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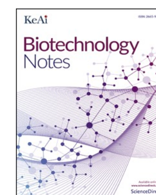

Deciphering *Rickettsia conorii* metabolic pathways: A treasure map to therapeutic targets

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ABSTRACT

Indian tick typhus is an infectious disease caused by intracellular gram-negative bacteria *Rickettsia conorii* (*R. conorii*). The bacterium is transmitted to humans through bite of infected ticks and sometimes by lice, fleas or mites. The disease is restricted to some areas with few cases but in last decade it is re-emerging with large number of cases from different areas of India. The insight in to genetic makeup of bacterial pathogens can be derived from their metabolic pathways. In the current study 18 metabolic pathways were found to be unique to the pathogen (*R. conorii*). A comprehensive analysis revealed 163 proteins implicated in 18 unique metabolic pathways of *R. conorii*. 140 proteins were reported to be essential for the bacterial survival, 46 were found virulent and 10 were found involved in resistance which can enhance the bacterial pathogenesis. The functional analysis of unique metabolic pathway proteins showed the abundance of plasmid conjugal transfer TrbL/VirB6, aliphatic acid kinase short chain, signal transduction response regulator receiver and components of type IV transporter system domains. The proteins were classified into six broad categories on the basis of predicted domains, *i.e.*, metabolism, transport, gene expression and regulation, antimicrobial resistance, cell signalling and proteolysis. Further, *in silico* analysis showed that 88 proteins were suitable therapeutic targets which do not showed homology with host proteins. The 43 proteins showed hits with the DrugBank database showing their druggable nature and remaining 45 proteins were classified as novel drug targets that require further validation. The study will help to provide the better understanding of pathogens survival and embark on the development of successful therapies for the management of Indian tick typhus.

1. Introduction

Indian tick typhus (ITT) is a spotted fever group Rickettsial disease caused by intracellular bacteria, *Rickettsia conorii* (*R. conorii*). Human acquire the pathogenic bacteria through bite from brown dog tick (*Rhipicephalus sanguineus*) which was first isolated from India in 1950. Dogs are the primary host associated with the brown dog tick and can serve as the competent reservoir of *R. conorii*.[1] Indian tick typhus is extremely prevalent in various regions of the country but mainly occurs in hilly forest areas. The cases of ITT have been reported from Maharashtra, Madhya Pradesh, Jammu and Kashmir, Uttarakhand, Haryana, Rajasthan, Tamil Nadu, Kerala, Sikkim, Manipur, Uttar Pradesh, Karnataka, and Himachal Pradesh.[2]

The symptoms of ITT include fever, skin rashes, and headache. The 'flu-like' symptoms that are the initial sign of rickettsial infection sometimes lead to a delayed or incorrect diagnosis. Misdiagnosis of ITT symptoms can lead to multiple diseases including abrupt kidney failure, pulmonary edema, interstitial pneumonia, neurological problems, and other multiorgan symptoms. The mortality rates for *R. conorii* infection can range from 3.2 to 32 % among infected humans.[3,4] The main target cells in humans for Rickettsial pathogens infection is endothelial cells where they multiply and start infecting. The bacteria pathogen then moves to lymph nodes through lymphatic vessels and to other visceral organs such as lungs, spleen, kidneys, and heart through circulation. According to CDC Yellow Book 2024 Rickettsial pathogens are highly virulent and can progress rapidly to severe illness. The immediate treatment with doxycycline or azithromycin is recommended for suspected patients and almost no other broad spectrum antibiotic provides

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effective treatment.[5–7] Chloramphenicol is the sole alternative antibiotic for treating Rickettsial infections but associated with high deaths (wwwnc.cdc.gov/travel/yellowbook/2024). Currently no vaccine is available for the prevention of disease and limited number of antibiotics for treatment of severe infection showed some necessity of developing alternative drug treatments. The metabolic pathways of an organism represents interactions between genes and their probable products which ultimately results in the formation of specific component of system.[8–10] These metabolic pathways contains considerable amount of information regarding their genetic makeup. The thoughtful elucidation of bacterial pathogens metabolic pathways can help in the identification of important genes involved in virulence and pathogenesis.[11,12] These genes may act as promising therapeutic candidates and can ultimately speed up the process of drug or vaccine development. Furthermore, *omics* and bioinformatics have gained much importance in biological research and attempts to extract the meaningful message for different datasets.[13–16] The current study reports the prediction of potential therapeutic targets by interpretation of *R. conorii* metabolic pathways using computational approach.

2. Methodology

2.1. Identification of metabolic pathways, analysis and protein retrieval

The Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used to download the metabolic pathways associated with *R. conorii* strain Malish 7 (pathogen) and *Homo sapiens* (host).[17] A comparison of the metabolic pathways between pathogen and host were performed to find that are specific to *R. conorii*. The pathways similar to both (pathogen and host) were excluded from the list and the rest were further analysed for the identification of genes involved in these pathways. The protein sequences involved in these pathways were retrieved which will be analysed for possible therapeutic targets. The detailed methodology is shown in Fig. 1, and Table 1 provides a sketch of the different bioinformatics tools and databases employed in the current study.

2.2. Essential, virulent and resistant genes analysis

The proteins present in specific metabolic pathways of *R. conorii* were evaluated for the essentiality using Database of Essential Genes version 15.2 (DEG 15.2).[18] The virulent proteins among metabolic pathway proteins were predicted at Virulence Factor Database version 6.0 (VFDB 6.0).[19] Resistance genes of *R. conorii* were predicted at Comprehensive Antibiotic Resistance Database 2023 (CARD 2023).[20] The blastp search was performed for similarity search with DEG, VFDB, and CARD at 10^{-5} e-value and 100 bit score as cut-off parameters.

Table 1
The various software's and databases used in the current research.

