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, cefotaxime, 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
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 pneumoniae* 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
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 (ptsl) (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 (recX) (S.No. 11 in table 1) had been implanted in many of these repair pathways [49]. RecX produces ssDNA tails, which are required to initiate recombination from a double-stranded break [50]. RuvA and recX 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].

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**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.
| 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            | No       | No               |
| 2    | SP0175 | K00794     | 6,7-dimethyl-8-ribiliumazine synthase | ribH       | 2.5.1.78   | Riboflavin metabolism | Cytoplasmic            | No       | Yes              |
| 3    | SP0179 | K03550     | Holiday junction DNA helicase RuvA | ruvA       | 3.6.4.12   | Homologous recombination | Cytoplasmic            | No       | No               |
| 4    | SP0193 | K07447     | Putative Holiday junction resolvase | ruvX       | 3.1.-      | No pathway        | Cytoplasmic            | No       | No               |
| 5    | SP0369 | K05366     | Penicillin-binding protein 1A | ponA       | 2.4.1.-    | Peptidoglycan biosynthesis | Cytoplasmic            | No       | No               |
| 6    | SP0387 | K11618     | DNA-binding response regulator | rpoD       | -          | Two-component system | 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-methyltetrahydroleptoprotoglutamate-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 | pisY       | 2.3.1.15   | Glycerolipid and glycerophospholipid metabolism | Membrane               | No       | Yes              |
| 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 | rsml       | 2.1.1.196  | No pathway        | Cytoplasmic            | No       | No               |
| 18   | SP0991 | K01243     | 5-methyladenosine/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     | Phosphatase acetyltransferase | pta        | 2.3.1.8    | Taurine and hypotaurine metabolism, Pyruvate metabolism, Propanoate metabolism, Methane metabolism | Cytoplasmic            | No       | Yes              |
| 22   | SP1105 | K02888     | Ribosomal protein L21 | rplU       | -          | Ribosome          | Cytoplasmic            | No       | Yes              |
| 23   | SP1113 | K03530     | DNA-binding protein HU | hup        | -          | No pathway        | Cytoplasmic            | No       | No               |
| 24   | SP1176 | K08483     | Phosphonopyruvate-protein phosphotransferase | psl        | 2.7.3.9    | Phosphotransferase system | Cytoplasmic            | No       | No               |
| 25   | SP1285 | K03501     | Glucose-inhibited division protein B | rsmG       | 2.1.1.170  | No pathway        | Cytoplasmic            | No       | No               |
| 26   | SP1412 | K13292     | Prolipoprotein diacylglycerol transferase | lgt        | 2.-.-      | No pathway        | Cytoplasmic            | 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       | No               |
| 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       | No               |
| 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 | Cytoplasmic            | No       | Yes              |
| 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, isoleucine and leucine biosynthesis, pantothenate and CoA biosynthesis | Cytoplasmic            | No       | No               |
| 37   | SP2203 | K02314     | Replicative DNA helicase | dnaC       | 3.6.4.12   | DNA replication | Membrane               | No       | No               |

*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 II alpha subunit (dnaE) (S.No. 15 in table 1), DNA translocase (HsK) (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 methylations 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 biosynthetic pathways of cobalamin (vitamin B12), haem, sirohaem, tetrapyrroles, which are large macrocyclic compounds derived from enzymatic conversions to diacylglycerol. Glycerophospholipid biosynthesis is essential for forming numerous constituents of the bacterial cell wall [56,57].

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-tetrahydropridine-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 typhimurium [61].

