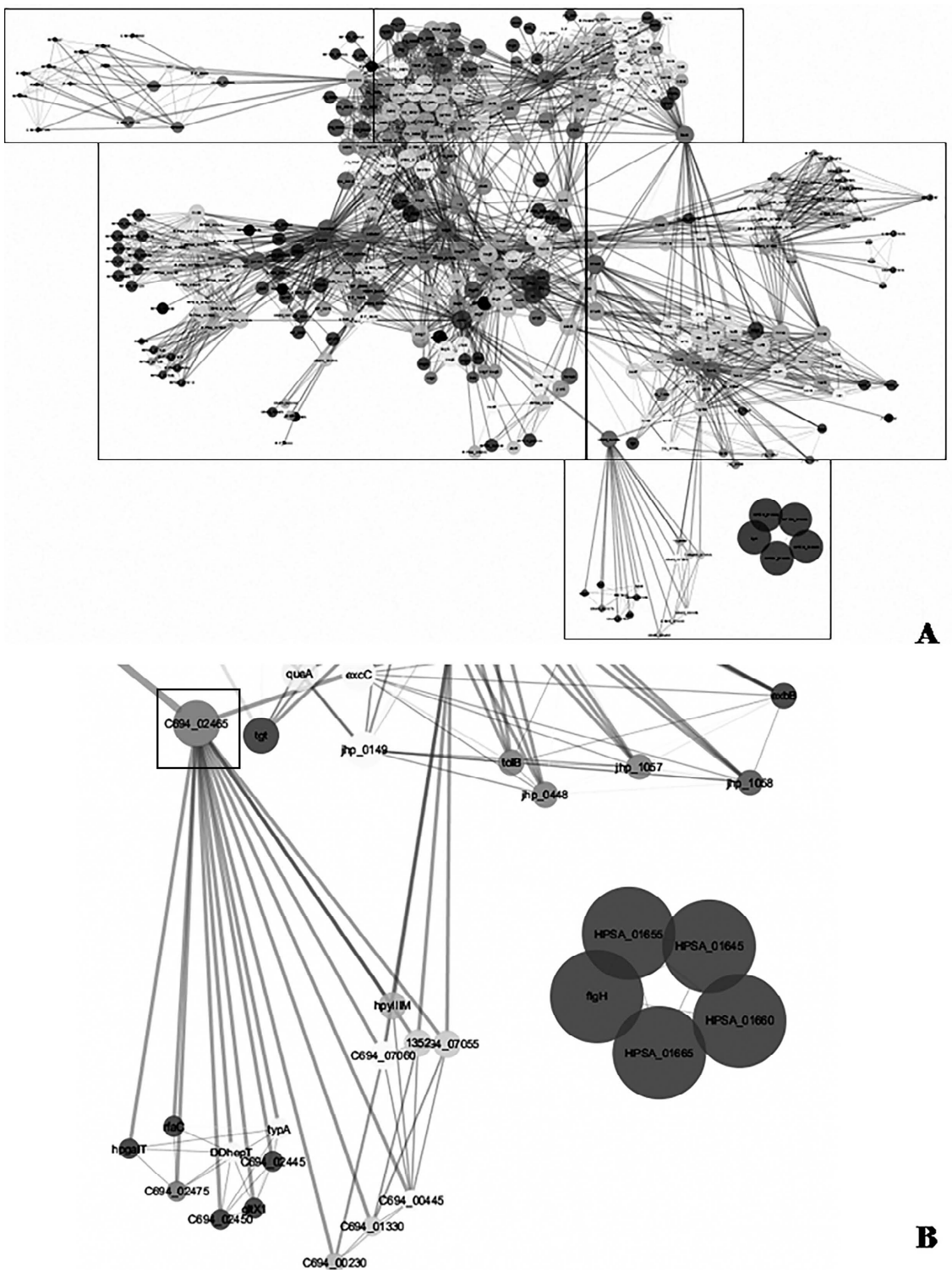

Fig. (2). A) Bird eye view of protein-protein interactions with 361 nodes and 3146 edges of 609 interacting partners as revealed by cytoscape. **B**) Protein-protein interactions of drug target C694_01330 (Type II adenine specific DNA methyl tranferase) highlighted in rectangle box as revealed by cytoscape.

pathogenic islands as the potential source for novel drug targets.