S. No.	Software/Databases	Link
1	Kyoto Encyclopedia of Genes and Genomes (KEGG)	https://www.genome.jp/kegg/
2	UniProt	https://www.uniprot.org/
3	Basic Local Alignment Search Tool (BLAST)	https://blast.ncbi.nlm.nih.gov/Blast.cgi
4	Database of Essential Genes (DEG)	http://origin.tubic.org/deg/public/index.php
5	Virulence Factor Database (VFDB)	http://www.mgc.ac.cn/VFs/main.htm
6	Comprehensive Antibiotic Resistance Database (CARD)	https://card.mcmaster.ca/
7	PsortB	https://www.psort.org/psortb/
8	CELLO	http://cello.life.nctu.edu.tw/
9	SOSUI GramN	https://harrier.nagahama-i-bio.ac.jp/sosui/sosuigramn/sosuigramn_submit.html
10	InterPro	https://www.ebi.ac.uk/interpro/
11	Expasy ProtParam	https://web.expasy.org/protparam/
12	Drug Bank	https://go.drugbank.com/

2.3. Subtractive genomics approach

The shortlisted essential, virulent or resistant proteins of *R. conorii* were evaluated for the homology analysis with the host proteins (*Homo sapiens*) using blastp search at National Centre for Biotechnology Information with e-value of 0.00001[59,21]. The protein sequences showing hits with the host proteins at set threshold were considered homologous proteins and rest were considered as non-homologous proteins which were considered for further evaluation.

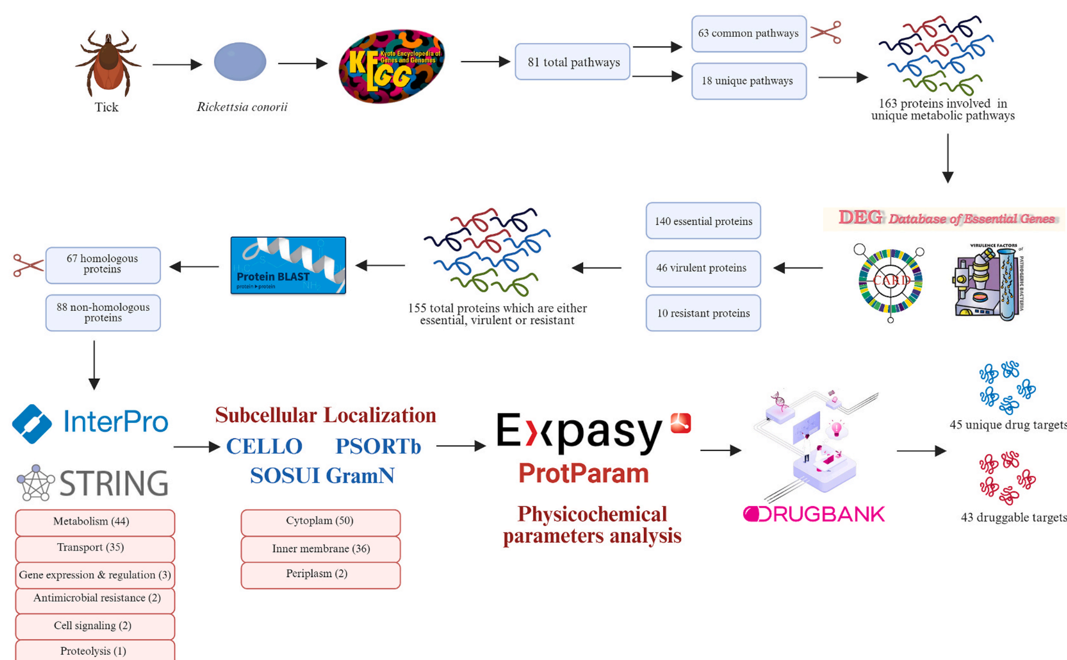

Fig. 1. The overall methodology and statistics of the study was depicted in the figure.

2.4. Subcellular localization of proteins

The identification of proteins location in bacterial cells helps in the precise function prediction. The location of protein sequences were predicted by CELLO v.2.5,[22] SOSUIGramN[23] and PsortB 3.0.[24] These tools are specialized and provide effective results for the subcellular localization of gram-negative bacterial proteins. These tools distinguish proteins according to their location in the bacteria, *i.e.*, extracellular, outer membrane, inner membrane, periplasm, and cytoplasm. Protein sequences were assigned to their specific locations based on the consensus of at least two of the utilized programs.

2.5. Functional annotation of metabolic pathway proteins

The InterproScan version 5.68–100.0 was utilized to analyse the conserved domain among the shortlisted proteins sequences.[25] The identification of conserved domains enables to classify proteins in to different functional classes.[26] InterproScan adds useful information from the InterPro database to enable users to gain a more comprehensive understanding of the significance of results.

2.6. Physiochemical characterization

ExPASy Protparam online tool was used for identification of different physiochemical properties of the proteins.[27] The tool helps to predict protein sequences number of amino acid residues, molecular weight, aliphatic index, theoretical pI, instability index, and grand average of hydrophaticity (GRAVY). All of these properties of proteins have an impact to scrutinize potential therapeutic targets.

2.7. Druggability analysis

DrugBank is a comprehensive online database that contains quantitative information on thousands of well-researched drugs and therapeutic targets, including chemical, physical, pharmacological, and biological data.[28] Homologous proteins that bind to drugs and drug-like compounds help to determine the ability of proteins to act as potential druggable targets. The druggability test for every shortlisted protein sequence was analysed using DrugBank. The search against DrugBank version 5.1.12. was performed at an expect value of 0.0001 for the prediction of potential druggable targets. Furthermore, the similarity search of shortlisted protein sequences was performed with the 17 strains of *R. conorii* (054, BIME, CH8-1, Sendai-29, Sendai-58, HCN-13, B8, Khabarovsk, IM16, A-167, TIGMIC, ITTR, XinjiangF3, XinjiangF2, XinjiangF1, XinjiangM1, and ISTTCDC1) to identify their presence across these strains.

