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In Silico Identification of Common Putative Drug Targets among the Pathogens of Bacterial Meningitis

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Abstract

Monitoring infectious emerging diseases, especially the central nervous system infections, has become one of the important priorities in health care system. Epidemiological, serological and bacteriological studies revealed that *Streptococcus pneumonia, Neisseria meningitidis, Haemophilus influenzae* type b and *Staphylococcus aureus* are common pathogens of bacterial meningitis. Therefore, identification of common drug targets in these pathogens would be crucial to overcome drug resistance to existing antibiotic therapy. In the present study, comparative proteome analysis, subtractive genomic approach and metabolic pathway analysis were implemented to propose common potential drug targets for pathogens of bacterial meningitis. *Streptococcus pneumonia* was selected as reference organism, and the common proteins of the pathogens were verified for essentiality in pathogen's survival, using Database of Essential Genes (DEG). The 213 essential proteins identified were screened for human non-homology. Thirty seven unique essential proteins which are non-homologues to human were proposed as common potential drug targets for pathogens of bacterial meningitis. Pathway analysis revealed that 26 drug targets were enzymes, eight were non-enzymes, and three were conserved hypothetical proteins. Six enzymes were involved in pathways unique to the pathogens of bacterial meningitis. Furthermore, prediction of sub cellular localization and drug prioritization of 37 proteins affirmed that the drug targets would be useful in design and discovery of novel therapeutic compounds against bacterial meningitis.

Keywords: Bacterial Meningitis; Common Drug Targets; BLASTP; DEG; KEGG; Sub Cellular Localization; Putative Drug Targets

Introduction

Meningitis is an immediate effect of bacteria, virus, and fungi infection [1], or due to other microorganisms in the subarachnoid space, able to cause an inflammatory reaction in the dura, pia and arachnoid, as well as Cerebrospinal fluid (CSF) [1,2]. The infectious agents enter into any part of the space, spread rapidly, and cause meningitis. Infection also reaches the ventricles, either directly from choroid plexuses or by reflux through the foramina of Magendie and Luschka. Bacterial meningitis is much more serious and can cause severe disease that can result in brain damage, and even death [3]. Meningitis caused by pathogens other than bacteria are relatively mild and clears up within a week without specific treatment [1]. Hence, bacterial meningitis is the most critical form of the disease and requires special attention for designing therapeutic agents.

Causative organisms of bacterial meningitis differ with age groups of humans. *Streptococcus pneumonia, Neisseria meningitidis, Haemophilus influenza* type b, *Streptococcus agalactiae* (group B Streptococci), *Escherichia coli, Listeria monocytogenes* and *Staphylococcus aureus* are common in infants suffering with bacterial meningitis. In elderly individuals, *Streptococcus pneumonia, Neisseria meningitidis, Haemophilus influenza, Streptococcus aureus*, coagulase-negative staphylococci, aerobic Gram-negative bacilli, *Pseudomonas aeruginosa,* and *Propionibacterium acnes* are causative of bacterial meningitis. The pathogens causing bacterial meningitis in infants and adults are *Streptococcus pneumonia, Neisseria meningitidis, Haemophilus influenza, Staphylococcus aureus, etc.* [4,5]. Ten years retrospective study in south India [6], and from patient records of Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati (Rayalaseema region Andhra Pradesh, India) also reported that these organisms are common pathogens of bacterial meningitis [7].

The clinical significance of bacterial meningitis includes headache, fever, neck stiffness, nausea, vomiting, myalgia, photophobia, cerebral dysfunction manifested with confusion, delirium, ischemia, increased Intracranial Pressure (ICP), and declining consciousness ranging from lethargy to coma [8]. Available antibiotic therapy results in poor outcome in treatment of bacterial meningitis. The selection of antibiotic treatment mainly depends on local resistance pattern, clinical significance in conjunction with allergies, sensitivity to penicillin, vancomycin, and resistance to drugs like ofloxacin, cotrimxazole, cefrotaxime, ceftriaxone and trtracycline [6]. The poor outcome and drug resistance of existing drug molecules necessitate implementation of alternative strategy for designing drug molecules against bacterial meningitis.