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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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References

1. Tanve S, Wilson BC, Tuchin VV, Matthews D (2008) Biophotonics. Advances in Ginsberg L (2004) Difficult and recurrent meningitis. J Neurol Neurosurg Psychiatry 75: i6-i21.
2. Pérez-Llorens X, McCracken GH Jr (2003) Bacterial meningitis in children. Lancet 361: 2139-2148.
3. Tunkel AR, Hartman BJ, Kaplan SL, Kaufman BA, Roos KL, et al. (2004) Practice guidelines for the management of bacterial meningitis. Clin Infect Dis 39: 1267-1284.
4. Schiech WF 3rd, Ward JI, Band JD, Hightower A, Fraser DW, et al. (1985)
Bacterial meningitis in the United States, 1978 through 1981. The National Bacterial Meningitis Surveillance Study. JAMA 253: 1749-1754.

5. Narasinga RB, Mahdi KI, Mohamed SN, Mohamed EBS (1998) Etiology and occurrence of acute bacterial meningitis in children in Benghazi, Libyan Arab Jamahiriya. Eastern Mediterranean Health Journal. 4: 50-57.

6. Mani R, Pradhan S, Nagarathna S, Wasuilla R, Chandramuki A (2007) Bacteriological profile of community acquired acute bacterial meningitis: a ten-year retrospective study in a tertiary neurocare centre in South India. Indian J Med Microbiol 25: 108-114.

7. Munikumar M, Vani Priyadarshini I, Dibyabhabha P, Umamaheswari A, Vengamma B (2012) Computational approaches to identify common subunit vaccine candidates against bacterial meningitis. Interdisciplinary Sciences: Computational Life Sciences. (In press).

8. Misra UK, Kailita J, Prabhakar S, Chakravarty A, Kochar D, et al. (2012) Endoscopic third ventriculostomy in tuberculous meningitis needs more evidence. Ann Indian Acad Neurol 15: 233.

9. Wei L, Liu Y, Dubuchak I, Shon J, Park J (2002) Comparative genomics approaches to study organism similarities and differences. J Biomed Inform 35: 142-150.

10. Dutta A, Singh SK, Ghosh P, Mukherjee R, Mitter S, et al. (2006) In silico identification of potential therapeutic targets in the human pathogen Helicobacter pylori. In Silico Biol 6: 43-47.

11. http://en.searchcommons.org/59527897.

12. Amineni U, Pradhan D, Marisetty H (2010) In silico identification of common putative drug targets in Leptospira interrogans. J Chem Biol 3: 165-173.

13. Sakharkar KR, Sakharkar MK, Chow VT (2004) A novel genomics approach for the identification of drug targets in pathogens, with special reference to Pseudomonas aeruginosa. In Silico Biol 4: 355-360.

14. Perumal D, Lim CS, Sakharkar KR, Sakharkar MK (2007) Differential genome analyses of metabolic enzymes in Pseudomonas aeruginosa for drug target identification. In Silico Biol 7: 453-465.

15. Anishetty S, Pulim M, Pennathur G (2005) Potential drug targets in Mycobacterium tuberculosis through metabolic pathway analysis. Comput Biol Chem 29: 368-378.

16. Chong CE, Lim BS, Nathan S, Mohamed R (2006) In silico analysis of BHK-21 wildtype pseudomonal genome sequence for potential drug targets. In Silico Biol 6: 331-338.

17. Sharma V, Gupta P, Dixit A (2008) In silico identification of putative drug targets from different metabolic pathways of Aeromonas hydrophilia. In Silico Biol 8: 331-338.

18. Bharathi MJ, Ramakrishnan R, Vasu S, Meenakshi R, Shivkumar C, et al. (2003) Epidemiology of bacterial keratitis in a referral centre in south India. Indian J Med Microbiol 21: 239-245.

19. Butt AM, Tahir S, Nasrullah I, Idrees M, Lu J, et al. (2012) Mycoplasma genitalium: a comparative genomics study of metabolic pathways for the identification of drug and vaccine targets. Infect Genet Evol 12: 53-62.

20. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389-3402.

21. Kanehisa M, Araki M, Goto S, Hattori M, Hirakawa M, et al. (2008) KEGG for integrated genomic and chemical biology. Nucleic Acids Res 36: D428-D430.

22. Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M (2007) KAAS: an automatic genome annotation and pathway reconstruction server. Nucleic Acids Res 35: W182-W185.

23. Cai CZ, Han ZL, Chen X, Chen YZ (2003) SVMProt: Web-based support vector machine software for functional classification of a protein from its primary sequence. Nucleic Acids Research 31: 3992-3997.

24. Narayan SA, Rakesh A, Qamar R, Nidhi T (2009) Subtractive genomics approach for in silico identification and characterization of novel drug targets in Neisseria meningitidis segroup B. J Comput Sci Syst Biol 2: 255-258.

25. Umamaheswari A, Kumar MM, Pradhan D, Marisetty H (2011) Docking studies towards exploring antiviral compounds against envelope protein of yellow fever virus. Interdiscip Sci 3: 64-77.

26. Yu NY, Wagner JR, Laird MR, Melli G, Rey S, et al. (2010) PSORTb 3.0: improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. Bioinformatics 26: 1608-1615.

27. Yu CS, Chen YG, Lu CH, Hwang JK (2006) Prediction of protein subcellular localization. Proteins 64: 643-651.

28. Knox C, Law V, Jewison T, Liu P, Ly S, et al. (2011) DrugBank 3.0: a comprehensive resource for ‘omics’ research on drugs. Nucleic Acids Res 39: D1035-D1041.

29. Holman AG, Davis PJ, Foster JM, Carlow CK, Kumar S (2009) Computational prediction of essential genes in an unculturable endosymbiotic bacterium, Wolbachia of Brugia malayi. BMC Microbiol 9: 243.

30. van Sorge NM, Doran KS (2012) Defense at the border: the blood-brain barrier versus bacterial foreigner. Future Microbiol 7: 383-384.

31. Abadio AK, Kloshima ES, Teixeira MM, Martins NF, Maigret B, et al. (2011) Comparative genomics allowed the identification of drug targets against human fungal pathogens. BMC Genomics 12: 76.

32. Bakheet TM, Doig AJ (2009) Properties and identification of human protein drug targets. Bioinformatics 25: 451-457.

33. Ropp PA, Hu M, Olesky P, Nicholas RA (2002) Mutations in ponA, the gene encoding penicillin-binding protein 1, and a novel locus, penC, are required for high-level chromosomally mediated penicillin resistance in Neisseria gonorrhoeae. Antimicrob Agents and Chemother 46: 769-777.

34. Scheffers DJ, Errington J (2004) PBP1 is a component of the Bacillus subtilis cell division machinery. J Bacteriol 186: 5153-5156.

35. Pereira SF, Henriques AO, Pinho MG, de Lancastre H, Tomasz A (2009) Evidence for a dual role of PBP1 in the cell division and cell separation of Staphylococcus aureus. Mol Microbiol 72: 895-904.

36. Arbeloa A, Segal H, Hugonnet JE, Josseaume N, Dubost L, et al. (2004) Role of class A penicillin-binding proteins in PBP5-mediated beta-lactam resistance in Enterococcus faecalis. J Bacteriol 186: 1221-1228.

37. Madagi S, Patil VM, Sadegh S, Singh AK, Garwal B, et al. (2011) Identification of membrane associated drug targets in Borrelia burgdorferi ZST- subtractive genomics approach. Bioinformation 6: 356-359.

38. Cai XH, Zhang Q, Shi SY, Ding DF (2005) Searching for potential drug targets in two-component and phosphorylase signal-transduction systems using three-dimensional cluster analysis. Acta Biochim Biophys Sin (Shanghai) 37: 293-302.

39. Converse PJ, Karakousis PC, Klinkenberg LG, Kessavan AK, Ly LH, et al. (2009) Role of the dosR-dosS two-component regulatory system in Mycobacterium tuberculosis virulence in three animal models. Infect Immun 77: 1230-1237.

40. Li Y, Zeng J, Zhang H, He ZG (2010) The characterization of conserved binding motifs and potential target genes for M. tuberculosis MtrAB reveals a link between the two-component system and the drug resistance of M. smegmatis. BMC Microbiol 10: 242.