3. Results and discussion

3.1. Unique metabolic pathways of R. conorii

We have identified 346 human metabolic pathways and 81 *R. conorii* metabolic pathways at KEGG. The metabolic pathways of *R. conorii* and humans were manually compared and found 63 metabolic pathways common in both the pathogen and host, while 18 metabolic pathways were unique to *R. conorii*. The 18 unique metabolic pathways were mentioned in Table 2 with pathway ID and number of proteins involved in these pathways. The highest number of proteins are involved in biosynthesis of secondary metabolites (76 proteins) followed by microbial metabolism in diverse environment (50 proteins), and bacterial secretion system (29 proteins). The quorum sensing pathway has also been found unique to the pathogen which contains 9 proteins. The quorum sensing helps to share information between bacterial cells regarding cell density and alter gene expression accordingly leading in the increased virulence of bacteria.[29] The two-component system is also the unique pathway found in *R. conorii* with the involvement of 20 proteins. The two-component system involved mechanism which supports bacteria for adapting to diverse changes in their environment. It was reported earlier that two-component system is linked with drug resistance and microbial pathogenesis in bacterial pathogens.[30] Lipopolysaccharide biosynthesis is an indispensable pathway for gram-negative bacteria as it is responsible for the synthesis of lipopolysaccharides and required for the virulence of intracellular bacteria.[31] There are 15 proteins involved in the biosynthesis of peptidoglycan which is a sac-like protective exoskeleton that helps bacteria to maintain cell shape, resist cytoplasmic osmotic pressure, protect bacteria form various environmental threats and crucial for their survival.[32]

Table 2
List of unique metabolic pathways of *Rickettsia conorii* with number of proteins involved in the specific pathway.

S. No.	Pathway name	Pathway ID	Protein list
1	Monobactam biosynthesis	rco00261	4
2	Lysine biosynthesis	rco00300	9
3	Benzoate degradation	rco00362	3
4	Cyanoamino acid metabolism	rco00460	2
5	Streptomycin biosynthesis	rco00521	2
6	Lipopolysaccharide biosynthesis	rco00540	11
7	O-Antigen nucleotide sugar biosynthesis	rco00541	7
8	Peptidoglycan biosynthesis	rco00550	15
9	Methane metabolism	rco00680	6
10	Biosynthesis of secondary metabolites	rco01110	76
11	Microbial metabolism in diverse environments	rco01120	50
12	Degradation of aromatic compounds	rco01220	2
13	beta-Lactam resistance	rco01501	5
14	Vancomycin resistance	rco01502	5
15	Cationic antimicrobial peptide (CAMP) resistance	rco01503	3
16	Two-component system	rco02020	20
17	Quorum sensing	rco02024	9
18	Bacterial secretion system	rco03070	29

A total of 258 proteins have been identified in the 18 unique pathways of pathogen and sequences of these proteins were retrieved from KEGG. The removal of 95 duplicate sequences provided the final list of 163 unique proteins that are part of the unique pathways of *R. conorii*. Every step in the pathway has already been thoroughly established necessary for the growth of bacteria. These unique metabolic pathways of pathogen contain a significant amount of information about the organism's genetic composition.[11] The 18 pathways considered in the study was exclusive to the intracellular pathogen *R. conorii* and are absent in humans.

3.2. Essential, virulent and resistant proteins of R. conorii

The availability of pathogen genome sequences has provided an abundance of data that may be helpful in developing therapeutic targets. Essential proteins play a key role in the pathogen's ability to survive and develop inside the host which have been depicted in earlier reports by different experimental and computational studies.[11,33] A total of 140 proteins out of 163 unique pathway proteins were found to be crucial for the survival of pathogen and can play an important role in metabolic pathways (Fig. 2; Table S1). These essential proteins may be indispensable for endurance under different environmental conditions and play decisive roles in various genetic and biological processes.[33,34] Earlier studies have also depicted the importance of essential proteins in bacterial pathogenesis and drug discovery process.[8,12]

The identification of proteins to be virulent or resistant is crucial for the identification of probable therapeutic targets. The presence of virulent and resistant proteins increases the pathogens ability to overcome host defence system and enable bacteria to overcome antimicrobial remedies by adapting demanding and competitive niches.[35] The

