IN SILICO IDENTIFICATION OF NOVEL DRUG TARGETS IN ACINETOBACTER BAUMANNII BY SUBTRACTIVE GENOMIC APPROACH

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ABSTRACT

Objective: Multiple drug resistance (MDR) in bacteria, particularly Gram-negative bacilli, has significantly hindered the treatment of infections caused by these bacteria. This results in the need for identifying new drugs and drug targets for these bacteria. The objective of this study was to identify novel drug targets in Acinetobacter baumannii which has emerged as a medically important pathogen due to an increasing number of infections caused by it and its MDR property.

Methods: In our study, we implemented in silico subtractive genomics approach to identify novel drug targets in A. baumannii American type culture collection 17978. Various databases and online software were used to build a systematic workflow involving comparative genomics, metabolic pathways analysis, and drug target prioritization to identify pathogen-specific novel drug targets.

Results: First, 458 essential proteins were retrieved from a database of essential genes, and by performing BLASTp against Homo sapiens, 246 human non-homologous essential proteins were selected of 458 proteins. Metabolic pathway analysis performed by Kyoto Encyclopedia of Genes and Genomes–Kyoto Automatic Annotation Server revealed that these 246 essential non-homologous proteins were involved in 66 metabolic pathways. Among these metabolic pathways, 12 pathways were found to be unique to Acinetobacter that involved 37 non-homologous essential proteins. Of these essential non-homologous proteins, 19 proteins were found in common as well as unique metabolic pathways and only 18 proteins were unique to Acinetobacter. Finally, these target proteins were filtered to 9 potential targets, based on subcellular localization and assessment of druggability using Drugbank, ChEMBL, and literature.

Conclusion: Our study identified nine potential drug targets which are novel targets in A. baumannii and can be used for designing drugs against these proteins. These drugs will be pathogen specific with no side effects on human host, as the potential drug targets are human non-homologous.

Keywords: Acinetobacter baumannii, Multiple drug resistance, Essential proteins, Metabolic pathway analysis, Druggability, Novel drug target.

INTRODUCTION

Some strains of bacteria are resistant to almost all available antibiotics, such as Acinetobacter baumannii, which is the focus of the present study. Antimicrobial resistance has been identified as one of the most important problems facing human health [1]. The most common and serious multiple drug-resistant (MDR) pathogens have been assigned within the acronym "ESKAPE," standing for Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa, and Enterobacter spp. [2]. Treatment of infections due to these bacteria has become difficult, resulting in increased morbidity and mortality [3,4]. A. baumannii is one of these most problematic MDR species [5]. Therefore, the identification of new drug targets in A. baumannii is urgently needed.

A. baumannii is a Gram-negative bacillus that is aerobic, pleomorphic, and non-motile. It is an opportunistic pathogen which mainly affects immunocompromised individuals, particularly those who have experienced a prolonged (>90 day) hospital stay [6]. The respiratory tract, blood, pleural fluid, urinary tract, surgical wounds, central nervous system, skin, and eyes are sites for infection or colonization [7,8]. The types of infections caused by this pathogen include pneumonia, bacteremia, endocarditis, skin and soft tissue infections, urinary tract infections, and meningitis.

OmpA, a member of the outer membrane proteins (OMPs), contributes significantly to the disease-causing potential of A. baumannii [9]. OmpA, being the most abundant surface protein on the pathogen, is also involved in the formation of biofilms [10,11]. The ability to form biofilms allows it to grow persistently in unfavorable conditions and environments. Phospholipase D and C also contribute to virulence potential of A. baumannii [12,13].

A. baumannii is able to acquire antibiotic resistance mechanisms which allow this organism to persist in hospital environments and facilitated the global emergence of MDR strains. The rapid emergence of multi- and pandrug-resistant strains of Acinetobacter highlights the organism’s ability to quickly acclimatize to selective changes in environmental pressures. The three fundamental mechanisms of antimicrobial resistance are (1) enzymatic degradation of antibacterial drugs, (2) alteration of bacterial proteins that are antimicrobial targets, and (3) changes in membrane permeability to antibiotics. In recent years, it has been designated as a “red alert” human pathogen, arising largely from its extensive antibiotic resistance spectrum [14].

