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IN SILICO COMPARATIVE ANALYSIS OF METABOLIC PATHWAYS OF *Heamophilus influenzae* AND *Helicobacter pylori* TO IDENTIFY POTENTIAL DRUG TARGETS

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ABSTRACT

Multiple drug resistance of pathogenic bacteria is threat to human health and hence there is an urgent need to identify new drug targets. Due to advances in sequencing techniques complete genomic sequences and metabolic pathways of many bacterias are available. Comparative pathway analysis in pathogenic bacterias and Homo sapiens can lead to identification of new drug targets. In order to identify potential drug targets in *Heamophilus influenzae* and *Helicobacter Pylori* in silico metabolic pathways analysis was carried out. Metabolic enzymes of *Heamophilus influenzae* and *Helicobacter Pylori* with no homolog in *Homo sapiens* have been identified at E value 0.01 by BLASTP. Essential enzymes and choke point reactions were identified by using database of essential genes and Choke point reaction finder tool respectively. Metabolic enzyme which are essential to pathogen, show no homolog in *Homo sapiens* and catalyze choke point reactions have been identified as potential drug targets in current study.

KEYWORDS:Haemophilus influenza, Helicobacter Pylori, Metabolic enzyme analysis, Choke point reactions, Drug targets,Database of essential genes.

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INTRODUCTION

Haemophilus influenza, a gram negative bacterium is a resident of upper respiratory tract in humans. It is recognized as pathogen by its involvement in pneumonia, bacteremia, meningitis, epiglottitis, septic arthritis, cellulitis and lower respiratory tract infections along with *Streptococcus pneumonia* in humans. Meningitis and bactermia occurs in the area of developing countries where Hib vaccine is not used. *Haemophilues influenza* produces beta-lactamase enzyme and hence resistant to penicillin family of drugs. *Haemophilus influenza* has also shown to be resistant to the macrolide, azalide, and ketolide group of antibiotics as well as to tetracyclines and rare resistance to quinolone[1, 2]. It is also seen that due to use of pneumococcal conjugated vaccines there is an increase in occurrence of non type able *Heamophilus influnezae* in respiratory tract infections which is indirectly increasing drug resistance in different *Haemophilus* strains and hence there is an urgent need to develop drug against it. [3, 4] *Helicobacter pylori* is a gram negative bacterium which is found in stomach and is linked in the development of peptic ulcers and inflammation of the corpus leading to development of duodenal ulcers and sometimes this condition might lead to gastric carcinoma. Studies on antibiotic resistance of *Helicobacter pylori* has shown multiple drug resistance to clarithromycin, amoxicillin, tetracycline, metrozidazople, fluoroquinones, rifobutin.[5, 6] Most of the drugs against *Haemophilus influenza* and *Helicobacter pylori* target specific biological processes, viz replication, transcription and translation. But, there are several enzymes involved in other than these mentioned biological processes like cell wall synthesis, fatty acid synthesis, amino acid synthesis which are essential for survival and unique to these pathogenic bacteria.

Till date complete genome sequences of 2777 (http://www.ncbi.nlm.nih.gov/genome/browse/) bacteria are available and many bacterial genome projects are currently in progress. Availability of genomic sequences of pathogenic bacteria has provided huge information to identify drug and vaccine targets. Any protein which is essential for the pathogenic bacteria for its survival or it catalyses very important step in metabolic pathway giving rise to unique product and having no homologue in human might serve as best drug target[7]. Sarangi et al[7](2009), Reddy et al[8], (2010), Sakharkar et al[9](2004),Mandage et al[10](2010) have used subtractive genomics approach to find drug targets in different pathogenic bacteria. In the current study, comparison of metabolic pathway enzymes of *Haemophilus influenzae* and *Helicobacter pylori* with *Homo sapiens* has been carried out by using pathway tool of metacyc database which is non redundant, metabolic pathway and enzyme database. It contains information related to metabolic pathways which have been experimentally demonstrated in scientific literature[11].Comparision of metabolic pathways is followed by identification of essential enzymes by using database of essential gene(DEG)[12] and choke point reactions by choke point reaction finder at metacyc database. Choke point reaction can be defined as a reaction which uniquely consumes a specific substrate or uniquely produces specific product which means that consumption of unique substrate results in accumulation of unique product. Inhibition of enzyme catalyzing this reaction would result in stalling activities of the cell, essential for its survival.[13,14].Metabolic enzyme which are essential for pathogenic bacteria, catalyze choke point reactions and absent in Humans might serve as potential drug targets.