Whole genome sequences of *Streptococcus pneumonia, Neisseria meningitides, Haemophilus influenza,* and *Streptococcus aureus* are available in the Institute of Genomic Research Comprehensive Microbial Research (TIGR CMR). Whole genome sequencing technology provides expansive information for the identification of new therapeutic targets in pathogens. Comparative genomics is a large scale, holistic approach that compares two or more genomes of pathogens to discover the similarities [9]. Comparative studies can be performed at different levels of the genomes to obtain multiple perspectives about the organisms. Subtractive genomic approach is an extremely informative

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technique to identify the potential targets, which are expected to be essential for pathogen, but absent in host [10].

Comparative and subtractive genomic approaches, based on the strategy that the proteins encoded by essential genes of pathogen and non-homologous to the host can be used as drug targets [11-12]. Such an approach had been effectively used to identify drug targets in bacterial species such as *Pseudomonas aeruginosa* [13,14], *Helicobacter pylori* [10], *Mycobacterium tuberculosis* [15], *Burkholderia pseudomallei* [16] *Aeromonas hydrophila* [17] *and Leptospira interrogans* [12]. In the present study, a similar approach had been carried out to identify the common potential drug targets against bacterial meningitis. Furthermore, the predicted drug targets were validated through metabolic pathway analysis, subcellular localization and druggability.

Materials and Methods

Comparative analysis

Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae and *Staphylococcus aureus* are four common species causing bacterial meningitis in all age groups of human [4,5]. *Streptococcus pneumonia* was selected as reference organism, as it is the most predominant pathogen of bacterial meningitis in south India [6,18]. A similar report was also observed in Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati, of Rayalaseema region [7]. Multi-genome comparative analysis was applied to the four bacterial meningitis pathogens to identify 250 common proteins, and a dataset was created [7].

Identification of essential and non-human homologous proteins

The dataset was analyzed for essentiality by DEG (Database of Essential Genes), with cut off for e-value 10^{-10} and bit score>100 [12,19]. The obtained essential proteins were screened for non-homolog to human using NCBI BLASTP [20], with threshold expectation value>10^{-3} and bit score<100 [12].

Metabolic pathway analysis

KAAS (KEGG Automatic Annotation Server) server provides functional annotation of proteins by BLAST comparisons against the manually curated KEGG genes database. The result contains KEGG Orthology (KO) assignments and automatically generated KEGG pathways [21,22]. Comparative metabolic pathway analysis for the identified drug targets of pathogens of bacterial meningitis with human metabolic pathway was performed using KAAS to find unique pathways of the pathogens, as well as to trace the role of drug targets in various metabolic pathways.

Functional classification of hypothetical proteins

Support vector machine (SVMProt) is specific for classification of proteins into functional family from its primary sequence [23]. Scoring of SVMprot classification of proteins had been estimated by reliability index, and its usefulness has been demonstrated by statistical analysis. Functionality of the three hypothetical proteins were predicted using SVMProt [11,24,25].

Prediction of subcellular localization

Sub cellular localization of proteins could be used to obtain information about their potential functions. Sub cellular localization of the drug targets were carried out by PSORTb [26], and the results obtained were further validated with CELLO v2.5 [27].

Evaluating druggability of the targets

The Drug Bank (http://www.drugbank.ca) database is a distinctive bioinformatics and cheminformatics resource that combines detailed drug data (chemical, pharmacological and pharmaceutical) with comprehensive drug target information (sequence, structure and pathway). The database contains 6796 drug entries, including 1437 FDA-approved small molecule drugs, 134 FDA-approved biotech (protein/peptide) drugs, 83 nutraceuticals, and 5174 experimental drugs. Additionally, 4285 non-redundant protein (drug target/enzyme/transporter/carrier) sequences are linked to these drug entries [28,29]. Drug ability of the predicted 37 drug targets were further checked using Drug Bank.