41. Boynton ZL, Bennett GN, Rudolph FB (1996) Cloning, sequencing, and expression of clustered genes encoding b-hydroxybutyryl- co-enzyme A (CoA) dehydrogenase, crotonase, and butyryl-CoA dehydrogenase from Clostridium acetobutylicum ATCC 824. J Bacteriol 178: 3015-3024.

42. Morya VK, Dewaker V, Mecarty SD, Singh R (2010) In silico Analysis of metabolic pathways for identification of putative Drug targets for Staphylococcus aureus. J Comput Sci Syst Biol 3: 62-69.

43. Wickner W, Leonard MR (1996) Escherichia coli preprotein translocase. J Biol Chem 271: 29514-29516.

44. Chhabra G, Sharma P, Anant A, Deshmukh S, Kaushik H, et al. (2010) Identification and modeling of a drug target for Clostridium perfringens SM101. Bioinformation 4: 278-289.

45. Coombes BK, Valdez Y, Finlay BB (2004) Evasive maneuvers by secreted bacterial proteins to avoid innate immune responses. Curr Biol 14: R856-R867.

46. Finlay BB, McDaid (2005) Anti-immunology: evasion of the host immune response. Ann Indian Acad Acad Neurol 15: 233.

47. Yu NY, Wagner JR, Laird MR, Melli G, Rey S, et al. (2010) PSORTb 3.0: improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. Bioinformatics 26: 1608-1615.
48. Zylberman V, Klinke S, Haase I, Bacher A, Fischer M, et al. (2006) Evolution of vitamin B2 biosynthesis: 6,7-dimethyl-8-ribityllumazine synthases of Brucella. J Bacteriol 188: 6135-6142.

49. Wakahamaa T, Nakagawa N, Kuramitsu S, Masui R (2008) Structural basis for different substrate specificities of two ADP-ribose pyrophosphatases from Thermus thermophilus HB8. J Bacteriol 190: 1108-1117.

50. Haneda N, Morimoto K, Lovett ST, Kowalczykowski SC (2009) Reconstitution of initial steps of dsDNA break repair by the RecF pathway of E. coli. Genes Dev 23: 1234-1245.

51. Viswanathan M, Burdett V, Baille C, Modrich P, Lovett ST (2001) Redundant exonuclease involvement in Escherichia coli methyl-directed mismatch repair. J Biol Chem 276: 31053-31058.

52. Rideout MC, Boldt JL, Vahi-Ferguson G, Salamon P, Netzl A, et al. (2011) Potent antimicrobial small molecules screened as inhibitors of tyrosine recombinases and Holliday junction-resolving enzymes. Mol Divers 15: 989-1005.

53. Jasilionis A, Kaupinis A, Ger M, Vaisis M, Chitavichius D, et al. (2012) Gene expression and activity analysis of the first thermophilic U32 peptidase. Cent Eur J Biol 7: 587-595.

54. Mitsakos V, Dobson RC, Pearce FG, Devenish SR, Evans GL, et al. (2008) Inhibiting dihydrodipicolinate synthase across species: towards specificity for pathogens? Bioorg Med Chem 16: 842-844.

55. Voss JE, Scally SW, Taylor NL, Atkinson SC, Griffin MD, et al. (2010) Structure and evolution of a novel dimeric enzyme from a clinically important bacterial pathogen. J Biol Chem 285: 5188-5195.

56. Rathib B, Sarangi AN, Trivedi N (2009) Genome subtraction for novel target definition in Salmonella typhi. Bioinformation 4: 143-150.

57. Hyduke DR, Jarboe LR, Tran LM, Chou KJ, Liao JC (2007) Integrated network analysis identifies nitric oxide response networks and dihydroxyacid dehydratase as a crucial target in Escherichia coli. Proc Natl Acad Sci U S A 104: 8484-8489.