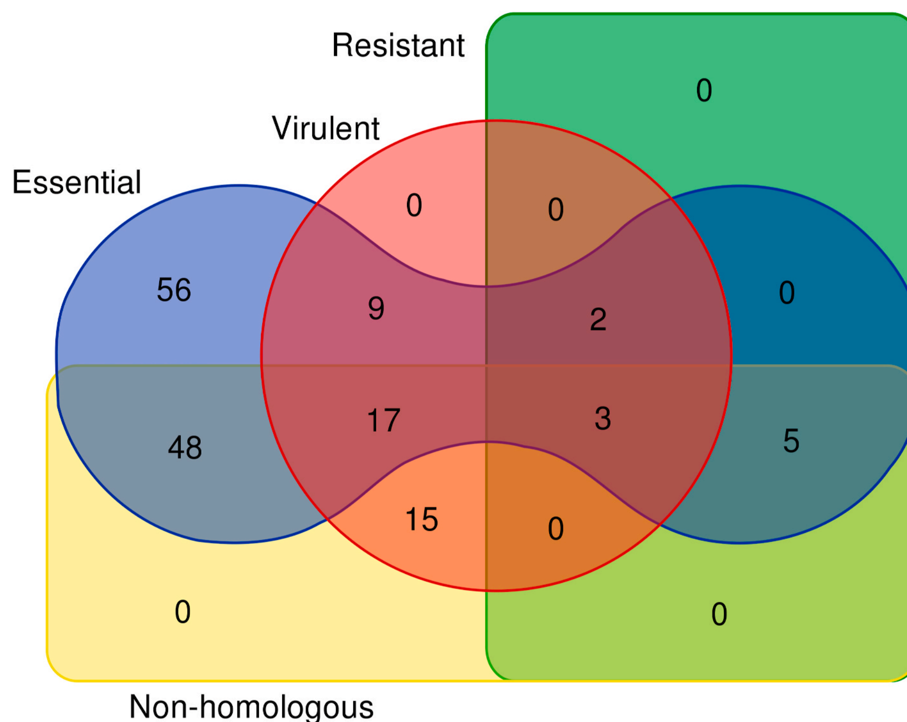

Fig. 2. The venn diagram represents the distribution of unique pathway proteins as essential, virulent, resistant and non-homologous to host proteins.

virulence factors and resistant proteins were examined among 163 unique pathway proteins by performing similarity search with VFDB and CARD respectively. A total of 46 proteins were predicted to be virulent and 10 proteins to be resistant (Fig. 2; Table S1). The predicted 46 virulent proteins may contribute to pathogenesis of *R. conorii*. Virulent proteins are secretory, cytosolic or membrane associated that support the bacteria to colonize the host at cellular level. The virulence factors enable bacteria to undergo fast adaptive shifts and aid in adhesion and evasion of bacterial cell.[36] The genomics studies have revealed that some lethal epidemic bacteria including *Rickettsia prowazekii* and *Mycobacterium leprae* which have restricted host range, holds reduced genome and contains large number of virulent proteins.[37] Furthermore, most of the virulent proteins identified in rickettsial pathogens are found to be the secretory in nature and part of Sec, T4SS and T5SS pathway.[38] The bacteria becomes less susceptible to different antimicrobial agents with the help of resistance proteins. The resistance to antimicrobial can be established through gene overexpression or the horizontal acquisition of resistance genes from other bacterial species.[39] According UN environment programme more than 1 million deaths were directly associated with drug resistance infections around the globe and can surge up to 10 million by 2050. Furthermore, World Health Organization (WHO) listed antimicrobial resistance among top 10 threats to health globally. Moreover, targeting the resistant proteins responsible for antimicrobial resistance in pathogenic bacteria can help to control the diseases.

3.3. Identification of R. conorii potential therapeutic targets

The proteins specific to pathogen were predicted using blastp search for 155 essential, virulent and resistant proteins against human proteome. A total of 67 proteins showed similarity with human proteins (homologous proteins) and 88 proteins did not showed any similarity with the human proteins (non-homologous proteins). The 88 non-homologous proteins presented the first set of therapeutic targets which can be used for drug discovery process (Fig. 2; Table S1). The remaining 67 proteins were eliminated since targeting them could cause the drug to become cross reactive and enhance cytotoxicity.[11]

3.4. Localization of metabolic pathway proteins

The subcellular localization of 88 non-homologous proteins showed 50 were cytoplasmic, 36 inner membrane and two periplasmic (Fig. 3; Table 3). The proteins found in cytoplasm and inner membrane was thought to be useful therapeutics for drug discovery process. The subcellular localization can reveal crucial details about protein function and interaction with other proteins and drug compounds. The precise location of proteins is also important for profoundly understanding function and construction of bacterial cell as these proteins are involved in various processes, *i.e.*, lipid biosynthesis, energy production, transport, protein secretion.[40,41]

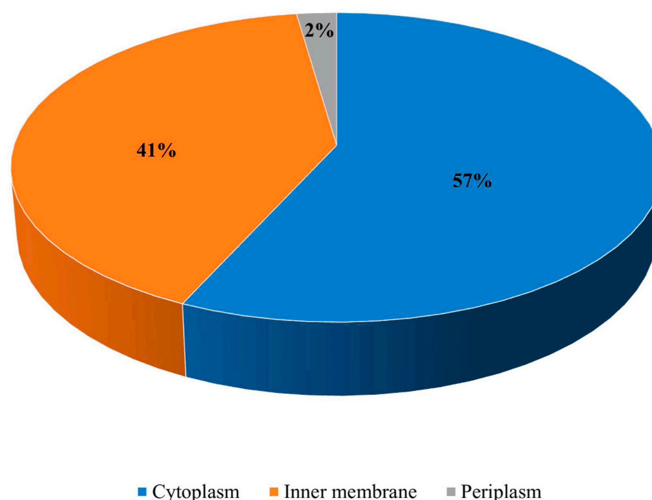

Fig. 3. Subcellular localization of shortlisted unique metabolic pathway proteins of *R. conorii*. Most of the proteins were localized in cytoplasm (57 %) followed by inner membrane (41 %) and periplasm (2 %).

Table 3
Functional classification of shortlisted metabolic pathway proteins which can serve as plausible therapeutic targets for drug discovery process. The table contains information regarding domain, function, broad class, subcellular localization and druggability.