In the present post-genomics era, the possibilities of selecting targets using computational approaches with integrated “omics” data, such as genomics, proteomics, and metabolomics have been increasing continuously. In silico methods like comparative and subtractive genomics are being widely used for the prediction and identification of potential drug targets in numerous pathogenic bacteria [15]. This technique relies on comparisons between the genomic sequences of the pathogen with the host to include the protein-coding genes sequences that are (a) absent in the host (non-homologous) and (b) indispensable for pathogen survival [16,17]. In the present study, a computational
comparative metabolic pathway analysis of host *Homo sapiens* and *A. baumannii* has been carried out to identify potential novel drug targets.

**METHODS**

The systematic identification and characterization of the potential drug targets of *A. baumannii* American type culture collection 17978 was done sequentially by the following methods.

Retrieval of essential proteins of *A. baumannii*

At first, according to the database of essential genes (DEG) [18], 458 essential proteins of *A. baumannii* were retrieved from NCBI in FASTA format.

Identification of non-human homologous essential proteins in *A. baumannii*

To identify human non-homologous essential proteins of *A. baumannii*, these 458 essential proteins were subjected to BlastP at NCBI server against *H. sapiens* with default parameters. Proteins having identity ≤38% and e>0.005 were considered as non-homologous proteins.

Metabolic pathway analysis

The human non-homologous essential proteins of *A. baumannii* obtained through BlastP were then subjected to metabolic pathway analysis, which was done by Kyoto Automatic Annotation Server (Kyoto Encyclopedia of Genes and Genomes [KEGG] automatic annotation server) [19] server at KEGG [20]

Unique pathway identification

After this, unique metabolic pathways of Acinetobacter were identified through the manual comparison of metabolic pathways of both *Acinetobacter* and *H. sapiens* using KEGG Database.

Subcellular localization

Subcellular localization of metabolic proteins (essential non-human homologous protein involved only in unique pathways) of *A. baumannii* was done by PSORTb [21] to identify the cellular localization of these putative therapeutic targets.

Drug target prioritization

Drug targets were prioritized by following three approaches:

**Drug bank**

Druggability of potential drug targets of *A. baumannii* was identified by sequence similarity to targets of the Food and Drug Administration (FDA) approved and small drug molecule by utilizing the Drug Bank [22].

ChEMBL

Druggability of potential drug targets of *A. baumannii* was identified by sequence similarity to targets of small drug molecule by utilizing the ChEMBL [23]. The default parameters for BLASTp were used to line up the potential drug targets from *A. baumannii* against the list of protein targets of compounds found within the Drug Bank and ChEMBL.

**LITERATURE**

Druggability of potential drug targets was also assessed based on information in the literature [24-32] about their efficacy as drug targets in other organisms.

**RESULTS AND DISCUSSION**

The present study was aimed to identify novel drug targets in *A. baumannii*. We followed subtractive genomic approach (Fig. 1) to identify the good therapeutic target proteins which are essential for bacterial survival but cannot be found in the host.