Results and Discussion

World health organization (WHO) and Centers for Disease Control and Prevention (CDC) reported that infectious diseases were the second leading cause of death worldwide. Bacterial meningitis being among the top ten causes of deaths related to infectious disease worldwide, and even survivors left suffer with permanent neurological sequelae [30]. Increasing emergence of antibiotic resistant pathogens is one of the biggest challenges for biomedical research and drug development [19]. Traditional drug discovery methods are time consuming, expensive, and often yield few drug targets [31]. The availability of complete genome sequences of several pathogenic microorganisms have been of enormous assistance in this endeavour. Combination of genome information with bioinformatics methods aims to reduce the problem of searching for potential drug targets from a large list in selecting drugs against pathogenic microorganisms [19]. Developments in bioinformatics have brought the development of integrated databases, algorithms, tools, comparison of genomes, and prediction of gene product function which paved way for development of antimicrobial agents and vaccines through rational drug design [12].

Identification of putative drug targets

Essential genes are indispensable to support cellular life, as they are necessary for survival, replication, and viability of the pathogen. Deletion, interruption or blocking of the protein expressed by an essential gene results in death of the organism, making them attractive targets for drug discovery [32]. Essential genes are conserved across bacterial genera, and have been proposed as promising candidates for broad spectrum drug targets, active against multiple bacterial species [32]. Therefore, identifying proteins essential for survival of bacteria causing meningitis and non homologous to host, could be proposed as novel drug targets. In the earlier work, 250 common proteins from pathogens of bacterial meningitis were reported [7]. Analysis of database of essential genes (DEG) analysis revealed that among the 250 common proteins; 213 proteins were vital for survival of the pathogens of bacterial meningitis, out of which 37 were non homologous to human (Table 1). These 37 proteins were considered as common putative drug targets for the pathogens of bacterial meningitis (Figure 1).

Metabolic pathway analysis

Metabolic pathway analysis of 37 drug targets revealed that 26 were enzymes, eight were non enzymes, and three were conserved hypothetical proteins. Six enzymes were involved in pathways unique to the pathogens and were involved in peptidoglycan biosynthesis, two-complement system, methane metabolism, phosphotransferase system and bacterial secretion system. Other target enzymes were found to be involved in important metabolic pathways like amino acid, carbohydrate, energy, lipid, nucleotide, cofactors, vitamins and genetic

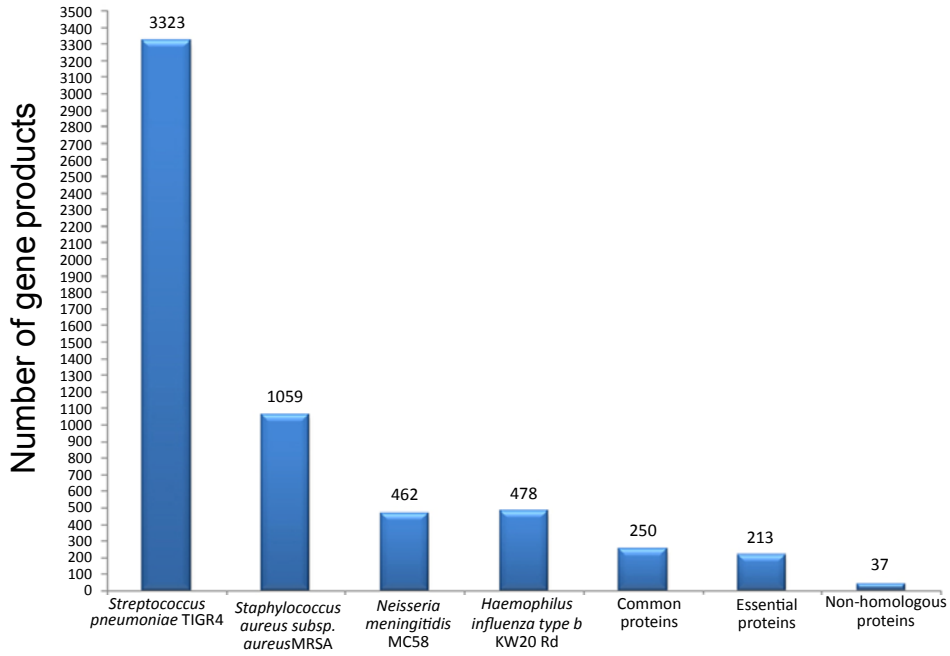

Figure 1: Summary of drug target identification. The plot detailed gene products of *Streptococcus pneumonia, Staphylococcus aureus, Neisseria meningitidis* and *Haemophilus influenzae* type b, 250 common proteins, 213 essential proteins and 37 drug targets against bacterial meningitis.

information processing. The eight non enzymes were involved in vital process, such as replication, transcription, translation, repair, cellular processes of cell growth, death, etc.