Sequence ID	Domain	Function	Broad class	Subcellular localization	Drugability
RC0333	*D_ala_D_ala*	Cell wall biosynthesis	Metabolism	Cytoplasm	Yes
RC0883	*UDP_GlcNAc_COvinyl_MurA*	Catalyses first step of bacterial peptidoglycan biosynthesis	Metabolism	Cytoplasm	Yes
RC0128	*Ala_racemase*	Peptidoglycan synthesis	Metabolism	Cytoplasm	Yes
RC0321	*Sig_transdc_resp-reg_receiver*	Transfers the phosphoryl group to an internal receiver domain	Transport	Cytoplasm	Yes
RC0592	*His_kinase_dom*	Involved in signal transduction systems	Cell signalling	Inner membrane	Yes
RC0593	*WalR-like*	Transcription regulation	Gene expression and regulation	Cytoplasm	Yes
RC0849	*Sig_transdc_resp-reg_receiver*	Involved in regulation of transcription process	Gene expression and regulation	Cytoplasm	Yes
RC0893	*Preprotein_translocase_YajC*	Protein secretion	Transport	Inner membrane	No
RC0190	*DHDPR_bac*	Catalyses the second step of lysine biosynthesis	Metabolism	Cytoplasm	Yes
RC0430	*Asp_semialdehyde_DH*	Several important metabolic intermediates are produced, bacterial cell wall biosynthesis,	Metabolism	Cytoplasm	Yes
RC1164	*Asp_kinase*	Metabolise aspartic acid to produce four amino acids -lysine, threonine, methionine and isoleucine, biosynthesis of proline, Aspartate kinase catalyses the first step in the biosynthesis of Lys,met, and ser	Metabolism	Cytoplasm	No
RC0243	*DapD_Trfase_Hexpep_rpt_fam*	Metabolise aspartic acid to produce four amino acids -lysine, threonine, methionine and isoleucine, bacterial cell wall biosynthesis	Metabolism	Cytoplasm	Yes
RC0579	*DAP_epimerase_DapF*	Bacterial cell wall biosynthesis, and dipicolinic acid	Metabolism	Cytoplasm	No
RC0911	*UDP-N-AcMur_synth*	Peptidoglycan synthesis	Metabolism	Cytoplasm	Yes
RC0912	*UDP-N-AcMur-Glu-dNH2Pim_ligase*	Peptidoglycan synthesis	Metabolism	Cytoplasm	Yes
RC1086	*Glyco_hydro_3_N*	Hydrolyse the glycosidic bond between two or more carbohydrates	Metabolism	Cytoplasm	Yes
RC0008	*Lipid_A_LpxA*	Lipopolysaacharide synthesis	Metabolism	Cytoplasm	Yes
RC0010	*LpxD*	Involved in signal transduction	Cell signalling	Cytoplasm	No
RC0090	*KDO8P_synthase*	Lipopolysaacharide synthesis	Metabolism	Cytoplasm	Yes
RC0118	*Kdotransferase*	Lipopolysaacharide synthesis	Metabolism	Inner membrane	No
RC0339	*UDP-acyl_GlcNac_deAcase*	Lipopolysaacharide synthesis	Metabolism	Cytoplasm	Yes
RC0440	*Glyco_trans_19*	Transfer of sugar moieties from activated donor molecules to specific acceptor molecules	Transport	Cytoplasm	No
RC0524	*Cytidylyl_trans, KdsB*	Lipopolysaacharide synthesis	Metabolism	Cytoplasm	Yes
RC0661	*KdsD/KpsF-type*	Capsular polysialic acid biosynthesis	Metabolism	Cytoplasm	No
RC1091	*LipA_acyltrans*	Lipopolysaacharide synthesis	Metabolism	Cytoplasm	No
RC1092	*LpxK*	Phosphorylates the 4′-position of a tetraacyldisaccharide 1-phosphate precursor (DS-1-P) of lipid	Transport	Inner membrane	No
RC1110	*LpxI_C, LpxI_N*	Lipopolysaacharide synthesis	Metabolism	Cytoplasm	No
RC0457	*Polysac_CapD-like,CapD_C*	Bacterial polysaccharide biosynthesis	Metabolism	Cytoplasm	Yes
RC0458	*WecB-like*	Common antigen biosynthesis	Metabolism	Cytoplasm	Yes
RC0657	*Alpha-D-phosphohexomutase_SF*	Phosphoryl transfer on their sugar substrates	Transport	Cytoplasm	Yes
RC1039	*RPE1, NTP_transferase_dom*	Protein translations, transfer nucleotides from one compound to another	Transport	Cytoplasm	No
RC0249	*PBP_1c*	Peptidoglycan synthesis	Metabolism	Inner membrane	Yes
RC0331	*UDP-N-AcMur_Ala_ligase_MurC*	Peptidoglycan synthesis	Metabolism	Cytoplasm	Yes
RC0332	*MurB*	Bacterial cell wall biosynthesis	Metabolism	Cytoplasm	Yes
RC0536	*Peptidase_S11*	Protein degration	Proteolysis	Periplasm	Yes
RC0560	*MurD*	Peptidoglycan synthesis	Metabolism	Cytoplasm	Yes
RC0562	*GlcNAc_MurG*	Peptidoglycan synthesis	Metabolism	Cytoplasm	Yes
RC0852	*Penicillin-binding_protein_2*	Peptidoglycan synthesis	Metabolism	Inner membrane	Yes
RC0855	*PBP_dimer*	Peptidoglycan synthesis	Metabolism	Inner membrane	Yes
RC0910	*Glycosyl_transferase_4*	Peptidoglycan synthesis	Metabolism	Inner membrane	No
RC1245	*PCB_OB*	Peptidoglycan synthesis, atalyse the transfer of sugar moieties from activated donor molecules to specific acceptor molecules	Metabolism	Inner membrane	Yes
RC0147	*PTA_PTB*	Catalyse the transfer of an acetyl or butaryl group to orthophosphate	Transport	Cytoplasm	Yes
RC0149	*Aliphatic_acid_kin_short-chain*	Facilitates the production of acetyl-CoA by phosphorylating acetate in the presence of ATP and a divalent cation	Transport	Cytoplasm	No
RC0150	*Aliphatic_acid_kin_short-chain*	Production of acetyl-CoA by phosphorylating acetate in the presence of ATP and a divalent cation	Transport	Cytoplasm	No
RC0169	*SuccDH_FuR_B_TM-su, Succ_DH_anchor*	Provides the membrane anchor protein	Metabolism	Inner membrane	No
RC0294	*SAICAR_synth*	Catalyses the seventh step in the *de novo* purine biosynthetic pathway	Metabolism	Cytoplasm	Yes
RC0325	*PS_Dcarbxylase*	Catalyses the formation of phosphatidylethanolamine (PtdEtn) from phosphatidylserine (PtdSer)	Metabolism	Inner membrane	No
RC0326	*CDP-OH_P_trans*	Phospholipid biosynthesis	Metabolism	Inner membrane	No

