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Construction of a comprehensive protein-protein interaction map for multi drug resistant *klebsiella pneumoniae* to identify drug targets: A computational approach

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Abstract: *Klebsiella pneumoniae* is an encapsulated rod-shaped, Gram-negative microbe that can form biofilm. It is an opportunistic Enterobacter usually involved in nosocomial infection, conferring resistance to almost all antibiotics and hence become therapeutically challenging. In the current study, the Protein Interaction Network (PIN) of MDR *K. pneumoniae* has been identified. The proteins are the building blocks of all organisms. Proteins interact with each other to carry out their physiological functions. The interactions are integrated to form Protein Interaction Network (PIN). The strain DA48896 has been selected as it was isolated from Pakistan and harboring bla-oxa-181, conferring resistance to carbapenem. Total 20,936 high confidence interactions of 3782 proteins have been predicted from the STRING database. The predicted interactions were annotated functionally and mapped on their corresponding pathways. The predicted PIN was verified using semantic similarity between the Gene Ontology. The topological properties were calculated and retrieved topologically significant proteins consisting of 390 proteins. Among them 49 proteins are non-homologous essential that can serve as the potential drug targets. These proteins were further explored for druggability, their association with pathways involved in drug resistance and eventually prioritized as potential drug targets. This study will be helpful to design drug candidates against prioritized proteins.

Keywords: *Klebsiella pneumoniae*; drug targets; protein-protein interactions (PPI); essentiality; genomics; drug resistance

INTRODUCTION

Klebsiella pneumoniae (Kp) is an encapsulated rod-shaped, Gram-negative microbe that can form biofilm. It is generally considered as an opportunistic Enterobacter causing infection in immunocompromised individuals and in hospital setting but studies have shown that hyper virulent strains are capable to infect healthy individuals (Roe, Vazquez, Esposito, Zarrilli, & Sahl, 2019). It is involved in multiple infections ranging from nosocomial infection, urinary tract infection, and bloodstream infection to pyogenic liver abscesses (Lery *et al*., 2014). Kp has shown resistance to nearly all available antibiotics regimen and heading towards pan-drug resistance as its strains are harboring Extended Spectrum β-lactamase (ESBL) and metallo-carbapenemase enzymes. The prevalence rate of these MDR bacteria become high and are of great concern worldwide as it becomes a great reservoir to spread resistance owing to the presence of drug-resistant plasmids and genes (Dunn, Connor, & McNally, 2019; Navon-Venezia, Kondratyeva & Carattoli, 2017). The biofilm formation as well as strong drug resistance mechanism turns this pathogen therapeutically challenging and exert adverse effects on the hospitalization cost and time (Oleksy-Wawrzyniak *et al*., 2022). Convergence between resistant and hyper-virulent strains is also observed which causes more invasive and life-threatening infection (L. Chen & Kreiswirth, 2018; Holt *et al*., 2015). There is a dire need of potential drug candidates as well as novel therapeutics to overcome the burden of infection due to MDR microbes. The hunting of novel drug targets is the most reasonable approach to prioritize potential antimicrobial compounds against the identified drug targets (Peraman *et al*., 2021).

In computational biology, different approaches which include comparative genomics and subtractive genomics are applied to find novel drug targets in which pathogen's proteome is scanned to find unique proteins that are not present in the host and essential for the pathogen (Fatoba, Okpeku, & Adeleke, 2021). However, the chances of development of antimicrobial drug resistance can never be ignored. This rise in resistance demands a systematic study which includes inter and intra Protein-Protein Interaction Network (PPIN) of pathogen that may lead to find possible solutions to overcome the burden of resistance (Kaur *et al*., 2021; Tan *et al*., 2021).

Proteins are the basic unit of all biological systems. Protein interactions are necessary in complex biological framework of cell and to carry out biological function. The PPIs are integrated to form Protein Interaction Network (PIN). The topological properties of proteins in PPIN with their essentiality, functional and pathways knowledge obtained from proteomic and/or genomic data

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mining of all available data resources has become a useful strategy to study a pathogen as system. The PPIN are also used to identify proteins that upon targeting could be lethal for pathogen (Kaur *et al*., 2021; Zahra, Jamil, & Uddin, 2021) . The topological analysis based on graph theory, which includes degree centrality, betweenness centrality, path length and closeness centrality are calculated to identify key proteins that play crucial role in PIN. Studies have shown that proteins tend to serve as 'hub' (proteins having large number of interacting partners) in interaction network are essential for the survival of the pathogen. These proteins are considered as potential drug targets (Folador *et al*., 2016).

In the current study, the drug targets against *Klebsiella pneumoniae* that is heading towards pan-drug resistance have been identified. The strain DA48896 was selected as it is in intermediate stage towards pan drug resistance (Nahid, Zahra, & Sandegren, 2017). The protein interaction map was constructed to identify potential drug targets whose inhibition could be lethal for pathogen. The topologically significant proteins were identified by considering the proteins that share the properties of hub and bottleneck nodes in the network. Further, topologically significant proteins that pass through the filter of essentiality and non-homology were prioritized as potential drug targets. These proteins were further elaborated as prioritized drug targets by considering the proteins that are sharing the pathways of resistant proteins and their druggability by comparing the sequence with already identified drug targets.

MATERIALS AND METHODS

The overall workflow of the current research is summarized in fig. 1.

Retrieval of proteome

In the current study, antimicrobial resistant isolates of *Klebsiella pneumoniae* originated from Pakistan were retrieved using the National Database of Antibiotic Resistant Organisms (NDARO), which is hosted by pathogen detection (https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/), NCBI. This database deals with the antimicrobial resistant genes and genomes that exhibit resistance profile. The strain of DA48896 was selected as it was isolated from Pakistan, having complete genome assembly and it is in intermediary towards pan-drug resistance (Nahid *et al*., 2017). The proteome of DA48896, having accession ID GCF_003006175.1_ASm3006177v1_protein.faa, was downloaded from NCBI reference sequence database (O'Leary *et al*., 2015).The sequences of interacting proteins were downloaded in FASTA format from STRING version 11 database for proteins interaction studies (Szklarczyk *et al*., 2018). The proteome of human host was downloaded from UniProt database (Apweiler *et al*., 2004) to identify non-homologous proteins.

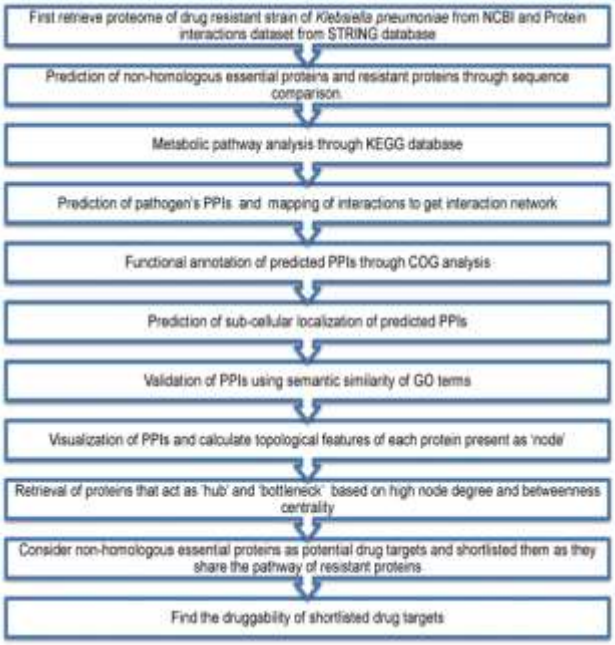

Fig. 1: Schematic representation of the current study showing all the steps of applied protocol

Identification of non-homologous essential proteins

The proteome of DA48896 was subjected to BLASTp (Johnson *et al*., 2008) against the human host to retrieve non-homologous proteins by selecting only those proteins that are absent in the human host. The whole proteome of DA48896 was examined to identify essential genes. For this purpose, the proteome of DA48896 was mapped on experimentally identified essential genes of KPNIH1 strain provided in an extensive study conducted by Ramage et.al. (Ramage *et al*., 2017). The Reciprocal Best Hit (RBH) BLASTp method at *e*-value 0.001 was applied to identify the homologs of experimentally identified essential proteins. In RBH method, the BLAST is run bi-directionally in which the query in first time is served as subject in the second cycle. Moreover, the proteome of DA48896 was subjected to BLASTp against DEG version 15.2 (Database of Essential Genes) (www.essentialgene.org) using *e*-value $1 \times 10^{-05}$to find out essential proteins. The DEG database covers large number of essential genes from 46 bacteria (Luo, Lin, Gao, Zhang, & Zhang, 2013). The RBH method was applied to identify homologs. The proteins obtained from any one source were considered as essential proteins.

Identification of resistant proteins

The CARD (Comprehensive Antibiotic Resistance Database) (Alcock *et al*., 2019) was used to find out the resistant proteins by BLASTp of the proteome of DA48896 against sequences present in CARD at *e*-value $1x10^{-3}$ with percent identity of 35% and query coverage of 70%. The CARD is a biological database that collects 2909 reference antibiotic resistance genes, their gene product and associated phenotype of 85 pathogens.

Metabolic pathway analysis

The KAAS (KEGG automatic annotation server) server(Moriya, Itoh, Okuda, Yoshizawa, & Kanehisa, 2007) executed by the KEGG (Kyoto Encyclopedia of Genes and Genomes) (Kanehisa, Furumichi, Tanabe, Sato, & Morishima, 2016) database was used to assign KO terms and pathway details of DA48896. The KAAS functionally annotates query proteins by bi-directional BLAST against manually curated KEGG genes database and assign KO (KEGG Orthology) terms and the pathways information in the resultant file. The result file was parsed to retrieve the details of pathways in which resistant and essential proteins are involved.

Protein-Protein Interactions studies

The STRING version 11 (Szklarczyk *et al*., 2018) database was used to predict the Protein-Protein Interactions of DA48896. The complete interactome of *Klebsiella pneumoniae* and their respective proteins' FASTA sequences were downloaded from STRING database. In the current study combined score of >=0.7 was used to retrieve the interactions. The proteome of DA48896 was mapped on the interaction network after removing redundancies using Interolog based method. Reciprocal Best hit (RBH) method was applied and the criterion of percent identity 35% and query coverage of 70% were used to obtain the homologs to avoid false positive interactions. Moreover, single protein was assigned as homolog.