Enzymes as a drug targets

Enzymes unique to pathogens: Among the 37 drug targets, six enzymes were involved in unique pathways of the pathogens. Penicillin binding protein 1A (ponA) (S.No. 5 in table 1), one of the common drug target in pathogens of bacterial meningitis, is involved in peptidoglycan biosynthesis which is unique to pathogens. Hence, selecting ponA as a potential target for designing inhibitors, would dissolve the structural integrity, flexibility and rigidity of the cell wall, and expose the pathogens to osmolysis in several pathogens. The existing literatures strengthen potentiality of ponA as common drug target against bacterial meningitis [33-37].

DNA-binding response regulator (rr03) (S.No 6 in table 1) belongs to two-component system. It serves as a basic stimulus-response coupling mechanism, to allow organisms to sense and respond to changes in many different environmental conditions [38]. These are sophisticated signaling systems, marked and integrated into a wide variety of cellular signaling protein like histidine kinase. The protein rr03 was reported as drug target in *Mycobacterium tuberculosis* [39-40].

Phosphoenolpyruvate protein phosphotransferase (ptsI) (S.No. 24 in table 1) involved in phosphotransferase system plays a major role in uptake of carbohydrates, particularly hexoses and disaccharides. Phosphate acetyltransferase (pta) (S.No. 21 in table 1), and acetate kinase (ackA) (S.No. 34 in table 1) belong to methane metabolism, which play central role in the conversion of complex organic matter to methane by carbon cycle. Preprotein translocase (SecA) (S.No. 29 in table 1) subunit involves in bacterial secretion system, and is necessary for virulence and survival against the host immune response [41,42]. Phosphotransferase, methane metabolism and bacterial secretion system are unique to pathogens. The metabolic pathways are critical for growth and survival of the organisms in extreme conditions. Therefore, proteins from the phosphotransferase, methane metabolism, and bacterial secretion system pathways would be of significant interest as potential drug targets in different bacterial pathogens like *Clostridium acetobutylicum* [43], *Staphylococcus aureus* [44], *Escherichia coli* [45], *Clostridium perfringens* [46] and *Borrelia burgdorferi* [37]. Hence, these proteins would become efficient common drug targets against bacterial meningitis.

Enzymes involved in common pathway: Amino acid metabolic pathway is an important pathway in identifying putative drug targets through computer aided drug discovery [44]. SdhA, metE and mtf (S.No. 1, 10 and 18 in table 1) were observed in amino acid metabolism, and were identified as novel drug target in common pathogens of bacterial meningitis. Mtf was identified as a drug target in number of bacterial pathogens of human; metE catalyzes the direct transfer of a methyl group from methyltetrahydrofolate to l-homocysteine to form methionin [47]. Drug target 6, 7-dimethyl-8-ribityllumazine synthase (ribH) (S.No. 2 in table 1) belongs to riboflavin metabolism [48,12]. Riboflavin is the central component of the cofactors of flavin adenine dinucleotide (FAD) and Flavin mononucleotide (FMN), and is therefore, required by all flavoproteins. As such, riboflavin is required for a wide variety of cellular processes and plays a key role in energy, fat and carbohydrate metabolism, hence was a good drug target for bacterial meningitis.

Homologous recombination pathway is essential for cell division in bacteria. It is observed in holliday junction DNA helicase (ruvA) (S.No. 3 in table 1), putative holliday junction resolves (ruvX) (S.No. 4 in table 1), and single stranded DNA specific exonuclease (recJ) (S.No. 11 in table 1) had been implanted in many of these repair pathways [49]. RecJ produces ssDNA tails, which are required to initiate recombination from a double-stranded break [50]. RuvA and recJ also act as exonuclease that mediates the excision step during mismatch repair [51]. RuvX was identified as a novel drug target in *Escherichia coli* [52].