MATERIALS AND METHODS

Comparision of metabolic pathway enzymes of Helicobacter pylori ,

Haemophilus influenza and Homo sapiens: Pathway tool available at Metacyc was used to compare metabolic pathways of *Helicobacter pylori , Haemophilus influenza* and *Homo sapiens*. Pathways which were unique to *Haemophilus influenza* and not present *Homo sapiens* were grouped under unique to *Haemophilus influenza* . Pathways which were unique to *Helicobacter pylori* but not present in the *Homo sapiens* were grouped under unique to *Helicobacter pylori*. Pathways which were present in both *Haemophilus influenza* and *Helicobacter pylori* but absent in the *Homo sapiens* were grouped as shared by *Haemophilus influenza* and *Helicobacter pylori*. The gene name, enzyme name and enzyme commission number of metabolic pathway enzymes were identified by cross species comparison.

***Retrieval of enzyme sequences in Swiss-Prot and BLAST*:** Protein sequences of metabolic enzymes unique to *Haemophilus influenza* and *Helicobacter pylori* and shared by both were retrieved from Uniprot database[15] by batch retrieval tool. Each protein sequence obtained was subjected to BLASTP search against *Homo sapiens* database with E-value cut-off of 0.01.[16]

***Identification of choke point reactions in Haemophilus influenzae and Helicobacter pylori*:** Choke point reactions in the *Helicobacter pylori* and *Haemophilus influezae* were identified by Choke Point finder tool available at Metacyc.

***Identification of the essential genes*:** Essential genes are the minimal set of the genes required by the living cell. By using database of essential genes(DEG), Essential metabolic enzymes in the *Homophilus influenzae* and *Helicobacter Pylori* have been identified.

RESULTS AND DISSCUSSION

***Metabolic pathway information*:** By using Metacyc, metabolic pathways of *Homo sapiens, Haemophilus influenza* and *Helicobacter pylori* were compared and following results were obtained. Table 1 shows number of metabolic pathways and metabolic enzymes present in the organisms taken in the study. Metabolic enzymes which are uniquely present in all the compared orgasnisms were identified and are shown in Table 2. Number of metabolic pathways shared by *Haemophilus influenzae, Helicobacter pylori and Homo sapiens* are shown in Table 3. Anishetty S et al[17]have carried out metabolic pathway comparison by using KEGG database for pathogen *Mycobacterium tuberculosis.*Similar study was carried out in case of *Mycobacterium leprea* and *Pseudomonas aeruginosa* by Anusuya Shanmugam et al [18] and Deepak Perumal et al[19] respectively.

Table 1
Metabolic pathways and enzymes present in the organism

Name of the organism	Metabolic pathway identified	Metabolic Enzymes
Homo sapiens	275	1028
Haemophilus influenza	189	843
Helicobacter pylori	144	570

Table 2
Metabolic enzymes uniquely present in the organism

Name of the organism	Metabolic enzymes uniquely present in the organism
Homo sapiens	820
Haemophilus influenza	296
Helicobacter pylori	172

Table 3
Metabolic enzymes shared by organism

Sharing of pathways between the organisms	No. of Pathways Shared	No. of enzymes shared
Shared by *Haemophilus influenza* and *Helicobacter pylori*	273	358
Shared in *Haemophilus influenza* and *Homo sapiens*	50	234
Shared in *Helicobacter pylori* and *Homo sapiens*	43	167

Choke Point reaction

Choke Point reactions were identified by choke point reaction finder tool available at Metacyc database. Number of enzymes catalyzing choke point reactions present in metabolic pathways of *Haemophilus influenzae* and *Helicobacter pylori* are shown Table 4.

Table 4
Choke Points Identified

Name of the organism	Choke Points Present in compared metabolic enzymes
Haemophilus influenza	**60**
Helicobacter pylori	**24**

Essential and non essential enzymes of *Haemophilus influenzae* and *Helicobacter Pylori*

Metabolic enzymes identified by comparing metabolic pathways were classified into essential and non essential by using information obtained from database of essential genes. Number of essential enzyme in *Haemophilus influenzae* and *Helicobacter pylori* identified have been shown in Table 5 and Table 6.

Table 5
Metabolic enzymes uniquely present in Haemophilus influenzae classified into essential and non essential enzymes.