Functional annotation of predicted PPIs

Proteins having similar functions tend to interact with each other. For functional analysis eggNOG mapper version 4.5.1(Huerta-Cepas *et al*., 2017) was used. The tool is synchronized with eggNOG database and assigns functional annotation to larger sets of proteins derived from pre-computed functional annotations of proteins based on orthology, as functional conservation is generally assumed among orthologous proteins and phylogenetic profiling available in eggNOG database (Huerta-Cepas *et al*., 2015). The output file includes COG (Clusters of Orthologous Groups) classification, GO-terms (Gene Ontology) and KO terms.

The COGs are classified in to 17 functional categories which also include one category belongs to generalize function and another one of unknown function(Galperin, Makarova, Wolf, & Koonin, 2014). The COG classification is widely applied to know the function of proteins based on their orthology. From COG the functional association of interacting partners were deduced.

Validation of predicted PPIs

The GO-terms provide the controlled vocabulary of genes and their gene products in terms of their molecular function (M.F), biological process (B.P) or cellular component (C.C). To deduce the function and to validate PPIs using GO-terms, semantic similarity score was calculated using GOGO algorithm (Zhao & Wang, 2018) which calculates functional similarity between the two genes. Further, to enhance the significance, the random predictions were made using PERL script provided in reference (Gupta, Srivastava, Osmanoglu, & Dandekar, 2020). The random dataset of PPIs was constructed such that it did not have any similar pair of proteins. The size of random interactions and predicted interactions were same. Semantic similarities of random interactions were also identified. The Wilcoxon Rank SUM test was used to find the function similarity distribution of predicted and random interactions dataset.

Sub-cellular localization prediction of PPIs

The PSORTb version 3.0 (N. Y. Yu *et al*., 2010) has been used to predict the sub-cellular localization (SCL) of the proteins present in interaction network. This tool incorporates several analytical methods like BLAST, SVM, outer membrane, motif and profile analysis using Moddhmm and signal peptide analysis to predict the SCL of archaea and bacteria having diversified cellular morphologies.

Visualization and retrieval of topologically significant proteins

The protein interaction network was visualized using Cytoscape version 3.7.2 (Su, Morris, Demchak, & Bader, 2014). The topological properties were calculated using network analyzer tool. To get the topologically significant proteins, two dataset containing top 20% proteins were constructed based on their node degree (number of interacting partner) refer as hub, betweenness centrality (shortest path between two proteins) refer as bottleneck. The common proteins present in both datasets were extracted and selected to pass the filter of essentiality and non-homology. Meanwhile CytoHubba tool (S.-H. Chen *et al*., 2009), a Cytoscape plugin was also utilized to validate the shortlisted target proteins. Further druggability were checked and their respective pathways were identified to shortlist the targets.

Retrieval of potential drug targets involved in resistance

All the shortlisted topologically significant proteins were analyzed for their involvement in resistance or sharing the pathways of resistant proteins. The proteins were considered as attractive drug targets if they are either resistant proteins or share the pathway of resistant proteins.

Prediction of druggability

The proteins were also scrutinized for their ability to bind drug like molecules. All topologically proteins were checked by sequence comparison with already identified drug targets present in DrugBank Database (Wishart *et al*., 2006). Currently, DrugBank database covered the large dataset and relevant information of about more than

4000 drug targets that are linked to 8261 drugs entities which included FDA approved small molecule drugs and biologics, nutraceutical drugs and experimental drugs.

RESULTS

The NDARO database has 189 clinical strains of *Klebsiella pneumoniae* having AMR (antimicrobial resistance) genotype isolated from Pakistan. Among them only DA48896 has shown complete assembly. The proteome of DA48896 was retrieved from Refseq NCBI. It has 5488 proteins. It is carbapenem resistant strain of *K. pneumoniae* that harbor blaOXA-181 and belongs to st147 pandemic lineage.

Identification of non-homologous essential proteins

Essential proteins are indispensable for the survival of the pathogen. The foremost criteria for any pathogen's protein to serve as potential drug target would be its absence from human host to avoid unwanted complications and its indispensability for the survival of pathogens. Total 822 proteins were shortlisted as essential proteins based on homology with essential proteins of KPNIH1 and from DEG database. Among them, 482 proteins were non-homologous to the human host.

Identification of resistant proteins and their pathways

The 295 proteins were shortlisted as resistant proteins retrieved after comparison of DA48896 proteome with CARD database. The KAAS server executed by KEGG was used to identify pathways of the resistant proteins. It is appeared that mostly proteins are involved in ABC transporter followed by two-component system, beta-lactam resistance, quorum sensing and biofilm formation. The resistant proteins and their respective pathways in which these proteins are involved is provided in Supplementary table S1 and the resistant pathways are summarized in fig. S1.

Identification of protein-protein interactions

The STRING version11 contains the interaction data of 5797 proteins of *K. pneumoniae*, among them 4544 proteins were considered as homolog of DA48896 obtained from BLASTp at *e*-value 0.001 using RBH method. Total 1.4 million redundant interactions were present in STRING database that become half after removing redundancies. In the current study, the proteome of DA48896 was mapped on the high confidence interactions that have combined score of ≥0.7 which also include sixty-seven experimentally proven PPIs of K. pneumoniae. The total number of interactions obtained was 20936 of 3782 proteins. The predicted interactions datas*et al*so included 61 experimentally identified interactions which increased the confidence of obtained PPIs. Among 67 experimentally identified interactions, the six interactions belong to macrolide 2'-phosphotransferase. Since, the homolog of macrolide 2'-phosphotransferase is not present in DA48896, so these interactions were not mapped.

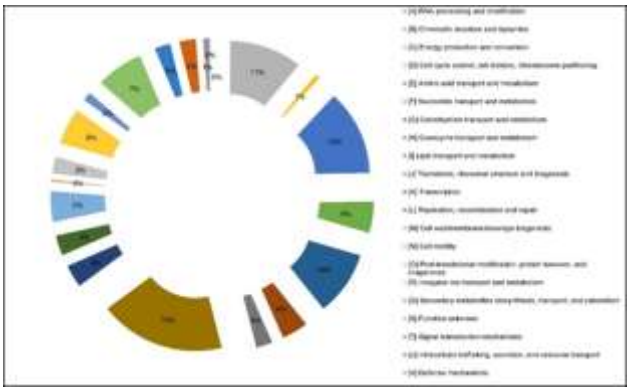

Fig. 2: Distribution of functionally annotated PPIs using COG functional classification

Functional annotation of predicted PPIs

The predicted PPIs were annotated with COG functional categories. It appears that majority of proteins involved in making interactions belong to 'J' category (translation, ribosomal structure and biogenesis) followed by E, C, G that code for amino acid transport and metabolism, energy production and conversion, and carbohydrate metabolism. The distribution of COG functional families among proteins involved in making interactions is summarized in fig. 2.

It is observed that proteins having same function tend to interact with each other which is also true in this study too, where PPIs belong to same COG category are majorly involved in making interactions as shown in fig. 3. The larger number of interactions among proteins involved in translation, ribosomal structure and biogenesis has been observed followed by amino acid and carbohydrate metabolism and transportation (E, G) and energy production and conversion (C). Beside this, the large number of interactions between 'C' and 'E' and 'C' and 'F' were also observed in heat map. It is reported in literature that the expression levels of proteins belong to 'C' (energy production and conversion), 'E' (amino acid transport and metabolism) and 'F' (carbohydrate metabolism) has become elevated during biofilm formation (Guilhen *et al*., 2016).

There are chances of these over-expressed gene could interact with each other to carry out complex biofilm formation process. The possible interactions between these proteins could also play role in bacterial resistance.

Validation of predicted PPIs

The predicted PPIs were validated using semantic similarity of annotated GO terms between two interacting proteins that were determined from GOGO algorithm (Zhao & Wang, 2018) that score the interactions from 0 to 1.

Table 1: Topologically significant proteins shortlisted as potential drug targets