S.No	CMR ID	KO number	Protein name	Gene name	E.C Number	Metabolic pathways	Subcellular localization	Structure	Drug prioritization
1	SP0105	K01752	L-serine dehydratase, iron-sulfur-dependent, alpha subunit	sdhA	4.3.1.17	Glycine, serine, threonine cysteine and methionine metabolism	Cytoplasmic membrane	No	No
2	SP0175	K00794	6,7-dimethyl-8-ribityllumazine synthase	ribH	2.5.1.78	Riboflavin metabolism	Cytoplasmic	No	Yes
3	SP0179	K03550	Holliday junction DNA helicase RuvA	ruvA	3.6.4.12	Homologous recombination	Cytoplasmic	No	No
4	SP0193	K07447	Putative Holliday junction resolvase	ruvX	3.1.-.-	No pathway	Cytoplasmic	No	No
5	SP0369	K05366	Penicillin-binding protein 1A	ponA	2.4.1.-	Peptidoglycan biosynthesis[a]			
6	SP0387	K11618	DNA-binding response regulator	rr03	-	Two-component system[a]	Cytoplasmic	No	Yes
7	SP0408	K03310	Sodium: alanine symporter family protein	-	-	No pathway	Membrane	No	No
8	SP0417	K00648	3-oxoacyl-(acyl-carrier-protein) synthase III	fabH	2.3.1.180	Fatty acid biosynthesis	Cytoplasmic	No	Yes
9	SP0424	K02372	(3R)-hydroxymyristoyl-(acyl-carrier-protein) dehydratase	fabZ	4.2.1.-	Fatty acid biosynthesis	Cytoplasmic	No	Yes
10	SP0585	K00549	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	metE	2.1.1.14	Cysteine and methionine metabolism, Selenocompound metabolism	Cytoplasmic	No	Yes
11	SP0611	K07462	Single-stranded-DNA-specific exonuclease RecJ	recJ	3.1.-.-	Base excision repair, mismatch repair, homologous recombination	Cytoplasmic	No	No
12	SP0775	K02959	Ribosomal protein S16	rpsP	-	Ribosome	Cytoplasmic	No	Yes
13	SP0851	K08591	Glycerol-3-phosphate acyltransferase	plsY	2.3.1.15	Glycerolipid and glycerophospholipid metabolism	Membrane	No	No
14	SP0878	K03466	DNA translocase	ftsK	-	No pathway	Membrane	No	No
15	SP0895	K02337	DNA polymerase III, alpha subunit	dnaE	2.7.7.7	Purine metabolism, pyrimidine metabolism, DNA replication, Mismatch repair, Homologous recombination	Cytoplasmic	No	No
16	SP0936	K02341	DNA polymerase III, delta prime subunit	holB	2.7.7.7	Purine metabolism, pyrimidine metabolism, DNA replication, Mismatch repair, Homologous recombination	Cytoplasmic	No	Yes
17	SP0938	K07056	Tetrapyrrole methylase family protein	rsmI	2.1.1.198	No pathway	Cytoplasmic	No	No
18	SP0991	K01243	5-methylthioadenosine/S-adenosylhomocysteine nucleosidase	mtf	3.2.2.9	Cysteine and methionine metabolism	Cytoplasmic	Yes	Yes
19	SP1072	K02316	DNA primase	dnaG	2.7.7.-	DNA replication	Cytoplasmic	No	No
20	SP1073	K03086	RNA polymerase sigma-70 factor	rpoD	-	No pathway	Cytoplasmic	No	Yes
21	SP1100	K00625	Phosphate acetyltransferase	pta	2.3.1.8	Taurine and hypotaurine metabolism, Pyruvate metabolism, Propanoate metabolism, Methane metabolism[a]	Cytoplasmic	No	Yes
22	SP1105	K02888	Ribosomal protein L21	rplU	-	Ribosome	Cytoplasmic,		
23	SP1113	K03530	DNA-binding protein HU	hup	-	No pathway	Cytoplasmic	No	No
24	SP1176	K08483	Phosphoenolpyruvate-protein phosphotransferase	ptsI	2.7.3.9	Phosphotransferase system[a]	Cytoplasmic	No	Yes
25	SP1285	K03501	Glucose-inhibited division protein B	rsmG	2.1.1.170	No pathway	Cytoplasmic	No	No
26	SP1412	K13292	Prolipoprotein diacylglyceryl transferase	lgt	2.-.-.-	No pathway	Cytoplasmic membrane	No	No
27	SP1429	K08303	Peptidase, U32 family		3.4.-.-	Epithelial cell signaling in Helicobacter pylori infection	Cytoplasmic	No	No
28	SP1517	K03624	Transcription elongation factor	greA	-	No pathway	Cytoplasmic	No	No
29	SP1702	K03070	Preprotein translocase, SecA subunit	secA1	-	Bacterial secretion systema	Cytoplasmic	No	Yes
30	SP1748	K07574	Conserved hypothetical protein		-	No pathway	Cytoplasmic	No	No
31	SP1777	K09457	Conserved hypothetical protein	queF	1.7.1.13	No pathway	Cytoplasmic	No	Yes
32	SP1910	K06878	Conserved hypothetical protein		-	No pathway	Cytoplasmic	No	No
33	SP2007	K02601	Transcription antitermination protein	nusG	-	No pathway	Cytoplasmic	No	No
34	SP2044	K00925	Acetate kinase	ackA	2.7.2.1	Taurine and hypotaurine metabolism, Pyruvate metabolism, Propanoate metabolism, Methane metabolism[a]	Cytoplasmic	No	No
35	SP2097	K00674	Putative 2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase	dapH	2.3.1.117	Lysine biosynthesis	Cytoplasmic	No	Yes
36	SP2126	K01687	Dihydroxy-acid dehydratase	ilvD	4.2.1.9	Valine, leucine and isoleucine biosynthesis, pantothenate and CoA biosynthesis	Cytoplasmic	No	No
37	SP2203	K02314	Replicative DNA helicase	dnaC	3.6.4.12	DNA replication	Membrane	No	No