The current study was the first report till date to employ successful systematic *insilico* analysis for identification of potential and novel drug target candidates from pathogenic islands of 23 *H. pylori* strains. Systematic *in silico* analysis included five steps - the first two steps were used to identify drug targets and the next three steps were used to characterize the drug targets. The initial step in the systematic analysis is to screen the genome of *H. pylori* strains to identify pathogenic islands. Screening the genome of *H. pylori* strains using islands viewer [37] identified 31 pathogenic islands Table **1**. The second step is to analyzae the pathogenic islands to identify the potential drug targets for

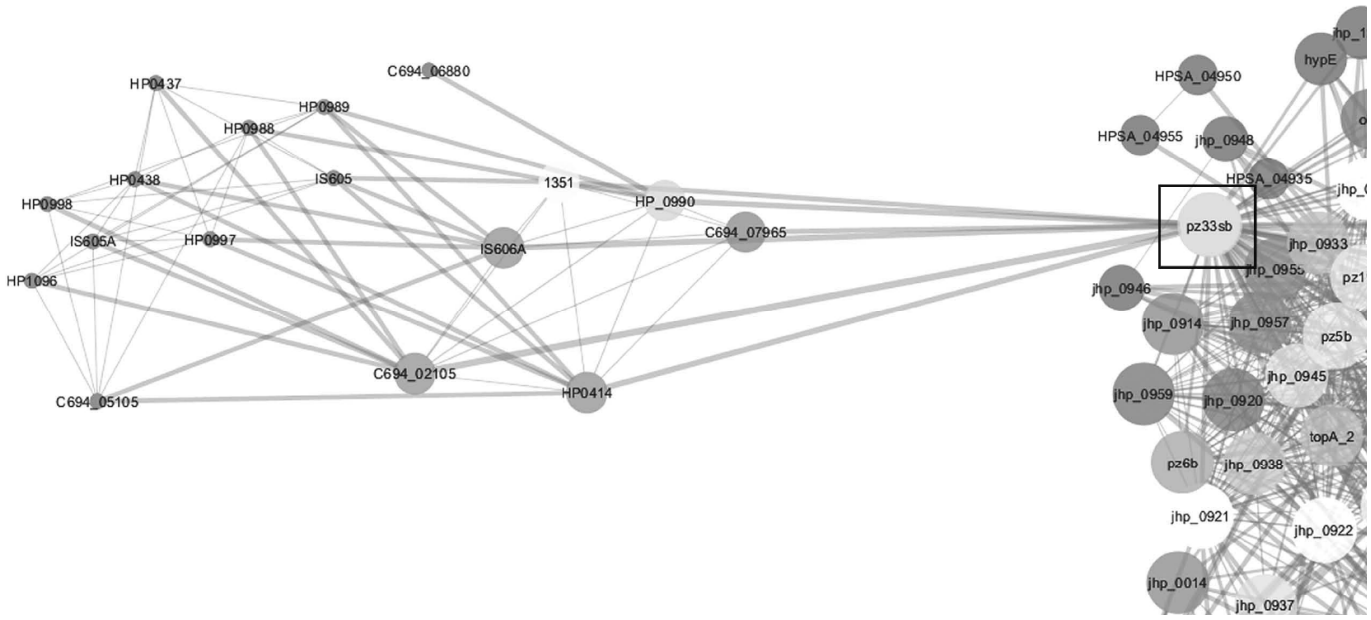

Fig. (3). Protein-protein interactions from the hub pz33sb highlighted in rectangle box with IS606A transposase and IS605A transposase as revealed by cytoscape.