NCBI ID	*Gene Name*	Protein Name	COG	Betweenness Centrality	Degree Centrality
WP_002919219.1	*rpoA*	MULTISPECIES: DNA-directed RNA polymerase subunit alpha [Bacteria]	K	0.006875	103
WP_000729185.1	*rplX*	MULTISPECIES:50 S ribosomal protein L24 [Proteobacteria]	J	0.001426	91
WP_002919515.1	*secY*	MULTISPECIES: preprotein translocase subunit SecY [Enterobacterales]	U	0.001752	91
WP_002885691.1	*rpsF*	MULTISPECIES:30S ribosomal protein S6 [Enterobacterales]	J	0.001738	85
WP_000290724.1	*rpmF*	MULTISPECIES:50S ribosomal proteinL32[Enterobacterales]	J	0.0032	78
WP_002892018.1	*rpmJ*	MULTISPECIES:50S ribosomal proteinL36 [Enterobacterales]	J	0.001477	72
WP_002888624.1	*ftsQ*	MULTISPECIES: cell division protein FtsQ [Enterobacterales]	M	0.005555	71
WP_002898148.1	*pflB*	MULTISPECIES: formate C-acetyl transferase [Enterobacteriaceae]	C	0.003395	60
WP_002898408.1	*ompA*	MULTISPECIES: porin OmpA [Enterobacterales]	M	0.015916	60
WP_002885722.1	*priB*	MULTISPECIES: primosomal replication protein N [Enterobacterales]	L	0.00322	48
WP_002888628.1	*ftsZ*	MULTISPECIES: cell division protein FtsZ[Enterobacterales]	D	0.014345	41
WP_004144748.1	*fbaA*	MULTISPECIES: classII fructose-bisphosphate aldolase[Enterobacterales]	G	0.002725	38
WP_002888638.1	*secA*	MULTISPECIES: protein translocase subunit SecA [Enterobacterales]	U	0.005092	37
WP_004151533.1	*dnaN*	MULTISPECIES: DNA polymerase III subunit beta [Klebsiella]	L	0.004262	35
WP_002913505.1	*ptsH*	MULTISPECIES: phosphor carrier protein HPr [Enterobacterales]	G	0.005269	33
WP_000522253.1	*Crr*	MULTISPECIES: glucose-specific phosphotransferase enzyme IIA component [Gamma proteobacteria]	G	0.005796	31
WP_002913342.1	*aroC*	MULTISPECIES: chorismite synthase [Enterobacterales]	E	0.00584	31
WP_002898160.1	*Cmk*	MULTISPECIES: cytidylate kinase [Enterobacterales]	F	0.004475	30
WP_004147526.1	*fepB*	MULTISPECIES: Fe2+-enterobactin ABC transporter substrate-binding protein [Enterobacterales]	P	0.012958	30
WP_004186365.1	*wzyE*	MULTISPECIES:O-antigen assembly polymerase [Enterobacteriaceae]	M	0.00264	29
WP_004177353.1	*dnaE*	MULTISPECIES: DNA polymerase III subunit alpha [Enterobacterales]	L	0.004185	28
WP_004224270.1	*trpB*	MULTISPECIES: tryptophan synthase subunit beta [Klebsiella]	E	0.00463	28
WP_002888816.1	*gcd*	MULTISPECIES: glucose/quinate/shikimate family membrane-bound PQQ-dependent dehydrogenase [Enterobacteriaceae]	G	0.00259	27
WP_002912260.1	*hisI*	MULTISPECIES: bifunctional phosphoribosyl-AMP cyclo hydrolase/phosphoribosyl-ATP diphosphatase HisIE [Klebsiella]	E	0.003416	27
WP_002888565.1	*ftsW*	MULTISPECIES: cell division protein FtsW [Enterobacterales]	D	0.002231	26
WP_004151712.1	*bssR*	MULTISPECIES: biofilm formation regulatory protein BssR[Enterobacterales]		0.002155	25
WP_002889306.1	*pyrH*	MULTISPECIES: UMP kinase [Bacteria]	F	0.001699	24
WP_002918420.1	*rpoN*	MULTISPECIES: RNA polymerase sigma-54factor [Enterobacterales]	K	0.008372	24
WP_004145860.1	*bamA*	MULTISPECIES: outer membrane protein assembly factor BamA [Enterobacterales]	M	0.004513	24
WP_009484128.1	*gshB*	MULTISPECIES: glutathione synthase [Klebsiella]	H	0.004891	24
WP_004176793.1	*ybhQ*	MULTISPECIES: membrane protein [Enterobacterales]		0.003888	23
WP_002898132.1	*lolA*	MULTISPECIES: outer membrane lipoprotein carrier protein LolA [Enterobacterales]	M	0.005979	22
WP_015874944.1	*yebY*	MULTISPECIES: DUF2511 domain-containing protein [Klebsiella]	S	0.003862	22
WP_032432809.1	*seqA*	Replication initiation negative regulator SeqA [Klebsiella pneumoniae]	L	0.008584	22
WP_002920815.1	*rpoH*	MULTISPECIES: RNA polymerase sigma factor RpoH [Enterobacterales]	K	0.001437	21
WP_002888632.1	*lpxC*	MULTISPECIES: UDP-3-O-[3-hydroxy myristoyl] N-acetyl glucosamine deacetylase [Enterobacterales]	M	0.002287	20
WP_002889327.1	*lpxD*	MULTISPECIES: UDP-3-O-(3-hydroxy myristoyl) glucosamine N-acyltransferase [Enterobacterales]	M	0.002583	20
WP_002899288.1	*fabH*	MULTISPECIES:3-oxoacyl-ACP synthase III [Enterobacterales]	I	0.002699	20
WP_004151534.1	*dnaA*	MULTISPECIES: chromosomal replication initiator protein DnaA [Enterobacterales]	L	0.002059	20

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WP_032433833.1	*lptD*	LPS assembly protein LptD [Klebsiella pneumoniae]	M	0.002928	20
WP_002911547.1	*yecS*	MULTISPECIES: cystine ABC transporter permease [Enterobacterales]	E	0.004245	19
WP_004178647.1	*lpxA*	MULTISPECIES: acyl-[acyl-carrier-protein] -- UDP-N-acetyl glucosamine O-acyltransferase [Enterobacterales]	M	0.00196	19
WP_032433763.1	*bamA*	Outer membrane protein assembly factor BamA [Klebsiella pneumoniae]	M	0.002752	19
WP_002918382.1	*murA*	MULTISPECIES: UDP-N-acetylglucosamine 1-carboxy vinyl transferase [Enterobacterales]	M	0.003203	18
WP_004145263.1	*kdtA*	MULTISPECIES:3-deoxy-D-manno-octulosonic acid transferase [Enterobacterales]	M	0.002611	18
WP_004146501.1	*wecF*	MULTISPECIES: TDP-N-acetyl fucosamine: lipid IIN-acetyl fucos aminyl transferase [Enterobacteriaceae]	M	0.001506	18
WP_002887979.1	*ispH*	MULTISPECIES:4-hydroxy-3-methylbut-2-enyl diphosphate reductase [Enterobacterales]	I	0.001536	17
WP_002888808.1	*yacC*	MULTISPECIES: hypothetical protein [Enterobacterales]		0.005419	17
WP_002910393.1	*kdsA*	MULTISPECIES:2-dehydro-3-deoxy phosphor octonate aldolase[Enterobacterales]	M	0.001531	17

Table 2: Proteins that are either resistant or involved in resistance pathway

NCBI Protein IDs	Protein name	COG	KO	Pathway	Subcellular localization
WP_000522253.1	*crr*	G	K02777	ko00010 Glycolysis / Gluconeogenesis	Cytoplasmic
				ko00500 Starch and sucrose metabolism	
				ko00520 Amino sugar and nucleotide sugar metabolism;	
				ko01100 Metabolic pathways	
				ko02026 Biofilm formation - Escherichia coli	
				ko05111 Biofilm formation	
				ko02060 Phosphotransferase system (PTS)	
WP_002888632.1	*lpxC*	M	K02535	ko00540 Lipopolysaccharide biosynthesis	Cytoplasmic
				ko01100 Metabolic pathways	
WP_002888638.1	*secA*	U	K03070	ko02024 Quorum sensing	Cytoplasmic
				ko03060Protein export	
				ko03070 Bacterial secretion system	
WP_002889327.1	*lpxD*	M	K02536	ko01100 Metabolic pathways	Cytoplasmic
				ko00540 Lipopolysaccharide biosynthesis	
WP_002898148.1	*pflB*	C	K00656	ko00620 Pyruvate metabolism	Cytoplasmic
				ko00640 Propanoate metabolism	
				ko00650 Butanoate metabolism	
				ko01100 Metabolic pathways	
				ko01120 Microbial metabolism in diverse environments	
WP_002910393.1	*kdsA*	M	K01627	ko00540 Lipopolysaccharide biosynthesis	Cytoplasmic
				ko01100 Metabolic pathways	
WP_002911547.1	*yecS*	E	K10009	ko0210 ABC transporters	Cytoplasmic Membrane
WP_002913505.1	*ptsH*	G	K02784	ko02060 Phosphotransferase system (PTS)	Cytoplasmic
WP_002918382.1	*murA*	M	K00790	ko00520 Amino sugar and nucleotide sugar metabolism	Cytoplasmic
				ko00550 Peptidoglycan biosynthesis	
				ko01100 Metabolic pathways	
WP_002918420.1	*rpoN*	K	K03092	ko02020 Two-component system	Cytoplasmic
				ko05111 Biofilm formation	
WP_002919219.1	*rpoA*	K	K03040	ko03020 RNA polymerase;	Cytoplasmic
WP_002919515.1	*secY*	U	K03076	ko02024 Quorum sensing	Cytoplasmic Membrane
				ko03060Protein export	
				ko03070 Bacterial secretion system	
WP_004144748.1	*fbaA*	G	K01624	ko00010 Glycolysis / Gluconeogenesis	Cytoplasmic
				ko00680 Methane metabolism	
				ko01130 Biosynthesis of antibiotics	
				ko00030 Pentose phosphate pathway	
				ko00051 Fructose and mannose metabolism	
				ko00710 Carbon fixation in photosynthetic organisms	
				ko01100 Metabolic pathways	
				ko01230 Biosynthesis of amino acids	
				ko01110 Biosynthesis of secondary metabolites	
				ko01120 Microbial metabolism in diverse environments	
				ko01200 Carbon metabolism	

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WP_004145263.1	*kdtA*	M	K02527	ko00540 Lipopolysaccharide biosynthesis ko01100 Metabolic pathways	Unknown
WP_004151534.1	*dnaA*	L	K02313	ko02020 Two-component system ko04112 Cell cycle	Cytoplasmic
WP_004178647.1	*lpxA*	M	K00677	ko00540 Lipopolysaccharide biosynthesis ko01100 Metabolic pathways ko01503 Cationic antimicrobial peptide (CAMP) resistance	Cytoplasmic
WP_004224270.1	*trpB*	E	K01696	ko00260 Glycine, serine and threonine metabolism ko00400 Phenylalanine, tyrosine and tryptophan biosynthesis ko01110 Biosynthesis of secondary metabolites ko01130 Biosynthesis of antibiotics ko01100 Metabolic pathways ko01230 Biosynthesis of amino acids	Cytoplasmic
WP_009484128.1	*gshB*	H	K01920	ko00270 Cysteine and methionine metabolism ko00480 Glutathione metabolism ko01100 Metabolic pathways	Cytoplasmic
WP_032433833.1	*lptD*	M	K04744	-	Outer Membrane
WP_002898408.1	*ompA*	M	K03286	-	Outer Membrane