Enzymes	Total	No homolog in *Homo sapiens* at E Value 0.001	Choke Point	No homolog in *Homo sapiens* at E Value 0.001 and choke present for the enzymatic reaction
Essential Enzymes	116	33	10	8
Non Essential Enzymes	180	64	50	11

Table 6
Metabolic enzymes uniquely present in Helicobacter pylori classified into essential and non essential enzymes

Enzymes	Total	No homolog in *Homo sapiens* at E Value 0.001	Choke Point	No homolog in *Homo sapiens* at E Value 0.001 and choke present for the enzymatic reaction
Essential Enzymes	73	23	6	4
Non Essential Enzymes	99	37	18	13

Identification of potential drug targets

Metabolic enzymes which are essential for bacteria to survive, show choke point reactions and unique to either *Haemophilus influenza, Helicobacter pylori* or present in both are identified as a potential drug targets and are shown in the Table 7-9.Most of the identified drug targets belong to amino acid biosynthesis pathways, Cell wall synthesis pathway, Carbohydrate synthesis pathway and fatty acid synthesis pathways.

Table 7
Enzymes which can be potential Drug targets identified in Haemophilus influenza

Pathway name	Name of the enzyme	Essential	Not homolog in Homo sapiens and choke point present
dTDP-L-rhamnose biosynthesis I	dTDP-4-dehydrorhamnose 3,5-epimerase	Yes	Yes
DNA Replication machinery	Uracil-DNA glycosylase	Yes	Yes
Cell wall synthesis	N-acetylmuramoyl-L-alanine amidase	Yes	Yes

Cell wall synthesis	murein transglycosylase	Yes	Yes
(KDO)2-lipid A biosynthesis I	lipid A biosynthesis (KDO)2-(lauroyl)-lipid IVA acyltransferase	Yes	Yes
Tryptophan Biosynthesis	menaquinone-specific isochorismate synthase	Yes	Yes

Table 8
Enzymes which can be potential Drug targets identified in Helicobacter Pylori

Pathway name	Name of the enzyme	Essential	Not Present in Homo sapiens and choke point present
Cofactor Biosynthesis	pantoate--beta-alanine ligase	Yes	Yes
	dihydrodipicolinate reductase	Yes	Yes
Amino acid Bioysnthesis	chorismate synthase	Yes	Yes
Flavin Biosynthesis	bifunctional riboflavin kinase/FMN adenylyltransferase	Yes	Yes

Table 9
Enzyme which can be a potential drug targets shared by Haemphilus influenzae and Helicobacter pylori

Pathway Name	Name of the enzyme	No homolog in *Homo sapiens* at E value-0.01 and Choke Point Present
Alanine and Cell wall synthesis	Alanine racemase	Yes
Homoserine Biosynthesis	Aspartate Kinase	Yes
Homoserine Biosynthesis	Aspartate-semialdehyde dehydrogenase	Yes
Tryptophan Biosynthesis	Indole-3-glycerol-phosphate synthase	Yes
Tryptophan Biosynthesis	Indole-3-glycerol-phosphate lyase	Yes
Tryptophan Biosynthesis	Phosphoribosylanthranilate isomerase	Yes

Valine Biosynthesis	Ketol-acid reductoisomerase	Yes
Valine Biosynthesis	Dihydroxy-acid dehydratase	Yes
Chorismate Biosynthesis	3-deoxy-7-phosphoheptulonate synthase	Yes
	3-dehydroquinate synthase	Yes
	3-dehydroquinate dehydratase	Yes
Carbohydrate Biosynthesis	D-sedoheptulose 7-phosphate isomerase	Yes
	3-deoxy-8-phosphooctulonate synthase	Yes
Cell Wall Synthesis	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	Yes
	glutamate racemase	Yes
	UDP-N-acetylmuramate dehydrogenase	Yes
	UDP-N-acetylmuramate—L-alanine ligase	Yes
Thiamin biosynthesis I	Thiamine-phosphate diphosphorylase	Yes
Fatty acid Synthesis	Beta-ketoacyl-acyl-carrier-protein synthase I	Yes
	Lipid IV(A) 3-deoxy-D-manno-octulosonic acid transferase.	Yes

CONCLUSION

Emergence of the multiple drug target resistance in pathogenic bacteria is common phenomenon. Therefore there is need to identify atypical drug targets. Large scale genomic information is available of prokaryotic genomes in public domain database and information related to the essential genes is also available in the form of Database of Essential Genes(DEG).In the present study we have identified potential drug targets which are unique *to Haemophilus influenzae* and *Helicobacter Pylori* and shared by both. Identification of common drug targets by metabolic pathway analysis in *Haemophilus influenzae , Helicobacter pylori* and other pathogenic bacteria can lead to development of broad spectrum antibiotics. Homology modeling of identified targets can be used to detect potential binding sites that can be targeted for drug designing. Virtual Screening against all these identified targets can lead to development of new therapeutics against pathogenic bacteria.

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