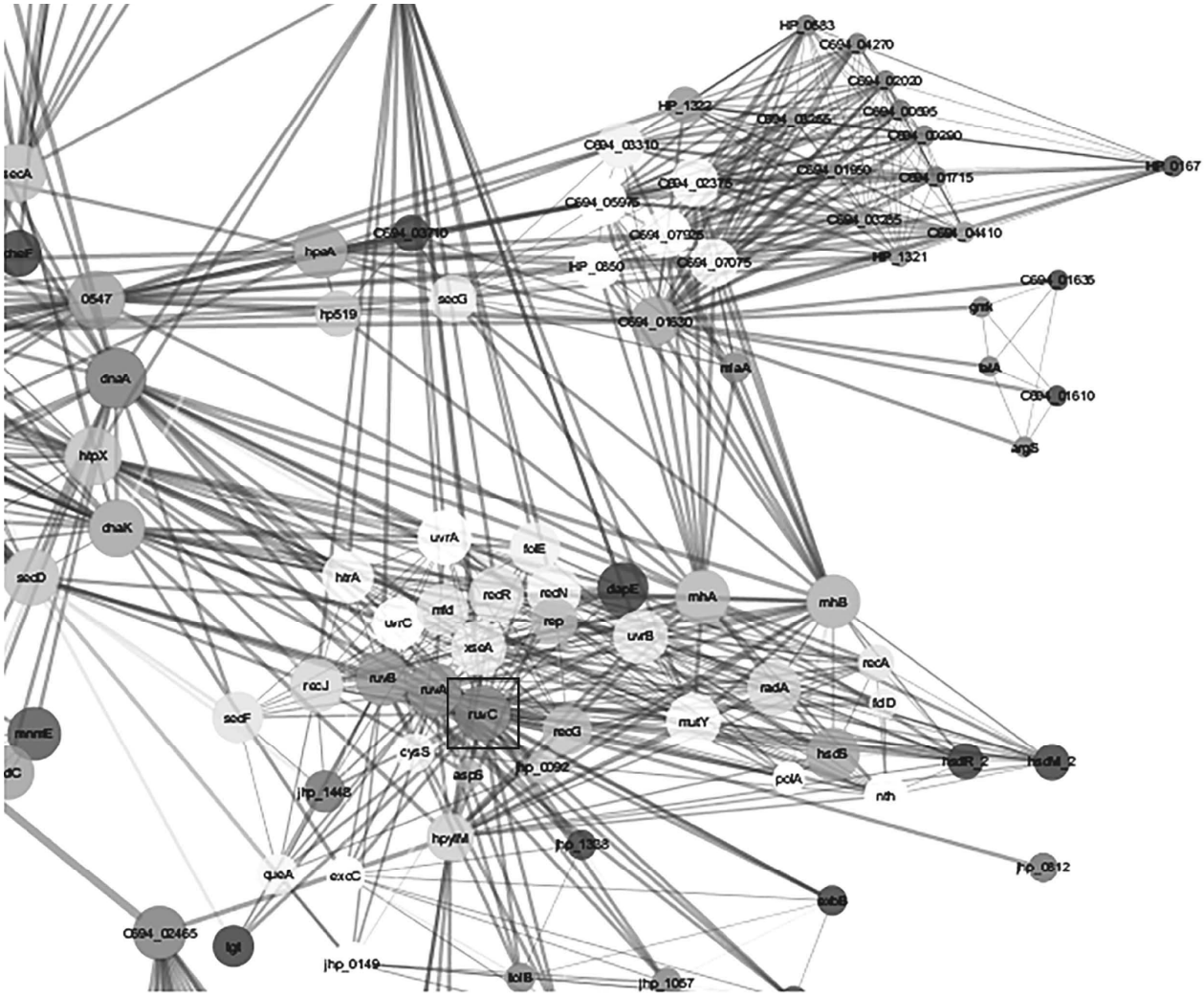

Fig. (4). Protein-protein interactions of drug target ruvC (Holiday junction resolvase) highlighted in rectangle box with other DNA repair system as revealed by cytoscape.