Table 3: Druggable Proteins Present in Resistant Pathways

NCBI Protein IDs	Gene Name	COG	Pathway	Subcellular localization	DrugBank ID
WP_002888632.1	*lpxC*	M	ko00540 Lipopolysaccharide biosynthesis ko01100 Metabolic pathways	Cytoplasmic	DB07861; DB01991; DB04257; DB07355; DB07536; DB08231
WP_002898148.1	*pflB*	C	ko00620 Pyruvate metabolism ko00640 Propanoate metabolism ko00650 Butanoate metabolism ko01100 Metabolic pathways ko01120 Microbial metabolism in diverse environments	Cytoplasmic	DB01992; DB03278; DB03940
WP_002910393.1	*kdsA*	M	ko00540 Lipopolysaccharide biosynthesis ko01100 Metabolic pathways	Cytoplasmic	DB01709; DB01819; DB02053; DB02433; DB02992; DB03248; DB03745; DB03937; DB03113; DB03936
WP_002913505.1	*ptsH*	G	ko02060 Phosphotransferase system (PTS)	Cytoplasmic	DB04522; DB01899
WP_002918382.1	*murA*	M	ko00520 Amino sugar and nucleotide sugar metabolism ko00550 Peptidoglycan biosynthesis ko01100 Metabolic pathways	Cytoplasmic	DB01879; DB02435; DB02995; DB04174; DB04474; DB00828; DB03397
WP_002919219.1	*rpoA*	K	ko03020 RNA polymerase;	Cytoplasmic	DB08266; DB00615; DB11753
WP_004144748.1	*fbaA*	G	ko00010 Glycolysis / Gluconeogenesis ko00680 Methane metabolism ko01130 Biosynthesis of antibiotics ko00030 Pentose phosphate pathway ko00051 Fructose and mannose metabolism ko00710 Carbon fixation in photosynthetic organisms ko01100 Metabolic pathways ko01230 Biosynthesis of amino acids ko01110 Biosynthesis of secondary metabolites ko01120 Microbial metabolism in diverse environments ko01200 Carbon metabolism	Cytoplasmic	DB03026
WP_004178647.1	*lpxA*	M	ko00540 Lipopolysaccharide biosynthesis ko01100 Metabolic pathways ko01503 Cationic antimicrobial peptide (CAMP) resistance	Cytoplasmic	DB01692; DB08558
WP_004224270.1	*trpB*	E	ko00260 Glycine, serine and threonine metabolism ko00400 Phenylalanine, tyrosine and tryptophan biosynthesis ko01110 Biosynthesis of secondary metabolites ko01130 Biosynthesis of antibiotics ko01100 Metabolic pathways ko01230 Biosynthesis of amino acids	Cytoplasmic	DB07732; DB07745; DB07748; DB07773; DB07890; DB07894; DB07925; DB07951; DB07952; DB07953
WP_002898408.1	*ompA*	M		Outer Membrane	DB04233

Among 20936 protein pairs, more than 12000 interactions were annotated with GO terms. It appears that 97% partners had functional similarity as either they were involved in same biological process, molecular function or a part of similar cellular component. To further validate the PPIs, the semantic similarities of random pairs were also determined and compared with the non-random interaction data. The semantic similarity of predicted pairs is higher than those of random pairs (Wilcox-Rank Sum Test; P-value>0.005) as shown in fig. 4. The Semantic similarity of majority of non-random interactions are ≥0.8 while in the case of random pairs, the majority of PPIs have low semantic similarity score that comprises of <0.4.

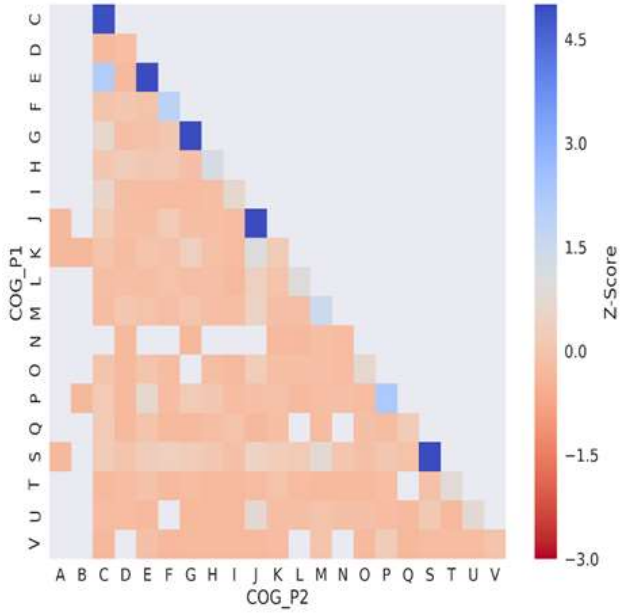

Fig. 3: Heat map of interactions between proteins based on their involvement in COG functional categories.

Identification of Sub-cellular localization of predicted PPIs

The sub-cellular localizations (SCL) of proteins were predicted using PSORTb tool. The SCLs were mapped on to the interacting partners to predict the localization of PPIs. The larger fractions of proteins pair reside in cytoplasm while interactions between proteins present in cytoplasm and cytoplasm membrane are also observed. Again, proteins interacting with each other mostly reside in the same compartment as shown in the fig. 5.

Visualization of PPIs

The Cytoscape version 3.7.2 was used to visualize the PPI network (PPIN). A PPIN is described as graph G (υ, E) where *υ* represent the nodes i.e., proteins and *E* represents the edges i.e., the interaction between proteins to calculate topological properties of network. The degree distribution in PPI network has followed the power law with correlation value (R=0.8) which indicates scale-free network, a common attribute of biological network where majority of nodes has fewer interaction with other nodes accompanied by small number of nodes interacting with large number of nodes in a network (Albert, 2005; Vallabhajosyula, Chakravarti, Lutfeali, Ray, & Raval, 2009; K. Vandereyken, J. Van Leene, B. De Coninck, & B. P. A. Cammue, 2018b).

Retrieval of topologically significant proteins

The topological properties of network play an important role to identify the influential proteins as disturbing those proteins could collapse the network in to small sub-networks or sub-modules and hence disturb the function (Jeong, Mason, Barabási, & Oltvai, 2001; Pan, Lahiri, Rajendiran, & Shanmugham, 2015). In the current study, topological analysis has been conducted using network analyzer tool to identify topologically significant proteins. For this purpose, node degree (*k*) of top 20% which comprised of 756 proteins ranging from 180 to 17 (*k*) were considered as hub proteins (proteins having large number of interacting partners). The hub proteins were enriched with essential proteins (Fisher exact test; *p*-value <0.005) as reported in literature, and in accordance with the concept of centrality and lethality (Jeong *et al*., 2001; Md Aksam, Chandrasekaran, & Pandurangan, 2017; Van Boeckel *et al*., 2019). Because of their interaction with numerous proteins, it can be a part of multiple cellular functions which gives the concept of centrality and pleiotropy (K. Vandereyken, J. Van Leene, B. De Coninck, & B. Cammue, 2018a). Only 2% of proteins in this group are involved in resistance. The bottleneck proteins are defined as the nodes that play central role in passing the information to other nodes and were identified by considering the top 20% proteins based on their high betweenness centrality. About 27% of bottleneck proteins are essential while 4% are involved in resistance. The common proteins between both datasets were retrieved to get the most significant proteins as drug gable targets. Approximately, 390 proteins were common between both data sets. The shortlisted proteins tend to serve as hub and bottleneck in PPIN and considered as topologically significant proteins.

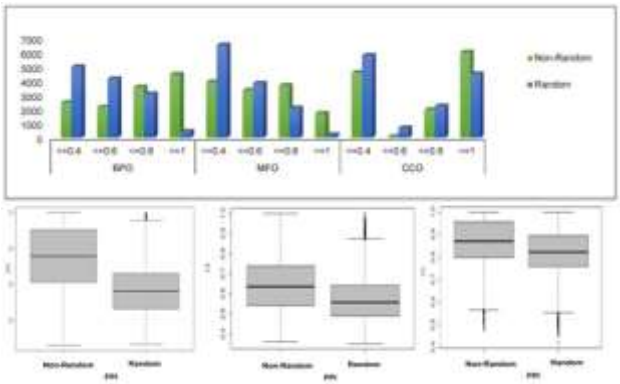

Fig. 4: Upper Panel: Semantic Similarity of Non-Random PPIs Compare with Random PPIs. Lower Panel: Box plot comparison of PPI (Random and Non-Random).

The topologically significant proteins are enriched with essential proteins (fisher exact test: p-value <0.005) as compared to their counterpart. Studies have shown that hub proteins that act as hub-bottleneck tend to be essential as compared to hub without bottleneck property (Boltz, Devkota& Wuchty, 2019; H. Yu, Kim, Sprecher, Trifonov, & Gerstein, 2007). Most proteins are involved in COG category of (E, F, G) which indicates amino acid transport and metabolism, nucleotide transport and metabolism, and carbohydrate transport and metabolism, respectively (fisher exact test: *p*-value <0.0001). Details are provided in Supplementary table 2. Among them, 142 proteins were identified as essential proteins, which further increased the confidence on these shortlisted proteins. Essential proteins were extracted and checked for their non-homology with host i.e., human, hence, further shortlisted them to 49 proteins. These 49 proteins are considered as potential proteins that could be lethal for pathogen upon target. The details of these 49 proteins have been provided in table 1 and their interaction network is visualized in fig. 6.

Among 49 proteins, 29 are localized in cytoplasm, six proteins reside in cytoplasmic membrane, four proteins are present in outer membrane and two proteins are localized in periplasm. However, the localizations of eight proteins are found to be unknown. On further investigation, it appears that the proteins having localization unknown are resided in inner cytoplasmic membrane. The shortlisted proteins are mostly enzyme in nature which highlights their role in bacterial system. Majority of proteins are involved in cell membrane biogenesis. Two proteins with same name i.e., BamA has been shortlisted. Both the proteins share 62% identity so both the proteins are screened as possible drug targets.

Involvement in resistant pathway

All the potential drug targets were checked for their involvement in resistant pathways. Total 20 out of 49 proteins are involved in resistance. The 3 proteins i.e., OmpA, MurA, LptD have direct role in resistance while 17 proteins sharing the pathway of other resistant proteins as provided in table 2. The rationale of targeting the proteins present in resistant pathways could increase the likelihood to disturb the resistant proteins and hence eventually become effective to overcome the resistance mechanism. In table 2, many proteins are belonged to 'M' COG category and involved in lipopolysaccharide biosynthesis pathway while proteins that are involved in quorum sensing, two component, peptidoglycan biosynthesis, pyruvate biosynthesis, biofilm formation are also present.

Identification of drug gable proteins

The drug gability is defined as the potential of protein to inhibit or modulate itself upon binding with small molecules. Among 49 proteins, 17 proteins have shown homology i.e., ≥35 %identity with already identified drug targets present in Drug Bank database. Whereas, other 4 proteins have shown little homology (having identity <35%). while other 28 targets have no homolog present in the Drug Bank Database. These proteins are considered as novel drug targets and can be explored structurally to identify drug pockets and protein binding interfaces to predict drug candidates against them. The drug gable proteins are summarized in supplementary table 3 along with drug candidates against them. Among 20 proteins, present in resistant pathways, 10 proteins were also shown homology with already identified drug targets. These proteins along with their respective drug candidates are summarized in table 3.