**Identification of non-homologous essential proteins**

Druggability of putative therapeutic targets

Druggability of each of the non-homologous essential proteins of *A. baumannii* was identified by sequence similarity to targets of small drug molecule by utilizing ChEMBL Literature.
| Essential non-homologous proteins | Involvement in pathways | Common pathway |
|-----------------------------------|-------------------------|----------------|
| **KO entry**                      | **Definition**          | **Unique pathway** | **Common pathway** |
| K01623                            | Fructose-bisphosphate aldolase, Class I | ko00680-Methane metabolism | ko00010-Glycolysis/gluconeogenesis ko00030-Pentose phosphate pathway ko00051-Fructose and mannose metabolism ko00710-Carbon fixation in photosynthetic organisms ko1200-Carbon metabolism ko1230-Biosynthesis of amino acids ko0010-Glycolysis/gluconeogenesis ko1230-Biosynthesis of amino acids ko00260-Glycine, serine, and threonine metabolism ko01200-Carbon metabolism ko00620-Pyruvate metabolism ko00720-Carbon fixation pathway in prokaryotes ko01200-Carbon metabolism |
| K15633                            | 2,3-Bisphosphoglycerate-independent phosphoglycerate mutase | ko00680-Methane metabolism | ko00010-Glycolysis/gluconeogenesis ko00030-Pentose phosphate pathway ko00051-Fructose and mannose metabolism ko00710-Carbon fixation in photosynthetic organisms ko1200-Carbon metabolism ko1230-Biosynthesis of amino acids ko0010-Glycolysis/gluconeogenesis ko1230-Biosynthesis of amino acids ko00260-Glycine, serine, and threonine metabolism ko01200-Carbon metabolism ko00620-Pyruvate metabolism ko00720-Carbon fixation pathway in prokaryotes ko01200-Carbon metabolism |
| K01007                            | Pyruvate, water dikinase | ko00680-Methane metabolism | ko00010-Glycolysis/gluconeogenesis ko00030-Pentose phosphate pathway ko00051-Fructose and mannose metabolism ko00710-Carbon fixation in photosynthetic organisms ko1200-Carbon metabolism ko1230-Biosynthesis of amino acids ko0010-Glycolysis/gluconeogenesis ko1230-Biosynthesis of amino acids ko00260-Glycine, serine, and threonine metabolism ko01200-Carbon metabolism ko00620-Pyruvate metabolism ko00720-Carbon fixation pathway in prokaryotes ko01200-Carbon metabolism |
| K00677                            | UDP-N-acetylglucosamine acyltransferase | ko00540-Lipopolysaccharide biosynthesis | ko01503-(cAMP) resistance ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis |
| K02535                            | UDP-3-O-[3-hydroxyxylristoyl] N-acetylglucosamine deacetylase | ko00540-Lipopolysaccharide biosynthesis |ko01503-(cAMP) resistance ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis |
| K02536                            | UDP-3-O-[3-hydroxyxylristoyl] glucosamine N-acetyltransferase | ko00540-Lipopolysaccharide biosynthesis | ko01503-(cAMP) resistance ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis |
| K03269                            | UDP-2,3-diacetylglucosamine hydrolase | ko00540-Lipopolysaccharide biosynthesis | ko01503-(cAMP) resistance ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis |
| K00748                            | Lipid-A-disaccharide synthase | ko00540-Lipopolysaccharide biosynthesis | ko01503-(cAMP) resistance ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis |
| K00912                            | Tetraacyldisaccharide 4'-kinase | ko00540-Lipopolysaccharide biosynthesis | ko01503-(cAMP) resistance ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis |
| K02527                            | 3-Deoxy-D-manno-octulosonic-acid transferase | ko00540-Lipopolysaccharide biosynthesis | ko01503-(cAMP) resistance ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis |
| K00979                            | 3-Deoxy-manno-octulosonate cytidylyltransferase | ko00540-Lipopolysaccharide biosynthesis | ko01503-(cAMP) resistance ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis |
| K03270                            | 3-Deoxy-D-manno-octulosonate 8-phosphate phosphatase | ko00540-Lipopolysaccharide biosynthesis | ko01503-(cAMP) resistance ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis |
| K06041                            | Arabinose-5-phosphate isomerase | ko00540-Lipopolysaccharide biosynthesis | ko01503-(cAMP) resistance ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis ko00540-Lipopolysaccharide biosynthesis |
| K01921                            | D-alanine-D-alanine ligase | ko00550-Peptidoglycan synthesis | ko01502-Vancomycin resistance ko00471-D glutamine and D glutamate metabolism ko00471-D glutamine and D glutamate metabolism ko00300-Lysine biosynthesis |
| K00790                            | UDP-N-acetylglucosamine 1-carboxyvinyltransferase | ko00550-Peptidoglycan synthesis | ko01502-Vancomycin resistance ko00471-D glutamine and D glutamate metabolism ko00471-D glutamine and D glutamate metabolism ko00300-Lysine biosynthesis |
| K00075                            | UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase | ko00550-Peptidoglycan synthesis | ko01502-Vancomycin resistance ko00471-D glutamine and D glutamate metabolism ko00471-D glutamine and D glutamate metabolism ko00300-Lysine biosynthesis |
| K00075                            | UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase | ko00550-Peptidoglycan synthesis | ko01502-Vancomycin resistance ko00471-D glutamine and D glutamate metabolism ko00471-D glutamine and D glutamate metabolism ko00300-Lysine biosynthesis |
| K00075                            | UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase | ko00550-Peptidoglycan synthesis | ko01502-Vancomycin resistance ko00471-D glutamine and D glutamate metabolism ko00471-D glutamine and D glutamate metabolism ko00300-Lysine biosynthesis |
| K00075                            | UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase | ko00550-Peptidoglycan synthesis | ko01502-Vancomycin resistance ko00471-D glutamine and D glutamate metabolism ko00471-D glutamine and D glutamate metabolism ko00300-Lysine biosynthesis |
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small molecule drugs by utilizing the Drug Bank database, ChEMBL, literature. This led to the identification of nine A. baumannii proteins that were highly similar to the binding partners of FDA approved and small experimental molecule drugs (Table 3), and these can act as potential novel drug targets.