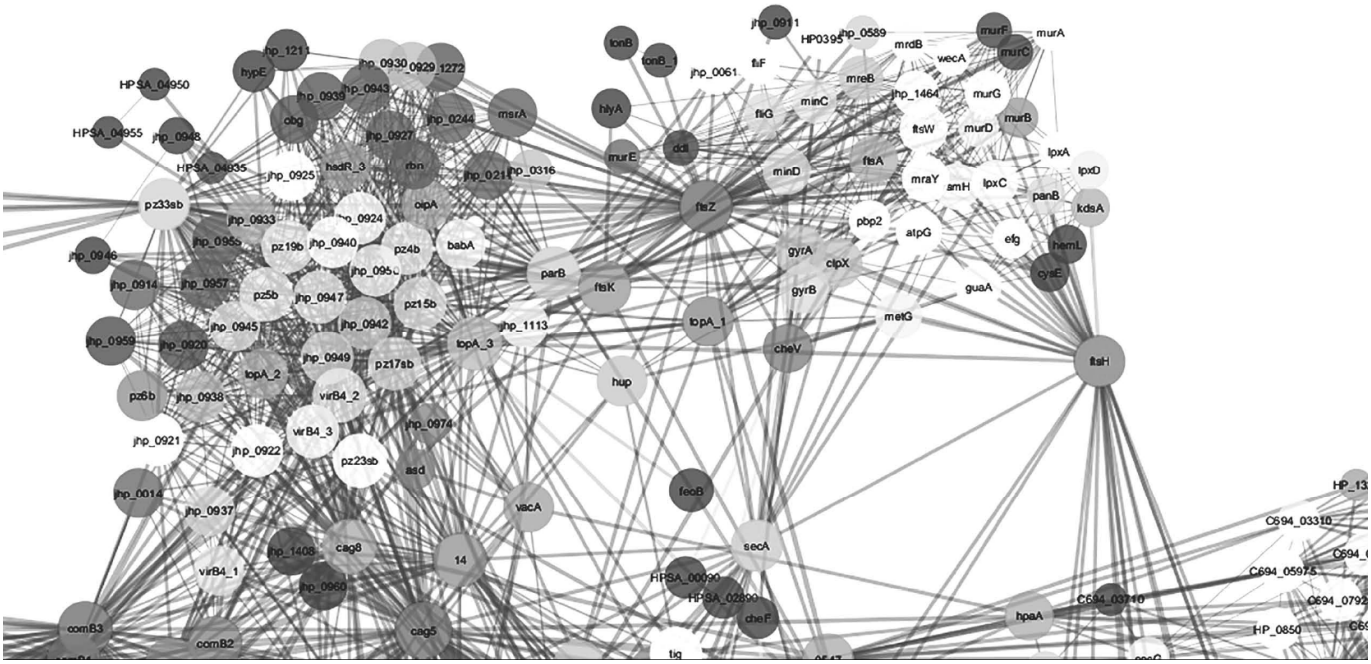

Fig. (5). Protein-protein interactions of drug targets Cag 5, Vir B, Vir B8, pz19b (mechanosenstive ion channel protein), ftsz/obg/GTPase, Com9 (DNA transformation competence protein), pz23sb (PARA protein), Vir B4, with Che V(chemotaxis proteins), adhesion proteins like BabA, and toxins like Vac A as revealed by cytoscape.