(*continued on next page*)

Table 3 (*continued*)

Sequence ID	Domain	Function	Broad class	Subcellular localization	Drugability
RC0402	*RpiB_LacA_LacB*	Synthesis of ribose from other sugars, as well as the recycling of sugars from nucleotide breakdown	Metabolism	Cytoplasm	Yes
RC0711	*Plipid/glycerol_acylTrfase*	Phospholipid biosynthesis	Metabolism	Inner membrane	No
RC0744	*IPdP_isomerase*	Biosynthesis of isoprenoids via the mevalonate pathway	Metabolism	Cytoplasm	Yes
RC0783	*Pyruvate_phosphate_dikinase*	Catalyses the reversible conversion of ATP and pyruvate to AMP and PEP (phosphoenolpyruvate)	Transport	Cytoplasm	Yes
RC0801	*UbiX-like*	Involved in the third step of ubiquinone biosynthesis	Metabolism	Cytoplasm	Yes
RC1270	*PLipase_D/ transphosphatidylase*	Produces phosphatidic acid from phosphatidylcholine	Metabolism	Cytoplasm	No
RC1271	*UbiD*	Involved in ubiquinone biosynthesis	Metabolism	Cytoplasm	No
RC1308	*Citrate_synthase*	Catalyses the initial reaction of the tricarboxylic cycle	Transport	Cytoplasm	Yes
RC1372	*HemJ-like*	Essential for protoporphyrinogen IX oxidase activity	Metabolism	Inner membrane	No
RC0507	ME_PTA	Catalyse the oxidative decarboxylation of malate into pyruvate	Metabolism	Cytoplasm	Yes
RC0671	*Gln_synth_cat_dom*	Plays an essential role in the metabolism of nitrogen by catalysing the condensation of glutamate and ammonia to form glutamine	Metabolism	Cytoplasm	Yes
RC1174	*NAD_Glu_DH_bac*	Catalyse the reversible oxidative deamination of glutamate to ketoglutarate and ammonia	Metabolism	Cytoplasm	No
RC0535	*MFS*	Represents a number of transmembrane transporters	Transport	Inner membrane	No
RC0288	*Cyt-d_ubiquinol_oxidase_su_1*	Oxidise ubiquinol and reduce oxygen as part of the electron transport chain	Metabolism	Inner membrane	Yes
RC0916	*Chromosome_initiator_DnaA-like*	Plays an important role in initiating and regulating chromosomal replication	Gene expression and regulation	Cytoplasm	No
RC0074	*YidC*	Involved in protein secretion processes	Transport	Inner membrane	No
RC0109	*SecG*	Protein secretion	Transport	Inner membrane	No
RC0175	*Translocase_SecE*	Protein secretion	Transport	Inner membrane	No
RC0879	*SecA*	Protein secretion	Transport	Cytoplasm	No
RC0986	*SecY*	Protein secretion	Transport	Inner membrane	No
RC0154	*SecD*	Protein secretion	Transport	Inner membrane	No
RC0390	*VirB11*	Involved in the type IV secretion system for DNA transfer	Transport	Cytoplasm	Yes
RC0894	*SecD*	Protein secretion	Transport	Inner membrane	No
RC1160	*TatA/E*	Translocation of proteins	Transport	Inner membrane	No
RC1215	*TatC*	Protein export	Transport	Inner membrane	No
RC1040	NTP_transferase_dom	Transfer nucleotides from one compound to another	Transport	Cytoplasm	No
RC0140	*T4SS_VirB3*	Transfer the DNA	Transport	Inner membrane	No
RC0141	*CagE_TrbE_VirB*	Export of proteins	Transport	Inner membrane	No
RC1217	*CagE_TrbE_VirB*	Export of proteins	Transport	Cytoplasm	No
RC0142	*Conjugal_tfr_TrbL/VirB6*	t-DNA transfer to plant cells	Transport	Inner membrane	No
RC0143	*Conjugal_tfr_TrbL/VirB6*	t-DNA transfer to plant cells	Transport	Inner membrane	No
RC0144	*Conjugal_tfr_TrbL/VirB6*	t-DNA transfer to plant cells	Transport	Inner membrane	No
RC0145	*Conjugal_tfr_TrbL/VirB6*	t-DNA transfer to plant cells	Transport	Inner membrane	No
RC0146	*Conjugal_tfr_TrbL/VirB6*	t-DNA transfer to plant cells	Transport	Inner membrane	No
RC0384	*Conjugal_tfr_TrbG/VirB9*	Export effector proteins to the extracellular milieu or the mammalian cell cytosol	Transport	Inner membrane	Yes
RC0388	*Conjugal_tfr_TrbG/VirB9/CagX*	Export effector molecules during infection	Transport	Periplasm	Yes
RC0385	*VirB8*	Bacterial virulence protein with cytoplasmic, transmembrane, and periplasmic regions	Antimicrobial resistance	Inner membrane	No
RC0387	*PtlE*	Bacterial virulence protein with cytoplasmic, transmembrane, and periplasmic regions	Antimicrobial resistance	Inner membrane	No
RC0389	*T4SS_VirB10/TraB/TrbI*	Transfer DNA to the plant host and the tra/trb systems are required for the conjugal transfer of the Ti plasmid between cells of Agrobacterium	Transport	Inner membrane	Yes
RC0391	*TraG/VirD4*	Essential for DNA transfer in bacterial conjugation	Transport	Inner membrane	No

3.5. Functional annotation of unique metabolic pathways proteins

InterproScan were utilized to further evaluate 88 unique pathway essential protein sequences for the existence of conserved and functional domains. Based on the functional annotation of predicted unique metabolic pathway proteins, the major domains found in proteins were Mur (6), conjugal_tfr_TrbL/VirB6 (5), aliphatic acid kin short-chain (2), Sig_transdc_resp-reg_receiver (2) and CagE_TrbE_VirB (2). The functional domain prediction of 88 unique metabolic pathways proteins and their literature study enabled the classification of these proteins into six broad functional classes, *i.e.*, gene expression and regulation (3), metabolism (45), cell signalling (2), transport (35), proteolysis (1), and antimicrobial resistance (2) (Fig. 4; Table 3).