The above listed nine potential druggable targets are involved in seven metabolic pathways (two-component system, D-alanine metabolism, lipopolysaccharide biosynthesis, geraniol degradation, beta-lactam resistance, vancomycin resistance, and peptidoglycan synthesis pathways) and their potential as novel drug targets is discussed as follows:

D-alanine ligase
D-alanine ligase is involved in 3 bacterial pathways, i.e., peptidoglycan synthesis, D-alanine metabolism, and vancomycin resistance. Due to involvement in multiple pathways, it is a very good target for drug discovery. This enzyme is ubiquitous among prokaryotes and is absent in eukaryotes making this a logical target for the development of antibiotics. This enzyme has been used as target for many drugs against infectious bacteria. Bruning et al. have used this enzyme as target for drug D-cycloserine in Mycobacterium tuberculosis [24].

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Table 1: (Continued)

| Essential non-homologous proteins | Involvement in pathways |
|-----------------------------------|-------------------------|
| K03507 Cell division protein Ftsz | ko00550-Peptidoglycan synthesis |
| K13779 Isohexenylglutaconyl-CoA hydratase | ko01501-Beta-lactam resistance |
| K00928 Aspartate kinase | ko00281-Geraniol degradation |
| K00133 Aspartate-semialdehyde dehydrogenase | ko00261-Monobactam biosynthesis |
| K00215 4-Hydroxy-tetrahydrodipicolinate reductase | ko00261-Monobactam biosynthesis |
| K03072 Preprotein translocase subunit SecD | ko003070-Bacterial secretion system |
| K03076 Preprotein translocase subunit SecF | ko003070-Bacterial secretion system |
| K03073 Preprotein translocase subunit SecE | ko02024-Quorum sensing |
| K03210 Preprotein translocase subunit YajC | ko003070-Bacterial secretion system |
| K03217 YidC/Oxa1 family membrane protein insertase | ko02024-Quorum sensing |
| K07659 Two-component system, OmpR family, phosphate regulon response regulator OmpR | ko003070-Bacterial secretion system |
| K07638 Two-component system, OmpR family, osmolarity sensor histidine kinase EnvZ | ko02020-Two-component system |
| K01497 GTP Cyclohydrolase II | ko02024-Quorum sensing |
| K03100 Signal peptidase I | ko02024-Quorum sensing |

K0: Guanosine triphosphate, cAMP: Cationic antimicrobial peptide

Table 2: Subcellular localization of proteins that are involved in only unique metabolic pathways

| KO entry | DEG No. | Subcellular localization | Whether druggable |
|----------|---------|--------------------------|-------------------|
| K01921   | DEG10430441 | Cytoplasmic | Yes |
| K00677   | DEG10430227 | Cytoplasmic | No |
| K02397   | DEG10430473 | Cytoplasmic | Yes |
| K02536   | DEG10430230 | Cytoplasmic | No |
| K00748   | DEG10430208 | Cytoplasmic | No |
| K00912   | DEG10430193 | Cytoplasmic | No |
| K02527   | DEG10430432 | Cytoplasmic | No |
| K00979   | DEG10430194 | Cytoplasmic | No |
| K03270   | DEG10430173 | Cytoplasmic | Yes |
| K02397   | DEG10430172 | Cytoplasmic | Yes |
| K03269   | DEG10430248 | Cytoplasmic | No |
| K01100   | DEG10430418 | Inner membrane | Yes |
| K02563   | DEG10430443 | Inner membrane | Yes |
| K03980   | DEG10430010 | Inner membrane | No |
| K03587   | DEG10430438 | Cytoplasmic | Yes |
| K13779   | DEG10430368 | Cytoplasmic | Yes |
| K07638   | DEG10430428 | Inner membrane | Yes |
| K07659   | DEG10430427 | Cytoplasmic | Yes |
This enzyme has also been used as drug target by other researchers [25,26,33,34].

**Osmolarity sensor histidine kinase EnvZ and phosphate regulon response regulator**

These proteins are involved in two-component system and belong to OmpR family. Two-component regulatory systems enable bacteria to detect physical or chemical changes and are mediators of signal transduction. This pathway is essential for the survival of bacteria and any disruption in this pathway leads to bacterial cell death. Two-component system pathway has been targeted by many inhibitors in different bacteria [35-40], and therefore, it can be very potent drug target in Acinetobacter too.