The 3 targets i.e., LpxA, LpxC, and KdsA belonged to lipopolysaccharide (LPS) biosynthesis. These are the key enzymes in the biosynthesis of lipopolysaccharide that covers outer membrane of Gram-negative bacteria (Pratap *et al*., 2017). The LpxA and LpxC are involved in the biosynthesis of Lipid A which is essential for the assembly of LPS system. Studies have shown that the Lipid A biosynthesis is heterogeneous process. It is not only involved in virulence as it interacts with the host cell and trigger immune response that's why also known as endotoxin but also have important role in the resistance against polymixin (Klein, Kobylak, Lindner, Stupak, & Raina, 2014; Pratap *et al*., 2017; Raetz & Whitfield, 2002). The proteins involved in LPS biosynthesis are extensively studied and considered as potential drug targets against Gram-negative bacteria (Cesur, Siraj, Uddin, Durmuş, & Çakır, 2020; Han *et al*., 2020; Ramos *et al*., 2018). The current study also authenticates these proteins as potential drug targets, which are shortlisted, based on their topological property in PIN. Currently, around 8 drug candidates are in experimental phase against LpxC, LpxA, LpxD and KdsA present in DrugBank Database. But still no antibiotics against these targets are in clinical use.

The FbaA is also shortlisted in the current study which encodes class II fructose bisphosphate aldolase. It is involved in energy metabolism. Its expression level become up-regulated in carbapenem resistance which highlights its role in resistance.(Khan, Sharma, Faheem, Bisht, & Khan, 2017). Moreover, FbaA has been reported to play a vital role in the pathogenesis of important human pathogens (Ziveri *et al*., 2017). It is involved in multiple pathways as appeared in table 2 and act as hub protein in PIN, which makes it suitable target against drug resistant *K. pneumoniae*.

The protein PflB which encodes formate acetyl transferase has also shown druggability and is present in resistance pathway. In Kp, the expression level of PflB has found to be up regulated in response to carbapenem resistance (Sharma, Garg, Kumar, & Khan, 2019). Targeting the protein could be the better solution to combat resistance.

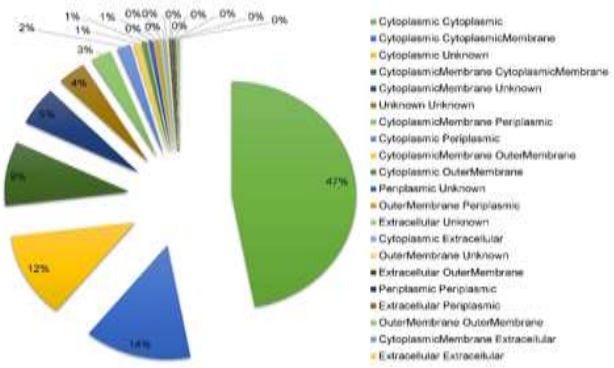

Fig. 5: Sub-cellular localization of predicted PPIs

Tryptophan synthase is an emerging target against *Mycobacterium tuberculosis* (Abrahams *et al*., 2017; Mohan *et al*., 2022) which is also shortlisted in the current study against drug resistant strain of Kp. Tryptophan synthase is involved in the final step of tryptophan biosynthesis. In Drug Bank database, 12 drugs against tryptophan synthase subunit B of *Salmonella typhimurium* are already in experimental phase.

The drug candidate against OmpA is currently in experimental phase. OmpA is an outer membrane protein and is directly involved in virulence, adhesion with host cell and in resistance mechanism which makes it suitable target for further research and development of new drugs. The expression level of OmpA has changed in polymixin resistant strains of *K. pneumonia* (Queiroz *et al*., 2021). The current study also authenticates it as suitable drug target based on intra PPIs of carbapenem resistant strain.

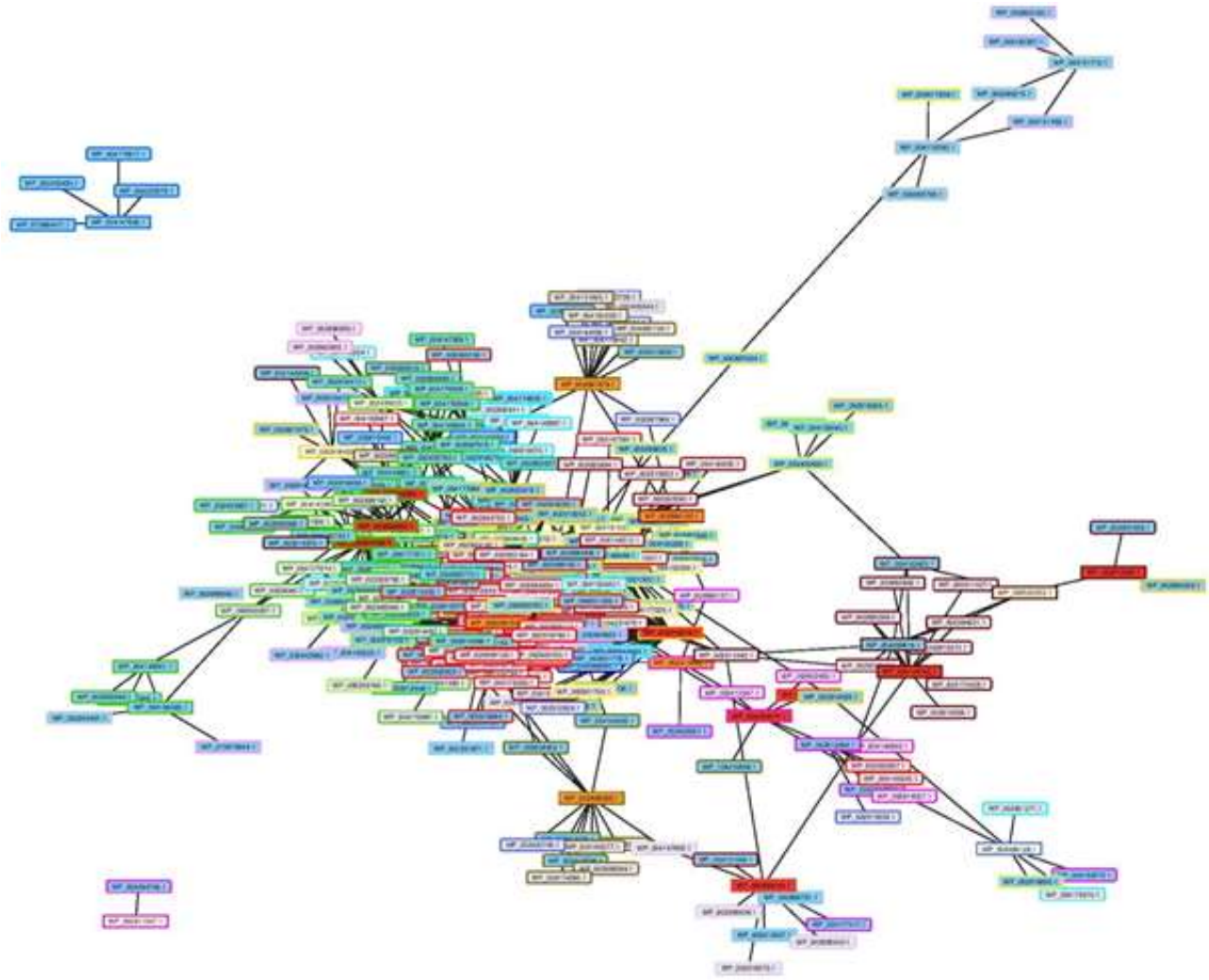

Fig. 6: Protein-Protein Interaction Network of Drug gable Targets. Only essential interacting partners are shown. Rectangular nodes represent topologically significant protein (TSP). Represent TSP sharing the pathway of resistant protein and no homolog in Drug Bank database. Represent TSP showing druggability and sharing resistant pathways. Represent proteins that are only drug gable. The border color represents COG families to which proteins belong. Represent interacting partners having homolog in Drug Bank Database.