The broad characterization of 88 unique pathway proteins were distributed across six different classes with metabolism (proteins involved in biosynthesis of cell wall, peptidoglycan, lipopolysaacharide, phospholipids and anabolic & catabolic process of various biomolecules) and transport (proteins involved in secretion, transfer of ions, nutrients & DNA and protein translocation) being the dominant categories. The metabolic pathway reconstruction analysis of 84 *Rickettsia* genomes documented earlier that most of the proteins involved in cell envelop biosynthesis, TCA cycle, secretion and protein translocation. Furthermore, the metabolic and transport network reconstruction identified 51 metabolites acquired from host by hijacking their metabolic pathways and required to compensate for degraded metabolic pathways.[42] A comparative analysis of *R. conorii* and non-pathogenic *R. montanensis* proteomes activate different proteomic expressions upon infection in macrophage cells. The *R. conorii* particularly triggered the synthesis of enzymes that plays roles in fatty acid β oxidation, tricarboxylic acid cycle oxidative phosphorylation, and membrane transporters.[43]

Majority of the proteins were found to be present in the category of metabolism (45 proteins). These proteins were found to be involved in the synthesis of peptidoglycan, phospholipid, lipopolysaccharides, bacterial cell wall, antigen, ubiquonone, isoprenoids and sugar. As an important glycopeptide polymer, the peptidoglycan layer of bacterial cell wall is crucial for maintaining cell structure and act as defence against osmotic pressure. A collection of Mur enzymes (Mur A, B, C, D, E, F, and G) is involved in the synthesis of peptidoglycan precursors that are absent in humans and are effective drug targets to block peptidoglycan synthesis.[32] Lipid_A_LpxA protein is involved in the catalysis of first step of lipid A biosynthesis and essential for the growth of gram-negative bacteria.[44] UbiD and UbiX are the proteins associated with decarboxylation of the 3-octaprenyl-4-hydroxybenzoate precursor resulting in the bacterial ubiquinone biosynthesis. The UniD function as decarboxylase whereas UbiX is involved in the production of cofactor precisely required for UbiD activity.[45,46]

A total of 35 proteins were found to function as transporters. These proteins were involved in transfer of DNA, sugar, export of proteins and other effector molecules during infection. The proteins involved in transport are crucial for the intracellular survival and making bacteria resistant as they are involved in efflux of drugs/antibiotics.[47] The Sec export pathway proteins (Sec A, D, E, G, and Y) were found as major proteins in the transport category. The Sec pathway in intracellular bacterial pathogens is highly conserved, ubiquitous and indispensable for the export of proteins in their unfolded state through SecYEG complex.[48] There are various bacterial pathogens (*Yersinia enterocolitica*, *Klebsiella pneumonia*, *Listeria monocytogenes* etc) which used Sec pathway for the transport of virulence factors across the cytoplasmic membrane.[49] The translocate of folded proteins occur through twin-arginine translocation (Tat) export pathway which is independent of Sec secretion system. TatA and TatC are the major subunits of Tat secretion system which together form complex machinery for Tat substrates. The proteins that are exported through Tat pathway have Tat signal sequence at N-terminus. The Tat pathway is functional in intracellular bacterial pathogens (*Mycobacterium tuberculosis* and *Rickettsia prowazekii*) and transport proteins that play prominent role in microbial pathogenesis.[50,51] The proteins VirB, VirB3, VirB6, VirB9, VirB10 and VirD4 were present in transport class. These proteins are the important constituents of bacterial type 4 secretory system (T4SS) which mediates the transport of DNA and proteins. The VirB proteins together assemble to form secretion machinery and VirD4 is responsible for recognizing and translocation of substrate. Antibiotic resistance genes are spread through T4SS between different bacterial species and influence adaptation of bacteria to various environmental changes.[52,53] Furthermore, *Ricketssia* and *Brucella* T4SS can mediate the translocation of effector molecules into the host cell cytoplasm.[54]

Three proteins have been found in the gene expression and regulation category having WalR-like, Sig_transdc_resp-reg_receiver and DnaA-like domains. These proteins are chiefly involved in regulation of replication, transcription and translation. WalR is a transcriptional regulatory protein involved in the modulating of ftsAZ operon ultimately regulating the bacterial cell division.[55] Furthermore, it was reported previously that WalR also plays an important role monitoring cell wall metabolism and formation of biofilm in bacterial pathogens.[56] Earlier it was documented that DnaA is responsible for the initiation of bacterial chromosomal replication which is a crucial step in the cell cycle.[57] VirB8 and PtlE are the two domains found in the antimicrobial resistance functional class. VirB8 is an essential assembly factor in gram-negative bacterial type IV secretion system (T4SS) which interacts with other proteins and directs assembly of membrane spanning complexes.[58] The PtlE protein possesses peptidoglycanase activity and this particular activity is required for bacterial toxin secretions.[59]

We have found two proteins that belong to the cell signalling category having His_kinase_dom and LpxD domain. The bacterial histidine