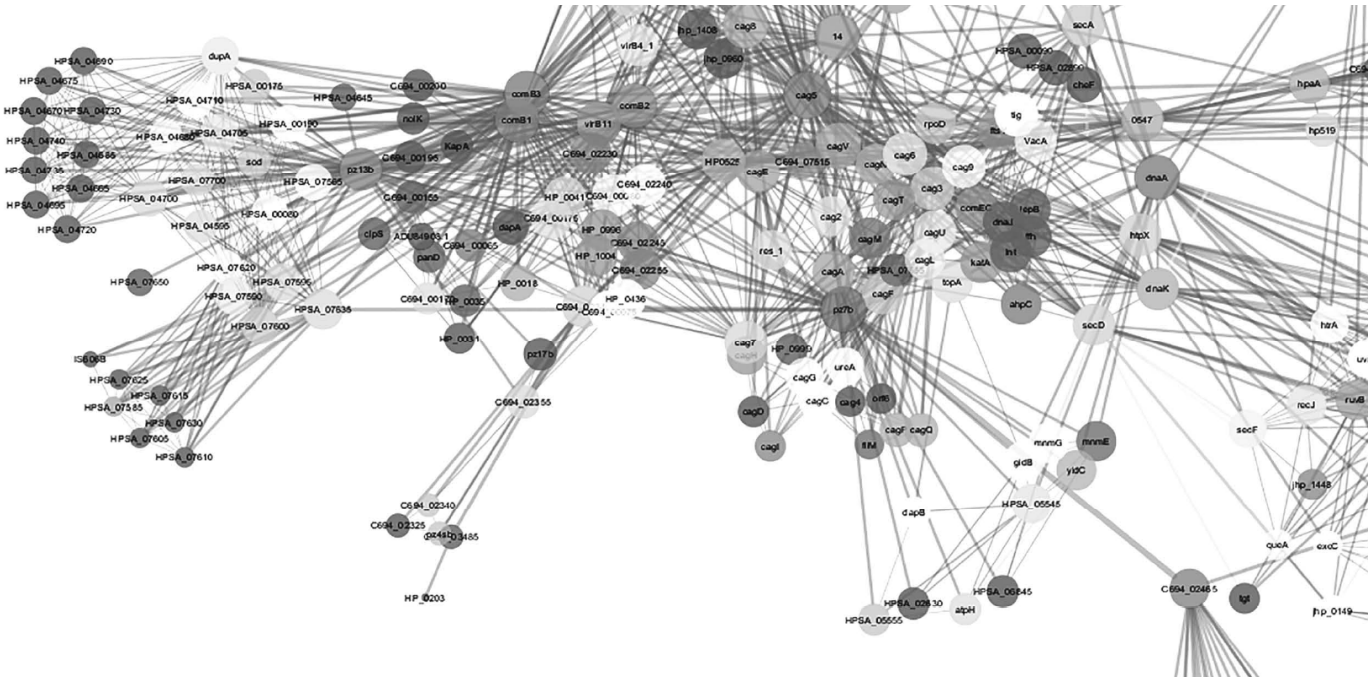

Fig. (6). Protein-protein interactions of drug targets Cag E, Vir B11, ISO606B transposase with Ure A (urease sububnit aplha) as revealed by cytoscape.

H. pylori. Analysis of the pathogenic islands resulted in identification of 642 virulence factors (bacterial genes) in 31 pathogenic islands Table **2**. The analysis of the 642 virulence factors identified 101 genes which were non-homologous to human and are essential for the survival of the pathogen. Further, analysis of 101 genes for gene property identified 31 novel and potential drug targets for *H. pylori* Table **3**. The third step in the systematic analysis is to implement protein-protein interactions to identify the interacting partners for the potential drug targets. STRING was used to study the protein-protein interactions and predicted 609 interacting partners for the 23 drug targets (Fig. **1**); Supplementary Table **1**. The fourth step is to accomplish network analysis on the interacting partners associated in the protein-protein interactions. Data of twenty three networks was exported and merged in cytoscape to perform network analysis. Data

Table 4. Host – pathogen interactions as predicted by tools PHISTO, PATRIC and HPIDB.