SUPPORTING INFORMATION

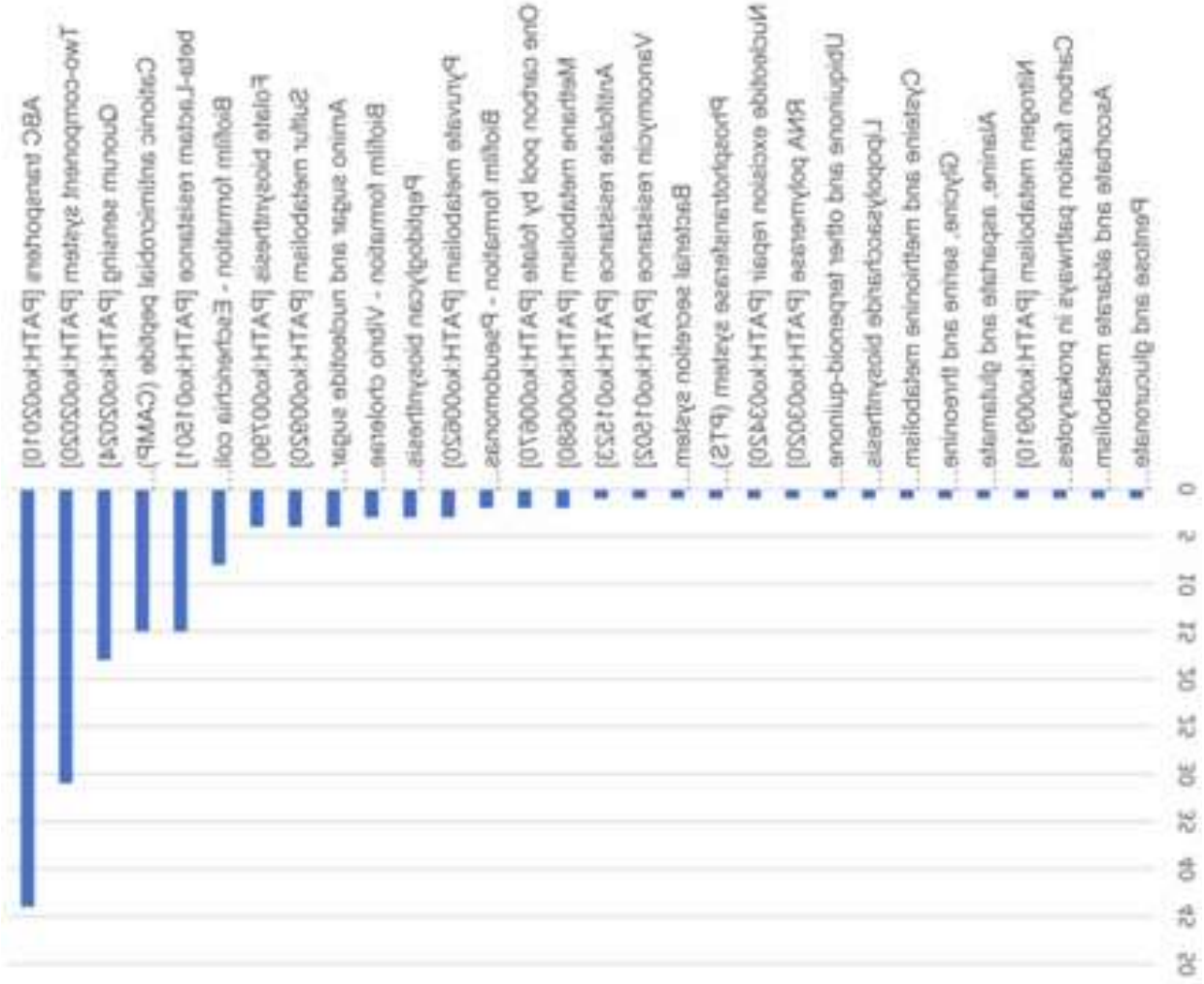

Fig. S1: Summary of the resistant Pathways.

Table S1: The resistant proteins and the pathway in which proteins are involved.

C	00040 Pentose and glucuronate interconversions [PATH:ko00040]	1
D	WP_039819510.1; K00012 UGDH, ugd; UDPglucose 6-dehydrogenase	
C	00053 Ascorbate and aldarate metabolism [PATH:ko00053]	1
D	WP_039819510.1; K00012 UGDH, ugd; UDPglucose 6-dehydrogenase	
C	00520 Amino sugar and nucleotide sugar metabolism [PATH: 00520]	4
D	WP_004151550.1; K04042 glmU; bifunctional UDP-N-acetylglucosamine pyrophosphorylase	
D	WP_023283047.1; K10011 arnA, pmrI; UDP-4-amino-4-deoxy-L-arabinose formyltransferas	
D	WP_002921035.1; K10012 arnC, pmrF; undecaprenyl-phosphate 4-deoxy-4-formamido-L-arabinose transferase	
D	WP_039819510.1; K00012 UGDH, ugd; UDPglucose 6-dehydrogenase	
C	00620 Pyruvate metabolism [PATH:ko00620]	3
D	WP_002902515.1; K03778 ldhA; D-lactate dehydrogenase	
D	WP_002898200.1; K01069 gloB, gloC, HAGH; hydroxyacylglutathione hydrolase	
D	WP_002909055.1; K01007 pps, ppsA; pyruvate, water dikinase	
C	00720 Carbon fixation pathways in prokaryotes [PATH:ko00 720]	1
D	WP_002909055.1; K01007 pps, ppsA; pyruvate, water dikinase	
C	00680 Methane metabolism [PATH:ko00680]	2
D	WP_002909055.1; K01007 pps, ppsA; pyruvate, water dikinase	
D	WP_002916493.1; K00058 serA, PHGDH; D-3-phosphoglycerate dehydrogenase	
C	00910 Nitrogen metabolism [PATH:ko00910]	1
D	WP_002918461.1; K00266 gltD; glutamate synthase	

C	00920 Sulfur metabolism [PATH:ko00920]	4
D	WP_002913623.1; K02045 cysA; sulfate/thiosulfate transferase	
D	WP_004183141.1; K10831 tauB; taurine transport system	
D	WP_004150838.1; K15555 ssuB; sulfonate transport system ATP binding protein	
D	WP_002922426.1; K00640 cysE; serine O-acetyltransferase	
C	00250 Alanine, aspartate and glutamate metabolism [PATH: ko00250]	1
D	WP_002918461.1; K00266 gltD; glutamate synthase	
C	00260 Glycine, serine and threonine metabolism [PATH:ko00260]	1
D	WP_002916493.1; K00058 serA, PHGDH; D-3-phosphoglycerate dehydrogenase	
C	00270 Cysteine and methionine metabolism [PATH:ko00270]	1
D	WP_002922426.1; K00640 cysE; serine O-acetyltransferase	
C	00540 Lipopolysaccharide biosynthesis [PATH:ko00540]	1
D	WP_032432825.1; K03760 eptA, pmrC; lipid A ethanolamine phosphotransferase	
C	00550 Peptidoglycan biosynthesis [PATH:ko00550]	3
D	WP_004151348.1; K01921 ddl; D-alanine-D-alanine ligase	
D	WP_004144532.1; K06153 bacA; undecaprenyl-diphosphatase	
D	WP_004152954.1; K05515 mrdA; penicillin-binding protein	
C	00790 Folate biosynthesis [PATH:ko00790]	4
D	WP_002918371.1; K00796 folP; dihydropteroate synthase	
D	WP_001043260.1; K18824 sul2; dihydropteroate synthase	
D	WP_004177432.1; K00287 DHFR, folA; dihydrofolate reductase	
D	WP_001083725.1; K18590 dfrA12, dhfr; dihydrofolate reductase	
C	00670 One carbon pool by folate [PATH:ko00670]	2
D	WP_004177432.1; K00287 DHFR, folA; dihydrofolate reductase	
D	WP_001083725.1; K18590 dfrA12, dhfr; dihydrofolate reductase	
C	00130 Ubiquinone and other terpenoid-quinone biosynthesis [PATH:ko00130]	1
D	WP_032433519.1; K18800 ubiI; 2-polyprenylphenol 6-hydroxylase	
C	03020 RNA polymerase [PATH:ko03020]	1
D	WP_004901914.1; K03043 rpoB; DNA-directed RNA polymerase	
C	03420 Nucleotide excision repair [PATH:ko03420]	1
D	WP_004146620.1; K03701 uvrA; excinuclease ABC subunit A	
C	02010 ABC transporters [PATH:ko02010]	44
D	WP_002913623.1; K02045 cysA; sulfate/thiosulfate transporter	
D	WP_004183141.1; K10831 tauB; taurine transport system	
D	WP_004150838.1; K15555 ssuB; sulfonate transport system	
D	WP_004224984.1; K15600 thiZ; putative hydroxymethylpyrimidine transport system ATP-binding protein	
D	WP_002895152.1; K05776 modF; molybdate transport system	
D	WP_004151229.1; K02010 afuC, fbpC; iron(III) transport system ATP-binding protein	
D	WP_004178579.1; K02062 thiQ; thiamine transport system	
D	WP_004188029.1; K11084 phnT; 2-aminoethylphosphonate	
D	WP_004149083.1; K05847 opuA; osmoprotectant transport system ATP-binding protein	
D	WP_004150950.1; K02065 mlaF, linL, mkl; phospholipid//cholesterol/gamma-HCH transport	
D	WP_032432790.1; K10441 rbsA; ribose transport system	
D	WP_004175074.1; K10542 mglA; methyl-galactoside transport system ATP-binding	
D	WP_002917730.1; K10558 lsrA, ego; AI-2 transport system	
D	WP_004150373.1; K10562 rhaT; rhamnose transport system	
D	WP_032433787.1; K05816 ugpC; sn-glycerol 3-phosphate transport system ATP-binding protein	
D	WP_004145006.1; K02036 pstB; phosphate transport system	
D	WP_004177295.1; K02041 phnC; phosphonate transport system	
D	WP_004142040.1; K10038 glnQ; glutamine transport system	
D	WP_002896392.1; K10000 artP; arginine transport system	
D	WP_002894706.1; K10004 gltL, aatP; glutamate/aspartate transport sistem ATP binding protein	
D	WP_004151455.1; K10010 tcyC, yecC; L-cystine transport system	
D	WP_004148803.1; K01995 livG; branched-chain amino acid transport system ATP-binding protein	
D	WP_004183132.1; K01995 livG; branched-chain amino acid system ATP_binding Protein	
D	WP_002890126.1; K01996 livF; branched-chain amino acid ATP_binding Protein	
D	WP_004175491.1; K01996 livF; branched-chain amino acid	
D	WP_004176216.1; K02071 metN; D-methionine transport s	
D	WP_032432966.1; K02071 metN; D-methionine transport s	
D	WP_004151853.1; K15583 oppD; oligopeptide transport s	
D	WP_004140266.1; K19229 sapD; cationic peptide transpo	
D	WP_004140269.1; K19230 sapF; cationic peptide transpo	
D	WP_004151710.1; K13892 gsiA; glutathione transport sy	
D	WP_032433341.1; K13896 yejF; microcin C transport sys	
D	WP_002908416.1; K02013 ABC.FEV.A; iron complex transp	
D	WP_004149516.1; K02013 ABC.FEV.A; iron complex transp	