[a]Represents targets from unique pathways of bacterial meningitis pathogens
CMR ID: Comprehensive Microbial Resource, KO ID: KEGG orthology, EC no: Enzyme Commission number.

Table1: Putative drug targets of common bacterial meningitis.

The DNA primase (dnaG) (S.No. 19 in table 1), replicative DNA helicase (dnaC) (S.No. 37 in table 1), DNA polymerase III alpha subunit (dnaE) (S.No. 15 in table 1), DNA translocase (ftsK) (S.No. 14 in table 1), and DNA polymerase III delta subunit (holB) (S.No. 16 in table 1) are critical intermediates in many recombination-dependent DNA repair and replication pathways [53]. The 3-oxoacyl-(acyl-carrier protein) synthase III (fabH) (S.No. 8 in table 1) and (3R)-hydroxymyristoyl-(acyl-carrier protein) dehydratase (fabZ) (S.No. 9 in table 1) catalyze the enzymatic reactions in fatty acid synthesis [12,54,55].

The glycerol-3-phosphate acyltransferase (plsY) (S.No. 13 in table 1) belongs to glycerolipid and glycerophospholipid biosynthesis. It shares two key enzymes, glycerol-3-phosphate acyltransferase and 1-acylglycerol-3-phosphate acyltransferase. Pathway of glycerolipid formation starts by converting glycerone phosphate (glycolysis intermediate) into glycerol-3-phosphate, followed by a number of enzymatic conversions to diacylglycerol. Glycerophospholipid biosynthesis is essential for forming numerous constituents of the bacterial cell wall [56,57].

Tetrapyrrole methylase family protein (rsmI) (S.No. 17 in table 1) catalyses the methylation of substrates S-adenosyl-L-methionine and tetrapyrroles, which are large macrocyclic compounds derived from biosynthetic pathways of cobalamin (vitamin B12), haem, sirohaem, chlorophyll, coenzyme F430, phytochromobilin. RsmI protein identified as drug target in *Clostridium perfringens* [44]. The prolipoprotein diacylglyceryl transferase (lgt) (S.No. 26 in table 1) catalyzes lipid modification reaction by transferring the diacylglyceryl group from phosphatidylglycerol to the sulfhydryl group of the invariant cysteine residue in the lipobox of prolipoproteins [58], and this modification is a prerequisite for the subsequent cleavage of the prolipoprotein signal peptide by prolipoprotein signal peptidase [59]. The prolipoprotein diacylglyceryl transferase (lgt) was also identified as a drug target in *Escherichia coli* [60] and *Salmonella typhimurium* [61].

