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Research Article

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 Drug bank, 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.

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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.

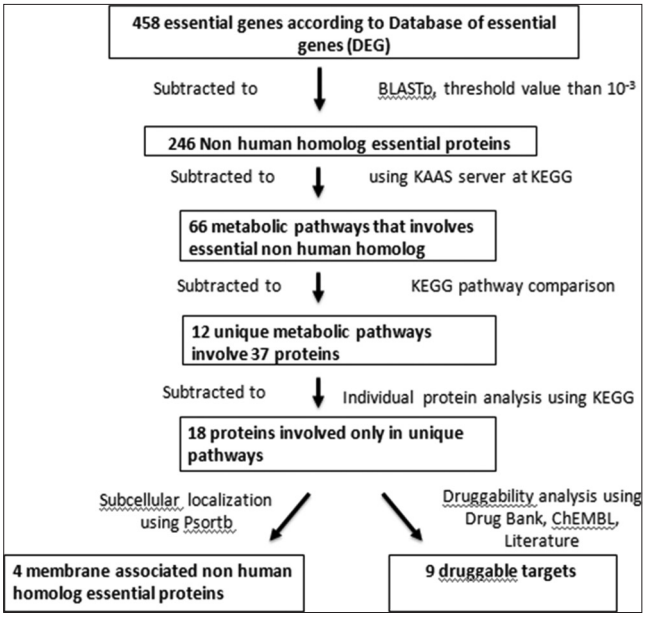

Fig. 1: A schematic representation of identification of novel drug targets in *Acinetobacter baumannii*

Identification of non-homologous essential proteins

At first, essential proteins of *A. baumanni* were retrieved from DEG database. To avoid any cross-reactivity of established drug with human host, 458 essential proteins were subjected to BLASTp against *H. sapiens*. Based on the result of BLASTp, 246 non-human homologous proteins were selected having identity ≤38% and e value cutoff >0.005.

Identification of essential non-homologous proteins involved in unique metabolic pathways

Metabolic pathway analysis of these 246 non-homologous proteins showed the involvement of these proteins in 66 pathways. Pathway comparison of *A. baumannii* and *H. sapiens* revealed 12 metabolic pathways that were unique to *A. baumannii,* and rest 54 pathways were common in pathogen and host. These 12 pathways involved 37 essential non-homologous proteins of *A. baumannii.*

These 37 essential non-homologous proteins were individually studied using KEGG database (Table 1). Of these 37 proteins, 18 proteins were found to be involved exclusively in unique metabolic pathways, while rest 19 proteins were involved in multiple unique pathways as well as in some common pathways between *H. sapiens* and *A. baumannii*. These 19 proteins were playing role in multiple pathways. Along with the involvement in unique pathways in unique pathways of pathogen, these 19 proteins were also involved in some pathways common between pathogen and host. Therefore, these were not considered for further analysis.

Subcellular localization

Until now, we have discussed about non-homologous essential proteins of the pathogen using computational comparative and subtractive genomics study. Although this is the major criterion in determining drug target from a pathogen, several other factors are taken into consideration for suitability of drug and vaccine targets. *In silico* prediction of subcellular localization provides a quick and inexpensive means for obtaining information regarding protein function. Cytoplasmic or membrane localization of the targets determines the ease of purification steps to be followed in experimental studies. In our study, of 18 essential protein targets, 4 are membrane proteins and rest fall into the category of cytoplasmic proteins, as predicted by PSORTb (Table 2).