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D	WP_032417273.1; K06074 ABC.VB12.A, btuD; vitamin B12	
D	WP_002912371.1; K09691 ABC-2.LPSE.A; lipopolysacchari	
D	WP_002900798.1; K09810 lolD; lipoprotein-releasing system	
D	WP_004175727.1; K02193 ccmA; heme exporter protein A	
D	WP_002920817.1; K09812 ftsE; cell division transport	
D	WP_002898170.1; K11085 msbA; ATP-binding cassette	
D	WP_004147344.1; K18889 mdlA, smdA; ATP-binding casset	
D	WP_016831378.1; K13409 raxB, cvaB; ATP-binding casset	
D	WP_004209695.1; K05685 macB; macrolide transport system	
D	WP_004175044.1; K06159 yojI; multidrug/microcin trans	
C	02060 Phosphotransferase system (PTS) [PATH:ko02060]	1
D	WP_002913506.1; K08483 PTS-EI.PTSI, ptsI; phosphotran	
C	03070 Bacterial secretion system [PATH:ko03070]	1
D	WP_032433534.1; K12340 tolC; outer membrane protein	
C	02020 Two-component system [PATH:ko02020]	31
D	WP_002890343.1; K07657 phoB; two-component system	
D	WP_004150807.1; K07660 phoP; two-component system,	
D	WP_001157751.1; K07659 ompR; two-component system,	
D	WP_060591393.1; K09475 ompC; outer membrane pore protein	
D	WP_002882898.1; K07640 cpxA; two-component system	
D	WP_002882901.1; K07662 cpxR; two-component system	
D	WP_002887820.1; K07663 creB; two-component system	
D	WP_004149058.1; K07642 baeS, smeS; two-component system	
D	WP_004175198.1; K07664 baeR, smeR; two-component system	
D	WP_039819862.1; K07799 mdtA; membrane fusion protein,	
D	WP_039819860.1; K07788 mdtB; multidrug efflux pump	
D	WP_002912617.1; K07789 mdtC; multidrug efflux pump	
D	WP_004185072.1; K18324 acrD; multidrug efflux pump	
D	WP_002895659.1; K07771 basR; two-component system	
D	WP_032105399.1; K07665 cusR, copR, silR; two-component sysstem	
D	WP_032431458.1; K07796 cusC, silC; outer membrane protein	
D	WP_002916836.1; K07666 qseB; two-component system	
D	WP_002894776.1; K07667 kdpE; two-component system	
D	WP_002918444.1; K07648 arcB; two-component system	
D	WP_002887843.1; K07773 arcA; two-component system	
D	WP_002888337.1; K07702 dpiA, citB; two-component system	
D	WP_002910198.1; K07684 narL; two-component system	
D	WP_065809691.1; K07677 rcsC; two-component system	
D	WP_032433494.1; K07678 barA, gacS, varS; two-component system	
D	WP_002911542.1; K07782 sdiA; LuxR family transcriptional regulator	
D	WP_023328343.1; K07679 evgS, bvgS; two-component system	
D	WP_004174237.1; K07690 evgA, bvgA; two-component system	
D	WP_002882749.1; K07712 glnG, ntrC; two-component system	
D	WP_032433750.1; K07713 zraR, hydG; two-component system	
D	WP_002894706.1; K10004 gltL, aatP; glutamate/aspartate transport sistem ATP binding protein	
D	WP_000242758.1; K10914 crp; CRP/FNR family transcriptional regulator	
C	02024 Quorum sensing [PATH:ko02024]	18
D	WP_002916836.1; K07666 qseB; two-component system,	
D	WP_002894776.1; K07667 kdpE; two-component system,	
D	WP_002911542.1; K07782 sdiA; LuxR family transcriptional regulator	
D	WP_002917730.1; K10558 lsrA, ego; AI-2 transport system ATP-binding protein	
D	WP_004148803.1; K01995 livG; branched-chain amino acid transport system ATP-binding protein	
D	WP_004183132.1; K01995 livG; branched-chain amino aciid transport system ATP-binding protein	
D	WP_002890126.1; K01996 livF; branched-chain amino aciid transport system ATP-binding protein	
D	WP_004175491.1; K01996 livF; branched-chain amino aciid transport system ATP-binding protein	
D	WP_000242758.1; K10914 crp; CRP/FNR family transcriptional regulator	
D	WP_004186228.1; K18139 oprM, emhC, ttgC, cusC, adeK,	
D	WP_004151853.1; K15583 oppD; oligopeptide transport system ATP-binding protein.	
D	WP_004180551.1; K02031 ABC.PE.A; peptide/nickel transport system ATP-binding protein	
D	WP_004191439.1; K02031 ABC.PE.A; peptide/nickel transport system ATP-binding Protein	
D	WP_032432760.1; K02031 ABC.PE.A; peptide/nickel transport system ATP-binding Protein	
D	WP_032433155.1; K02031 ABC.PE.A; peptide/nickel transport system ATP-binding Protein	
D	WP_004176939.1; K02032 ABC.PE.A1; peptide/nickel tranport system ATP-binding Protein	
D	WP_023286493.1; K02032 ABC.PE.A1; peptide/nickel tranport system ATP-binding Protein	
D	WP_039819854.1; K02032 ABC.PE.A1; peptide/nickel tranport system ATP-binding Protein	
C	05111 Biofilm formation - Vibrio cholerae [PATH:ko05111]	3

Continue…

D	WP_002922426.1; K00640 cysE; serine O-acetyltransferase	
D	WP_032433494.1; K07678 barA, gacS, varS; two-component system	
D	WP_000242758.1; K10914 crp; CRP/FNR family transcriptional regulator	
C	02025 Biofilm formation - Pseudomonas aeruginosa [PATH:k02025]	2
D	WP_000242758.1; K10914 crp; CRP/FNR family transcriptional regulator	
D	WP_032433494.1; K07678 barA, gacS, varS; two-component system	
C	02026 Biofilm formation - Escherichia coli [PATH:ko02026	8
D	WP_000242758.1; K10914 crp; CRP/FNR family transcriptional regulator	
D	WP_002916607.1; K04333 csgD; LuxR family transcriptional regulator	
D	WP_002911542.1; K07782 sdiA; LuxR family transcriptional regulator	
D	WP_032433494.1; K07678 barA, gacS, varS; two-component system	
D	WP_002918444.1; K07648 arcB; two-component system	
D	WP_002887843.1; K07773 arcA; two-component system	
D	WP_065809691.1; K07677 rcsC; two-component system	
D	WP_001157751.1; K07659 ompR; two-component system	
C	01501 beta-Lactam resistance [PATH:ko01501]	15
D	WP_060591393.1; K09475 ompC; outer membrane pore protein	
D	WP_004151853.1; K15583 oppD; oligopeptide transport system ATP-binding protein.	
D	WP_004177236.1; K03585 acrA, mexA, adeI, smeD, mtrC,	
D	WP_009485155.1; K03585 acrA, mexA, adeI, smeD, mtrC,	
D	WP_002892069.1; K18138 acrB, mexB, adeJ, smeE, mtrD,	
D	WP_020316808.1; K18138 acrB, mexB, adeJ, smeE, mtrD,	
D	WP_004186228.1; K18139 oprM, emhC, ttgC, cusC, adeK,	
D	WP_004184324.1; K18143 adeS; two-component system,	
D	WP_004151175.1; K18144 adeR; two-component system,	
D	WP_060591385.1; K18146 adeB; multidrug efflux pump	
D	WP_032433534.1; K12340 tolC; outer membrane protein	
D	WP_004152954.1; K05515 mrdA; penicillin-binding prote	
D	WP_000027057.1; K18698 blaTEM; beta-lactamase class A	
D	WP_004176269.1; K18699 blaSHV; beta-lactamase class A	
D	WP_000239590.1; K18767 blaCTX-M; beta-lactamase class	
C	01502 Vancomycin resistance [PATH:ko01502]	1
D	WP_004151348.1; K01921 ddl; D-alanine-D-alanine ligase	
C	01503 Cationic antimicrobial peptide (CAMP) resistance [ko1503]	15
D	WP_004150807.1; K07660 phoP; two-component system,	
D	WP_002921035.1; K10012 arnC, pmrF; undecaprenyl-phosphate 4-deoxy-4-formamido-L-arabinose transferase	
D	WP_023283047.1; K10011 arnA, pmrI; UDP-4-amino-4-deoxy-L-arabinose formyltransferase	
D	WP_002895659.1; K07771 basR; two-component system,	
D	WP_032432825.1; K03760 eptA, pmrC; lipid A ethanolamine phosphotransferase	
D	WP_004140266.1; K19229 sapD; cationic peptide transport	
D	WP_004140269.1; K19230 sapF; cationic peptide transport	
D	WP_002882898.1; K07640 cpxA; two-component system,	
D	WP_002882901.1; K07662 cpxR; two-component system,	
D	WP_002904397.1; K13632 marA; AraC family transcriptio	
D	WP_004177236.1; K03585 acrA, mexA, adeI, smeD, mtrC,	
D	WP_009485155.1; K03585 acrA, mexA, adeI, smeD, mtrC,	
D	WP_002892069.1; K18138 acrB, mexB, adeJ, smeE, mtrD,	
D	WP_020316808.1; K18138 acrB, mexB, adeJ, smeE, mtrD,	
D	WP_032433534.1; K12340 tolC; outer membrane protein	
C	01523 Antifolate resistance [PATH:ko01523]	1
D	WP_004177432.1; K00287 DHFR, folA; dihydrofolate reductase	

Table S2: The details of the proteins involved in COG category of E, F and G.

	COG distribution among Proteins in PPI network	cog distribution among non-important set-(3392)	COG distribution among proteins common in hub and bottleneck (390)	P-value
C	248	201	47	0.096754372
D	41	38	3	0.794769087
E	341	286	55	0.000502691
F	104	75	29	2.80591E-07
G	345	293	52	0.00381224
H	149	130	19	0.334412401
I	81	69	12	0.19292411
J	177	147	30	0.005049811
K	299	288	11	1.67593E-05
L	126	113	13	0.31004342

Continue…

M	182	160	22	0.384232313
N	15	11	4	0.060676911
O	128	120	8	0.139591281
P	269	259	10	6.3736E-05
Q	68	63	5	0.546702356
S	509	482	27	2.88619E-05
T	112	106	6	0.083215473
U	79	66	13	0.088736489
V	63	62	1	0.018779334
no cog	3336	2969	367	
	important		390	
	total no of proteins		3782	
			3392	

Table S3: The proteins showing homology with DrugBank database.