Peptidase U32 (S.No. 27 in table 1) plays a vital role in pathogenicity of *Streptococcus mutans* and Group B *Streptococcus* (GBS) strains. The putative 2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase (dapH) (S.No. 35 in table 1) belongs to lysine biosynthesis pathway and yields the *de novo* synthesis of lysine for illustration of peptidoglycan synthesis in bacteria [62-64], and also reported as drug target in *Salmonella typhi* [65]. The dihydroxy-acid dehydratase enzyme (ilvD) (S.No. 36 in table 1) belongs to the family of lyases; it catalyzes two similar dehydration reactions that convert 2, 3-dihydroxy-isovalerate into 2-keto-isovalerate, and 2, 3-dihydroxy-3-methylvalerate into 2-keto-3-methyl-valerate, which is a crucial drug target in E. coli and it carries out the penultimate step in biosynthesis pathways of isoleucine, leucine, lysine and valine [66].

Non-enzymes as drug targets: Eight non-enzymes (Sodium: alanine symporter family protein, DNA-binding protein HU, Transcription elongation factor, transcription anti-termination protein, Glucose-inhibited division protein B, tetrapyrrole methylase family protein, Ribosomal protein S16 and Ribosomal protein L21) were identified as drug targets. These drug targets were distinguished in cell signaling, cellular processes (Sodium: alanine symporter family protein (S.No. 7 in table 1)), replication, DNA repair (DNA-binding protein HU (S.No. 23 in table 1)), transcription (Transcription elongation factor (S.No. 28 in table 1), transcription anti-termination protein (S.No. 33 in table 1), translation (Glucose-inhibited division protein B (S.No. 25 in table 1)), and tetrapyrrole methylase family protein (S.No. 17 in table 1) ribosomal synthesis (Ribosomal protein S16 (S.No.12 in table 1) and ribosomal protein L21 (S.No. 22 in table 1).

Functional classification of putative uncharacterized proteins

The functional family of four hypothetical conserved proteins (S.No. 30, 31 and 32 in table 1) belong to protein families of zinc binding, DNA-binding, iron-binding, lipid-binding, metal-binding and transmembrane proteins (Supplementary material, table 1).

Prediction of subcellular localization

Computational prediction of subcellular localization provides a quick and inexpensive means for gaining insight into protein function, verifying experimental results, annotating newly sequenced bacterial genomes, and detecting potential cell surface/secreted drug targets. Among the 37 drug targets, 30 were cytoplasmic, six were membrane, and one was extracellular from PSORTb [27]. Similar results were also observed for 37 drug targets using CELLO v2.5 [26].

Druggable target prioritization

Fifteen common potential drug targets were found to be highly similar to the target proteins in Drug Bank (Table 1). Further, the common drug targets were explored for presence of 3D structures and were explored for the structure based drug designing (SBDD), to propose novel inhibitor molecules against bacterial meningitis.

Conclusion

Despite improvements in technology, treatments and understanding of how bacterial meningitis develops, the disease remains a potentially life-threatening emergency, capable of causing significant morbidity and mortality. *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae* type b and *Staphylococcus aureus* are common pathogens of bacterial meningitis of all age groups. *Streptococcus pneumonia* was selected as reference organism, as it is the most predominant pathogen of the bacterial meningitis. The large scale genome sequencing projects have increased the availability of completely sequenced genomic and proteomic data in public domain. In the present study, systematic processes of comparative analysis, subtractive genomic approaches and metabolic pathway analysis were defined for the identification of novel therapeutic drug targets against common pathogens of bacterial meningitis. Thirty seven common putative drug targets were successful in listing out as novel targets for the pathogens. The inhibitors designed against the targets will be specific to the pathogens, and therefore not toxic to the host. Identified targets were further characterized and verified for their role in the survival of the bacteria. Homology modeling of these targets will help to identify the best possible sites that can be targeted for drug design. Virtual screening of the novel targets might be useful in the discovery of novel therapeutic compounds against common pathogens of bacterial meningitis.

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