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

Table 1: Essential non-homologous proteins involved in 12 unique metabolic pathways

Essential non-homologous proteins		Involvement in pathways	
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 ko01200-Carbon metabolism ko1230-Biosynthesis of amino acids
K15633	2,3-Bisphosphoglycerate-independent phosphoglycerate mutase	ko00680-Methane metabolism	ko00010-Glycolysis/gluconeogenesis ko1230-Biosynthesis of amino acids ko00260-Glycine, serine, and threonine metabolism ko01200-Carbon metabolism
K01007	Pyruvate, water dikinase	ko00680-Methane metabolism	ko00620-Pyruvate metabolism ko00720-Carbon fixation pathway in prokaryotes ko01200-Carbon metabolism
K00677	UDP-N-acetylglucosamine acyltransferase	ko00540-Lipopolysaccharide biosynthesis ko01503-(cAMP) resistance	
K02535	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase	ko00540-Lipopolysaccharide biosynthesis	
K02536	UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase	ko00540-Lipopolysaccharide biosynthesis	
K03269	UDP-2,3-diacylglucosamine hydrolase	ko00540-Lipopolysaccharide biosynthesis	
K00748	Lipid-A-disaccharide synthase	ko00540-Lipopolysaccharide biosynthesis	
K00912	Tetraacyldisaccharide 4'-kinase	ko00540-Lipopolysaccharide biosynthesis	
K02527	3-Deoxy-D-manno-octulosonic-acid transferase	ko00540-Lipopolysaccharide biosynthesis	
K00979	3-Deoxy-manno-octulosonate cytidylyltransferase	ko00540-Lipopolysaccharide biosynthesis	
K03270	3-Deoxy-D-manno-octulosonate 8-phosphate phosphatase	ko00540-Lipopolysaccharide biosynthesis	
K06041	Arabinose-5-phosphate isomerase	ko00540-Lipopolysaccharide biosynthesis	
K01921	D-alanine-D-alanine ligase	ko00550-Peptidoglycan synthesis ko01502-Vancomycin resistance ko00473-D-alanine metabolism	
K00790	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	ko00550-Peptidoglycan synthesis	ko00520-Amino sugar and nucleotide sugar metabolism
K00075	UDP-N-acetylmuramate dehydrogenase	ko00550-Peptidoglycan synthesis	ko00520-Amino sugar and nucleotide sugar metabolism
K01924	UDP-N-acetylmuramate--alanine ligase	ko00550-Peptidoglycan synthesis	ko00471-D glutamine and D glutamate metabolism
K01925	UDP-N-acetylmuramoylalanine--D-glutamate ligase	ko00550-Peptidoglycan synthesis	ko00471-D glutamine and D glutamate metabolism
K01928	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase	ko00550-Peptidoglycan synthesis	ko00300-Lysine biosynthesis
K01929	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase	ko00550-Peptidoglycan synthesis ko01502-Vancomycin resistance	ko00300-Lysine biosynthesis
K01000	Phospho-N-acetylmuramoyl-pentapeptide-transferase	ko00550-Peptidoglycan synthesis ko01502-Vancomycin resistance	
K02563	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase	ko00550-Peptidoglycan synthesis ko01502-Vancomycin resistance	
K03980	Putative peptidoglycan Lipid II flippase	ko00550-Peptidoglycan synthesis	

(Contd...)

Table 1: ***(Continued)***

Essential non-homologous proteins		Involvement in pathways	
K03587	Cell division protein Ftsz	ko00550-Peptidoglycan synthesis ko01501-Beta-lactam resistance	
K13779	Isohexenylglutaconyl-CoA hydratase	ko00281-Geraniol degradation	
K00928	Aspartate kinase	ko00261-Monobactam biosynthesis	ko001210-2-Oxocarboxylic acid metabolism ko00300-Lysine biosynthesis ko1230-Biosynthesis of amino acids ko00260-Glycine, serine and threonine metabolism ko00270-Cysteine and methionine metabolism
K00133	Aspartate-semialdehyde dehydrogenase	ko00261-Monobactam biosynthesis	ko001210-2-Oxocarboxylic acid metabolism ko00300-Lysine biosynthesis ko1230-Biosynthesis of amino acids ko00260-Glycine, serine and threonine metabolism ko00270-Cysteine and methionine metabolism
K00215	4-Hydroxy-tetrahydrodipicolinate reductase	ko00261-Monobactam biosynthesis	ko00300-Lysine biosynthesis ko1230-Biosynthesis of amino acids
K03072	Preprotein translocase subunit SecD	ko03070-Bacterial secretion system	ko03060-Protein export
K03076	Preprotein translocase subunit SecY	ko03070-Bacterial secretion system ko02024-Quorum sensing	ko03060-Protein export
K03073	Preprotein translocase subunit SecE	ko03070-Bacterial secretion system ko02024-Quorum sensing	ko03060-Protein export
K03210	Preprotein translocase subunit YajC	ko03070-Bacterial secretion system ko02024-Quorum sensing	ko03060-Protein export
K03217	YidC/Oxa1 family membrane protein insertase	ko03070-Bacterial secretion system ko02024-Quorum sensing	ko03060-Protein export
K07659	Two-component system, OmpR family, phosphate regulon response regulator OmpR	ko02020-Two-component system	
K07638	Two-component system, OmpR family, osmolarity sensor histidine kinase EnvZ	ko02020-Two-component system	
K01497	GTP Cyclohydrolase II	ko02024-Quorum sensing	ko00740-Riboflavin metabolism
K03100	Signal peptidase I	ko02024-Quorum sensing	ko03060-Protein export