**Cell division protein FtsZ**

This protein is involved in 2 important metabolic pathways of *A. baumannii*, namely, peptidoglycan synthesis and beta-lactam resistance pathways. Beta-lactam resistance pathway codes for beta-lactamases which play role in degradation of beta-lactam antibiotics and makes them inactive. Drugs targeting this protein can inactivate beta-lactam resistance pathway and hence making the pathogen sensitive to beta-lactam antibiotics. Furthermore, disruption of peptidoglycan synthesis leads to bacterial cell death. Hence, this protein is an effective drug target as it is involved in two crucial pathways of the bacterial pathogen. This is supported by many wet laboratory studies where drugs have been used against FtsZ [41-46]. Sun et al. [47] used berberine-based FtsZ inhibitors with broad-spectrum antibacterial activity.

**Arabinose-5-phosphate isomerase and UDP-3-O-[3-hydroxymyristoyl]-N-acetylglucosamine deacetylase**

These proteins are involved in lipopolysaccharide synthesis. Lipopolysaccharides (e.g., lipid A) are essential constituents of bacterial endotoxin. Chemical inhibitors which can disrupt lipid A biosynthesis have the potential to act as antimicrobial agents. Lipid A biosynthesis occurs on the cytosolic surface of the inner membrane and is catalyzed by 10 unique enzymes. Arabinose-5-phosphate isomerase catalyzes first step in the synthesis of lipopolysaccharide and has been used as a drug target in many pathogens [27,48-50]. UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase catalyzes the hydrolysis of UDP-3-O-myrstyl-N-acetylglucosamine to form UDP-3-O-myrstoylglucosamine and acetate, the committed step in lipid A biosynthesis, and has been exploited as a drug target by various workers [31,32]. Inhibition of lipopolysaccharide biosynthesis, leading to a truncated lipopolysaccharide molecule, is a strategy for antibacterial drug development in which vital cellular structure is weakened [51].

**Table 3: Proteins highly similar to the targets of FDA approved and small experimental molecule drugs**

| DEG Number | KO entry | Drug | Organism | Source |
|------------|----------|------|----------|--------|
| DEG10430441 | K01921 | d-Cycloderine | *M. tuberculosis* | Bruning et al. [24] |
| DEG10430441 | - | Diazenedi carboxamides phosphonic acid | *E. coli* | Kovac et al. [25] |
| DEG10430172 | K06041 | 4-Phosphoerthryroinic acid | *P. aeruginosa, S. faecalis* | Lacoste et al. [26] |
| DEG10430418 | K01000 | Amphinomyein Mureidomycin A | *B. megaterium* | Woodruff and Wolfenden [27] |
| DEG10430438 | K03587 | S'-Guanosine-diphosphate-monothiophosphate and citric acid | *E. coli* | Tanaka et al. [28] |
| DEG10430428 | K07638 | Thiopropylene | *P. aeruginosa* | ChEMBL |
| DEG10430437 | K02535 | DPA | - | Drug bank |
| DEG10430427 | K07659 | Ethylene diaminetetraacetic acid | - | Drug bank |
| DEG10430443 | K02563 | - | Zheng et al. [31] |
| DEG10430368 | K13779 | Quercetin | ChEMBL |

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**AUTHOR CONTRIBUTIONS**

Meenu Goyal designed the overall methodology, revised manuscript and supervised the carried out work. Citu carried out the work and helped in preparing the first draft of manuscript. Nidhi Singh assisted in designing methodology, conducting data analysis and participated in critically reviewing the manuscript.

**CONCLUSION**

As resistant to all available antibiotics is reported in most of Gram-negative bacteria, especially in *A. baumannii*. Hence, there is a need to develop antibiotics against new drug targets. Our study found nine potential druggable drugs that are novel drug targets in *A. baumannii* and can be used for designing drugs against them. All of these have the potential to be used as drug targets as these are involved in crucial metabolic pathways of the pathogen and have been targeted successfully in other organisms. The drug would be specific for the pathogen and would not be lethal to the host as subtractive genomic approach applied in this case which includes human non-homologous proteins only. Molecular modeling of the targets will help in drug discovery by in silico methods. Virtual screening against these novel drug targets might be useful in the discovery of potential therapeutic agents against *A. baumannii* and can help in dealing with MDR.
CONFLICT OF INTERESTS

The authors declare no conflicts of interest in this work.

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