S. No	Host ID	Host	Pathogen ID	Pathogen	Interaction Type	Method	Reference
PHISTO							
1	Q06124	Tyrosine-protein phosphatase non-receptor type 11	P80200	Cytotoxicity-associated immunodominant antigen	-	anti bait coimmuno-precipitation	Higashi *et al.* [43]
2	Q9NZQ3	NCK-interacting protein with SH3 domain	Q8RNU1	Vacuolating cytotoxin A	-	two hybrid/coimmunoprecipitation	de Bernard *et al.* [44]
3	Q7KZI7	Serine/threonine-protein kinase MARK2	P55980	Cytotoxicity-associated immunodominant antigen	-	molecular sieving	Nesić *et al.* [45]
4	O43318	Mitogen-activated protein kinase kinase kinase 7	B5Z6S0	Cytotoxicity-associated immunodominant antigen	-	anti tag coimmuno-precipitation	Lamb *et al.* [46]
5	Q9Y4K3	TNF receptor-associated factor 6	Q9ZLT1	Cytotoxicity-associated immunodominant antigen	-	Other methods	Zhu *et al.* [47]
PATRIC							
1	Q06124	Tyrosine-protein phosphatase non-receptor type 11	B5Z6S0	Cytotoxicity-associated immunodominant antigen	Physical association	Anti-tagcoimmuniprecp-tion	Higashi *et al.* [43]
2	O43318	Mitogen-activated protein kinase kinase kinase 7	P80200	Cytotoxin-associated protein A	Physical association	Anti-tagcoimmuniprecp-tion	Lamb *et al.* [46]
3	Q06124	Tyrosine-protein phosphatase non-receptor type 11	P80200	Cytotoxin-associated protein A	Physical association	Anti-tagcoimmuniprecp-tion	Higashi *et al.* [43]
4	Q7KZI7	Serine/threonine-protein kinase MARK2	P80200	Cytotoxin-associated protein A	Direct interaction	Molecular seiving	Nesić *et al.* [45]
5	Q9Y4K3	TNF-receptor associated factor 6	P80200	Cytotoxin-associated protein A	Direct interaction	Anti-tagcoimmuniprecp-tion	Lamb *et al.* [46]
HPIDB							
1	Q9NZQ3	NCK-interacting protein with SH3 domain	P80200	Cytotoxin-associated protein A	Physical association	colocalization	de Bernard *et al.* [44]
2	Q06124	Tyrosine-protein phosphatase non-receptor type 11	B5Z6SO	Cytotoxin-associated protein A	Physical association	anti bait coimmuno-precipitation	Higashi *et al.* [43]
3	O43318	Mitogen-activated protein kinase kinase kinase 7	B5Z6SO	Cytotoxin-associated protein A	Physical association	Anti-tagcoimmuniprecp-tion	Lamb *et al.* [46]

visualization and analysis on the merged network demonstrated bird eye view of different protein hubs in the merged network with 361 nodes and 3146 edges of 609 interacting partners (Fig. **2A**). And the fifth and final step in the systematic analysis is to proceed with the host-pathogen interactions based on tools and literature mining. PHISTO, PATRIC and Host Pathogen Interaction Database were used to predict the host pathogen interactions for predicted interacting partners. Host-pathogen interactions identified important molecules which are closely associated with gastric cancer. These studies persuaded us to ascertain key molecules in *H. pylori* and their counter interacting molecules in the host leading to gastric cancer.