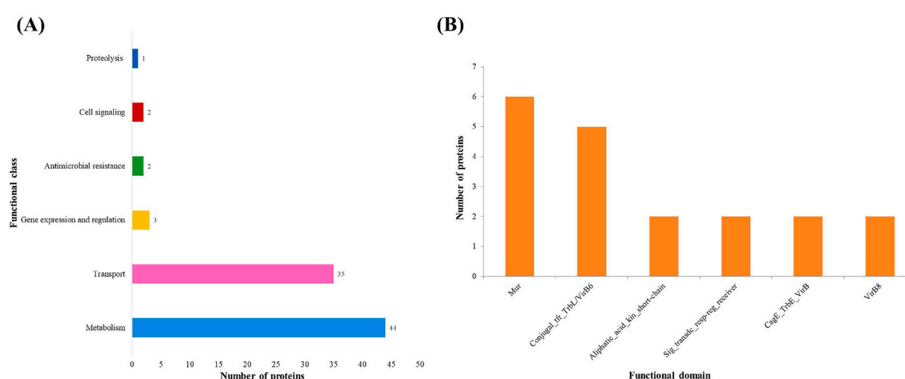

Fig. 4. Functional classification of 88 shortlisted unique metabolic pathway proteins which will serve as potential therapeutic targets for drug discovery process. The proteins are classified in to six broad functional classes, i.e., Metabolism, Transport, Gene expression and regulation, Antimicrobial resistance, Cell signalling and Proteolysis. (A) represents broad functional class and (B) represents major functional domains present in the unique metabolic pathway proteins.

kinase contains signalling components and is the integral component of signal transduction pathways in bacteria, regulating secretion systems, antimicrobial resistance and virulence.[60] LpxD in *Chlamydia trachomatis* catalyses the transfer of 3-hydroxy-arachidic acid onto UDP-3-*O*-myristoyl glucosamine for the biosynthesis of lipopolysaccharide.[61] In the class of proteolysis we have found only one protein containing Peptidases_S11 domain. The peptidase S11 have proteolytic activity that breakdown protein in to smaller polypeptides by cleavage of peptide bonds. It is involved in the biosynthesis of bacterial cell wall by cleaving the D-ala-D-ala cross links in the peptidoglycan.[62] There are many other important proteins which were listed with domains and function in Table 3. The human skin is home to vast array of microorganisms including bacteria, fungi and viruses known as skin microbiome. The skin microbiome have central role in maintaining health, breakdown of natural products and acting as protecting barrier to prevent the invasion of various pathogens.[63,64] The pathogenic bacteria able to adhere, grow, invade the human host and cause wide range of infections. The pathogenic bacteria have specific metabolic pathways which are absent in the skin microbiota that they utilize to enter, survive and colonize the host.[65,66]

3.6. Analysis of physiochemical characteristics

The analysis of physiochemical characteristics of 88 metabolic pathway proteins of *R. conorii* showed that molecular weight of proteins ranged from 30,030 Da to 181366.05 Da. The theoretical pI of 51 proteins was greater than 7 and 37 have lower than 7. The aliphatic index ranges from 78.67 to 143.57, which was thought to be a positive factor for the stability of globular proteins at high temperatures. GRAVY's highest value was 0.988 (RC1215), and its lowest value was −0.023 (RC1091) (Table S2). Proteins that have a low GRAVY score may interact with water molecules more effectively.[67] The molecular weight and physiochemical properties of the protein sequences play a crucial role in determining the final therapeutic targets. Proteins with molecular weights of 110 kDa are considered to be more suitable targets for drug development since they are easier to purify and function well under all conditions.[68]

3.7. Druggable targets prediction

An essential molecule that interferes with particular protein or some biological pathways specific to a disease state is known as a druggable compound. A therapeutic target differes from other biomolecules due to their respective location and precise function.[69] The DrugBank database was queried to find comparable druggable targets using the 88 unique pathway proteins of *R. conorii* that play a significant role in the metabolic pathways. A total of 43 proteins were predicted as druggable targets because they exhibited similarity with the drug targets present in the DrugBank database and rest 45 proteins were thought to be novel targets that require further validation (Fig. 1; Table 3). The identified druggable proteins may serve as the starting primers in the development of anti-*R conorii* drugs to treat Indian tick typhus. Moreover, the similarity search of shortlisted 88 proteins was performed across 17 other strains of *R. conorii*. We have identified large number of similar genes with high identity in these 17 strains. The detailed information was shown in Table S3. The identification of similar and unique genes across other *R. conorii* strains could be valuable for developing treatments targeting specific strains or broader range of *R. conorii* strains.[12]

4. Conclusion

In the present study, bioinformatics exploration of the metabolic pathways of *R. conorii* was performed to find best promising therapeutic candidates. There are 18 metabolic pathways which were unique to the pathogen and involved in the progression of bacterial pathogenesis. Moreover, the estimation of essentiality, resistant, virulent and homology search with host proteome assisted in the prioritization of therapeutic candidates. The functionality analysis of potential therapeutic candidates indicates the significance presence in peptidoglycan and lipopolysaccharides biosynthesis, export of effector molecules, breakdown of proteins into smaller polypeptides, signal transduction and antimicrobial resistance. The physiochemical properties and druggability analysis play a crucial role in determining the final therapeutic targets. To the best of our knowledge, this study represents an effort to shortlist proteins that could be effective candidates in designing diverse antimicrobials to address Indian tick typhus.

CRediT authorship contribution statement

Brijesh Prajapat: Writing – original draft, Investigation, Formal analysis. **Ankita Sharma:** Writing – original draft, Visualization, Validation, Methodology, Investigation. **Sunil Kumar:** Writing – review & editing, Resources, Project administration, Investigation, Funding acquisition. **Dixit Sharma:** Supervision, Software, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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