NCBI Id	Gene Name	Protein Name	DrugBank ID	COG	Sub-Cellular Localization
WP_002919219.1	rpoA	MULTISPECIES:DNA-directedRNApolymerasesubunitalpha[Bacteria]	DB08266;DB00615;DB11753	K	Cytoplasmic
WP_000290724.1	rpmF	MULTISPECIES: 50SribosomalproteinL32[Enterobacterales]	DB13179	J	Cytoplasmic
WP_002898408.1	ompA	MULTISPECIES: porinOmpA[Enterobacterales]	DB04233	M	Outer Membrane
WP_002888628.1	ftsZ	MULTISPECIES: celldivisionproteinFtsZ [Enterobacterales]	DB03532	D	Cytoplasmic
WP_004144748.1	fbaA	MULTISPECIES: classIIfructose-bisphosphatealdolase [Enterobacterales]	DB03026	G	Cytoplasmic
WP_004151533.1	dnaN	MULTISPECIES: DNApolymeraseIIIsubunitbeta[Klebsiella]	DB06998	L	Cytoplasmic
WP_002913505.1	ptsH	MULTISPECIES: phosphocarrierproteinHPr [Enterobacterales]	DB04522; DB01899	G	Cytoplasmic
WP_002913342.1	aroC	MULTISPECIES: chorismatesynthase[Enterobacterales]	DB03247; DB03350	E	Cytoplasmic
WP_002898160.1	cmk	MULTISPECIES: cytidylatekinase[Enterobacterales]	DB02456; DB02883; DB03403; DB04555	F	Cytoplasmic
WP_004224270.1	trpB	MULTISPECIES: tryptophansynthasesubunitbeta[Klebsiella]	DB07732; DB07745; DB07748; DB07773; DB07890;DB07894; DB07925;DB07951; DB07952; DB07953	E	Cytoplasmic
WP_002888816.1	gcd	MULTISPECIES: glucose/quinate/shikim atefamilymembrane-boundPQQ-depende ntdehydrogenase[Enterobacteriaceae]	DB01942	G	Cytoplasmic Membrane
WP_002920815.1	rpoH	MULTISPECIES: RNApolymerasesigmafactorRpoH[Enterobacterales]	DB08226; DB08266	K	Cytoplasmic
WP_002888632.1	lpxC	MULTISPECIES: UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosaminedeac etylase[Enterobacterales]	DB07861;DB01991; DB04257; DB07355:; DB07536;DB08231	M	Cytoplasmic
WP_002899288.1	fabH	MULTISPECIES: 3-oxoacyl-ACPsynthaseIII[Enterobacterales]	DB01034; DB01992; DB02039; DB02316; DB03661;DB04524;DB07429	I	Cytoplasmic
WP_004178647.1	lpxA	MULTISPECIES: acyl-[acyl-carrier-protein]--UDP-N-acetylglucosamineO-acyltransferase [Enterobacterales]	DB01692; DB08558	M	Cytoplasmic
WP_002918382.1	murA	MULTISPECIES:UDP-N-acetylglucosamine1-carboxyvinyltransferase [Enterobacterales]	DB01879; DB02435; DB02995; DB04174; DB04474; DB00828; DB03397	M	Cytoplasmic
WP_002887979.1	ispH	MULTISPECIES:4-hydroxy-3-methylbut-2-enyldiphosphatereductase[Enterobacterales]	DB01785;DB04714	I	Cytoplasmic
WP_002910393.1	kdsA	MULTISPECIES:2-dehydro-3-deoxyphosphooctonatealdolase [Enterobacterales]	DB01709;DB01819;DB02053; DB02433; DB02992;DB03248;DB03745;DB03937; DB03113;DB03936	M	Cytoplasmic

DISCUSSION

Antibiotic drug resistance among Gram-negative microorganism is a major concern worldwid*e* as the antibiotics that once used for treatment now become ineffective. *Klebsiella pneumonia* is a Gram-negative microbe that has shown resistance to almost all antibiotics. Because of its severity, the WHO has placed carbapenem resistance Kp among the priority list of pathogens for the research and development of new drug candidates to cope the menace (WHO, 2017). *K. pneumoniae* is usually considered as opportunistic but the hyper-virulent strains are enabled to infect healthy individuals as well. The convergence between hyper-virulent and hyper resistance strains has been reported, making it more therapeutically challenging (Holt *et al*., 2015; Lee *et al*., 2016).

In the current study, PPIs network of carbapenem resistant Kp strain namely DA48896 that is heading towards pan

drug resistant has been explored to identify the potential drug targets. More than 20,000 interactions of 3782 proteins covering 68% of total proteome have been predicted using interolog based method and validated using semantic similarity between gene ontology terms of two interacting partners as it is the most reported method to validate the interactions (Jain & Bader, 2010; Zhang & Tang, 2016) and by sub-cellular localization prediction of interacting proteins. Here, the potential drug targets have been predicted by integrating the network topology with essentiality and non-homology. The proteins, playing the role of both hub and bottleneck in PIN has been considered as topologically significant proteins. The retrieved proteins were not only enriched with essential proteins but majority are also involved in multiple pathways which satisfies both the concept i.e., centrality and lethality, and centrality and pleiotropy (Vandereyken *et al*., 2018b; H. Yu *et al*., 2008). Moreover, the topologically significant proteins were compared with the hub proteins extracted from cytoHubba tool and found that these topologically significant proteins were also present in hub protein dataset extracted from cytoHubba.

Total 49 proteins were shortlisted that are not only topologically significant but also essential and non-homologous. Large number of these proteins are involved in cell membrane and cell wall biogenesis. While, the proteins involved in translation, transcription, replication and pathways involved in energy metabolism are also present in the list of topologically significant proteins.

These proteins were further evaluated for their presence in resistant pathway and their homology with druggable targets present in Drugbank database. 20/49 proteins either share the pathway of resistant proteins or directly involved in resistance. Total 10 out of 20 proteins, present in resistant pathways, have shown homology with druggable targets, the drugs against these targets could be repurposed against carbapenem resistant strain of Klebsiella. Nonetheless, all of the drugs against these targets are in the experimental phase except the target MurA and RpoA. MurA is the natural target of antibiotic Fosfomycin in which Fosfomycin make irreversible covalent interaction with Cys115 which is present in enzyme's active site and hence inactivate the enzyme. The bacteria have already developed resistance against Fosfomycin (Eschenburg, Priestman, & Schönbrunn, 2005). The approved drug against RpoA is Rifamycin and Rifabutin. Rifamycin is used as first line of drug against *Mycobacterium tuberculosis* and *Staphylococcus aureus* where it inhibits DNA dependent RNA polymerase. Since Arr-2 protein which is involved in catalyzing ADP ribosylation of Rifamycin making it ineffective, is not only present in DA48896 but also widely dispersed in Gram-Negative microbe (Baysarowich *et al*., 2008; Zheng & Lupoli, 2021). Recently, Rifabutin has been used against carbapenem resistant *Acinetobacter baumannii* (Trebosc, Kemmer, Lociuro, Gitzinger, & Dale, 2021) giving the hope to inhibit these targets using drugs with novel mechanism of action.

The current study focuses on targets present in resistant pathways resulting in targets present in unconventional pathways like lipopolysaccharide biosynthesis, quorum sensing, biofilm formation, two-component system, pyruvate metabolism etc.Since, the conventional drug targets which include cell wall biosynthesis, nucleic acid biosynthesis and protein synthesis have been explored excessively so there comes a time to explore targets that are involved in other than conventional metabolic pathways (Belete, 2019).

Currently, targeting the proteins present in LPS biosynthesis pathway are considered as prolific approach to combat Gram-negative bacteria, as this pathway is not only essential for bacteria but also has role in virulence. Lipopolysaccharide is major constituent of outer membrane of Gram-negative organisms (Cesur *et al*., 2020; Ramos *et al*., 2018). In the current study, 5 proteins (LpxA, LpxC, LpxD, Kdsa, Kdta) have been shortlisted. Among them 3 i.e LpxA, LpxC and Kdsa have shown druggability while LpxD has shown little homology with druggable target while Kdta has shown no homology with already identified drug targets.

In the same way, the protein PflB, involved in pyruvate pathway, is also shortlisted. Studies have shown that pyruvate metabolism has been up regulated in carbapenem resistant pathogens owing to the consequence of fluctuation in central energy metabolism (Cheng *et al*., 2018; Sharma *et al*., 2019). Targeting the proteins involved in pyruvate metabolism and other metabolic pathways acquired by bacteria in diverse environment could be the possible solution to inhibit drug resistant pathogen. One such pathway is amino acid metabolism. The amino acid biosynthesis pathway of Kp is studied as crucial for its survival in lungs and manifestation of infection (Silver *et al*., 2019). Currently, proteins involved in amino acid synthesis are being considered as attractive targets against *Mycobacterium tuberculosis* and searching of inhibitors against tryptophan biosynthesis are being in progress (Abrahams *et al*., 2017; Consalvi, Scarpecci, Biava, & Poce, 2019). The current study also suggests tryptophan biosynthesis as an attractive target against carbapenem resistant *K. pneumoniae* Overall, the proteins that are not reported in DrugBank database also have equal potential to serve as drug target. The only necessity is to design the inhibitors that could be small molecule or peptidomimetics that inhibit the targeted protein and ultimately block the pathway. Likewise, the proteins involved in Sec pathway are also attractive targets as their homologs are not present in Human and the pathway is essential for pathogen. The protein SecA is a major enzyme in sec pathway where it functions as an ATPase that provides energy for the Sec-dependent protein translocation which constitute the bacterial translocation

system (Chaudhary, Chen, Jin, Tai& Wang, 2015). Similarly, the protein GshB help bacteria to survive in high oxidative stress and are involved in virulence (El Qaidi *et al*., 2020). Attenuating the target could be the solution to combat resistivity. However, there are other proteins that not only share important position in PIN but also show druggability like FtsZ which is the key cell division protein involved in cytokinesis (Kusuma, Payne, Ung, Bottomley& Harry, 2019).

CONCLUSION

In the current study, antimicrobial drug targets against carbapenem resistant strain of *Klebsiella pneumoniae* were identified by parsing the Protein Interaction network. The essential proteins that are non-homologous to the human host were identified based on the topological properties i.e., degree and betweenness centrality of proteins that are acting as nodes in the interaction network. The topologically significant proteins were further elaborated for their druggability and their involvement in the pathways in which resistant proteins are present. Total 20 proteins are either resistance proteins or present in resistance pathways. Among them, 10 proteins are homologs to the druggable targets. The obtained results further increased the confidence to the credibility of other proteins to act as druggable targets. The only need is to identify and design the inhibitors against them that could be a solution to combat drug resistance. This study will be helpful for scientific community to explore such proteins as drug targets and find novel solution to combat resistance. In addition, the methodology can be applicable to all other pathogens.

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ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

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