Data on protein-protein interactions, network analysis and host-pathogenic interactions provided few insights and understanding of *H. pylori* associated gastric cancer. As revealed by the data in the present study *H. pylori* uses cytokines, gastrin and toxin VacA to weaken the gastric mucosal barrier and colonize in the submucous. After the gastric mucosal barrier is weakened BabA facilitates *H. pylori* in adhering to the epithelial lining of the stomach [58-62]. T4SS system coded by cytotoxin associated gene pathogenicity island

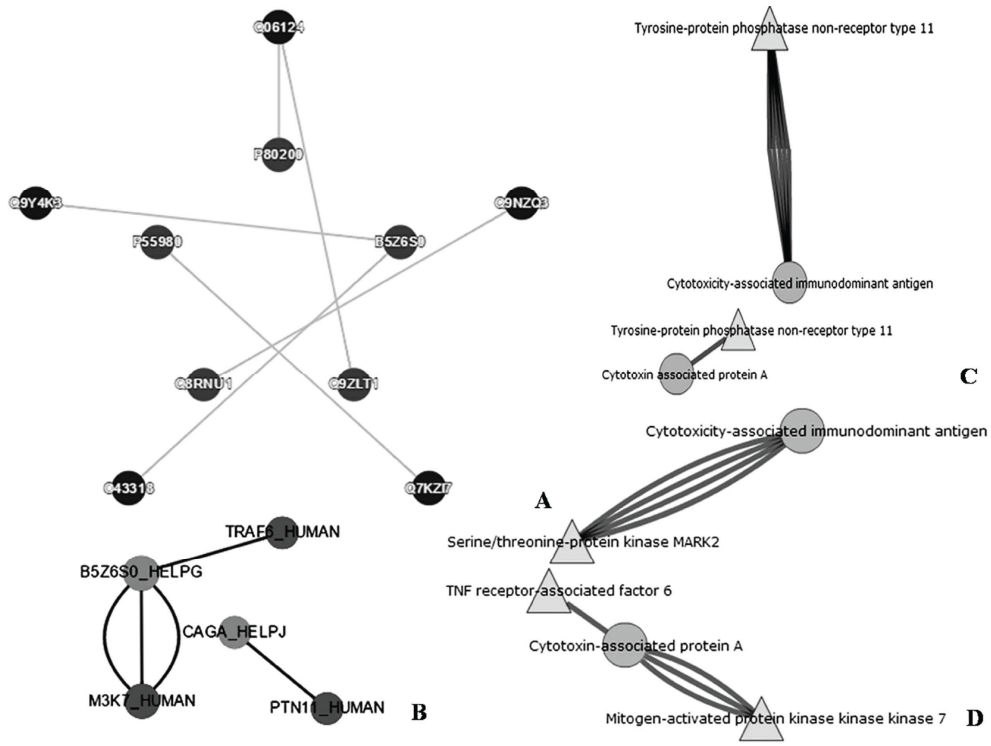

Fig. (7). Host and pathogen interactions as visualized by **A**) PHISTO **B**) HPIDB **C&D**) PATRIC.

Table 5. Host – pathogen interactions revealed from the text mining of the literature.

S. No	Host Pathogen Interactions		Interactions Causes	Reference
	Pathogen Proteins	Human Proteins		
1	CagA	E-cadherin	Gastric cancer	Murata-Kamiya *et al.* [48]
		Erk mitogen-activated protein kinase	Gastric cancer	Zhu *et al.* [47]
		Transforming growth factor-b-activated kinase 1 (TAK1)	Gastric cancer	Lamb *et al.* [46]
		Src family kinases	Gastric cancer	Higashi *et al.* [49]
		human kinase PAR1b/MARK2	Gastric cancer	Nesić *et al.* [45]
2	CagE type IV secretion system	Dendritic cells	MALT and Gastric cancer	Donald *et al.* [50]
		NF- B activator. TAK1, TRAF6, and MyD88	Intestinal metaplasia and Gastric cancer	Hirata *et al.* [51]
3	Holliday junctions resolves	Mus81	block DNA replication	Chen *et al.* [52]
4	Mechanosensitive ion channel protein	integrin-b-catenin	human articular chondrocyte (HAC) responses	Lee *et al.* [53]
		Focal Adhesion Kinase pp125FAK	osteoblast activation	Rezzonico *et al.* [54]
5	Type IV secretion system	protein kinase B, PKB	Gastric cancer	King *et al.* [55]
6	GTPase	Guanine Nucleotide Exchange FactorSec7 Domain	IL-8 expression	Mossessova *et al.* [56]
7	Transpoase	RNA-proteins	To regulate the RNA-proteins net-work	Kelley *et al.* [57]
8	VacA	VIP54	Infection/Gastric cancer	de Bernard *et al.* [44]