GTP: 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
K02535	DEG10430437	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	No
K06041	DEG10430172	Cytoplasmic	Yes
K03269	DEG10430248	Cytoplasmic	No
K01000	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

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].

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-Cycloserine	*M. tuberculosis*	Bruning *et al*. [24]
		Diazenedi carboxamides phosphinic acid	*E. coli*	Kovac *et al.* [25]
		Aminoethylphosphonic acid	*P. aeruginosa, S. faecalis*	Lacoste *et al*. [26]
DEG10430172	K06041	4-Phosphoerythronic acid	-	Woodruff and Wolfenden [27]
DEG10430418	K01000	Amphomycin Mureidomycin A	*B. megaterium*	Tanaka *et al*. [28]
		Tunicamycin Liposidomycin B	*E. coli*	Brandish *et al.* [29]
DEG10430438	K03587	5'-Guanosine-diphosphate-monothiophosphate and citric acid	-	Drug bank
		-	*P. aeruginosa*	ChEMBL
DEG10430428	K07638	Thienopyridine	-	Gilmour *et al*. [30]
		Radicicol	-	Drug bank
DEG10430437	K02535	DPA	-	Zhang *et al.* [31]
		Ethylenediaminetetraacetic acid	-	Jackman *et al*. [32]
DEG10430427	K07659	-	*A. platensis*	ChEMBL
		Glycerol	-	Drug bank
DEG10430443	K02563	-	*E. coli*	ChEMBL
DEG10430368	K13779	Quercetin	-	Drug bank

FDA: Food and drug administration, DPA: Dipicolinic acid, *M. tuberculosis: Mycobacterium tuberculosis, E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa, S. faecalis: Streptococcus faecalis, B. megaterium: Bacillus megaterium, A. platensis: Arthrospira platensis*

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-lactamase which plays 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. Furthremore, 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-myristoyl-N-acetylglucosamine to form UDP-3-O-myristoylglucosamine 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].

UDP-N-acetylglucosamine-N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase (MurG) and phospho-N-acetylmuramoyl-pentapeptide-transferase (MraY)

These proteins are involved in peptidoglycan synthesis and vancomycin resistance pathways. The bacterial cell is surrounded by layers of peptidoglycan, a covalently cross-linked polymer matrix composed of peptide-linked β-(1–4)-N-acetyl hexosamine. Peptidoglycan provides mechanical strength to bacteria helping it to survive under varying osmotic pressures. The proteins such as MurG and MraY, involved in peptidoglycan synthesis, can be good targets of inhibitors against bacteria and their role as drug targets has also been reported in previous studies on other organisms [52-55].

The potential drug targets identified in this study have been successfully used as drug targets in other pathogens and hence have the potential to be exploited for designing new antimicrobial agents against *A. baumannii.* Computer-aided drug design approach can further be used to identify hit and lead compounds against these targets [56].

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 proteins 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.

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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.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest in this work.

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