(cag PAI) injects CagA, peptidoglycan and VacA into the host to establish interaction with the host leading to inflammation, a condition known as gastritis.

Cag A changes the expression of host cells; induces elongation of cell, loss of cell polarity and cell proliferation; decreases acid secretion; and degrade cell-cell junctions [63]. CagA is phosphorylated and activated by src/Lyn kinase disturbing mitogen-activated protein kinase (MAPK) signaling in host cells through NCK-interacting with SH3/SH2 domain to modify cellular responses [64]. Cell focal adhesions are disrupted by CagA by binding and activating SHP2 phosphates/Tyrosine protein phosphatase non receptor type 11 [64]. Normal epithelial architecture is disrupted when polarity regulator PAR1b/MARK2 kinase is inhibited by CagA leading to loss of polarity in epithelial cells [64]. Another surface receptor protein in *H. pylori* Toll like receptor (TLR)-2 disrupts adherin junctions within gastricepithelial cells. TLR-2 activates protease calpain cleaving E-cadherin and allows increased β-catenin signaling to disrupt adherin junctions [65]. CagA-dependent, TRAF6-mediated Lys 63-ubiquitination and activation of TAK1 activate transcription factor NF-κβ, resulting in chronic inflammation and cancer when is constitutively expressed [66-68]. CagA and COX-2 were known for cell proliferation, prostaglandin biosynthesis and angiogenesis. Cag A induces proteasome mediated degradation directly by inactivating gastric tumor suppressor gene RUNX3 [69, 70] or indirectly p53 to modulate ASPP2 tumor suppressor genes [71].

Vacuolating cytotoxin (Vac) A induce ROS at the site of infection damaging mitochondrial DNA of gastric epithelial cells [44]. Vac A interact with a number of host surface receptors to trigger responses such as poreformation, cell vacuolation, endolysomal functions modification, immune inhibition and apoptosis [72-74]. VacA along with other virulence factors such as γ-glutamyl transpeptidase, and cholesterol α-glucosides modulate responses of T cells. Cag A and Vac A induce ROS and NF-κβ, along with cytokines, and chemokines.

Cytokines (IL-1, 6, 8), chemokines (CXCL8, CCL3, 4), metalloproteinases (MMPs), prostaglandin E2 (PGE2) and reactive oxygen nitrogen species (RONS) prolong inflammation inducing G cells to secrete the hormone gastrin in turn stimulating loads of acid damaging duodenum a condition known as ulcers [75, 76]. NF-κβ and β-catenin signaling pathways induce double stranded breaks, defective mitotic checkpoints, deregulate HR pathway of DSB repair and DNA repair enzymes leading to genetic diversification randomly heading towards activation of oncogenes and inactivation of tumor suppressor genes leading to gastric cancer [77].

CONCLUSION

Pathogenic islands are the good source for drug targets. Analysis of genomes in 23 *H. pylori* strains identified 31 pathogenic islands of them 29 bacterial genes which are nonhomologous to humans and are essential for pathogen. All the drug targets were found to be critical for the species and are already experimentally validated lending credence to our approach. PHISTO, HPIDB, PATRIC tools visualized host-pathogen interactions directly or indirectly predicting the role of certain pathogen molecules (drug targets) in gastric cancer. These novel drug targets may have possible therapeutic implications for gastric cancer.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

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