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Research article

Subtractive proteomics approach to Unravel the druggable proteins of the emerging pathogen *Waddlia chondrophila* and drug repositioning on its MurB protein

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ABSTRACT

Waddlia chondrophila is an emerging pathogen that has been implicated in numerous unpropitious pregnancy events in humans and ruminants. Taking into account its association with abortigenic events, possible modes of transmission, and future risk, immediate clinical measures are required to prevent widespread damage caused by this organism and hence this study. Here, a subtractive proteomics approach was employed to identify druggable proteins of *W. chondrophila*. Considering the essential genes, antibiotic resistance proteins, and virulence factors, 676 unique important proteins were initially identified for this bacterium. Afterward, NCBI BLASTp performed against human proteome identified 223 proteins that were further pushed into KEGG Automatic Annotation Server (KAAS) for automatic annotation. Using the information from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database 14 *Waddlia* specific metabolic pathways were identified with respect to humans. Analyzing the data from KAAS and KEGG databases, forty-eight metabolic pathway-dependent, and seventy metabolic pathway independent proteins were identified. Standalone BLAST search against DrugBank FDA approved drug targets revealed eight proteins that are finally considered druggable proteins. Prediction of three-dimensional structures was done for the eight proteins through homology modeling and the Ramachandran plot model showed six models as a valid prediction. Finally, virtual screening against MurB protein was performed using FDA approved drugs to employ the drug repositioning strategy. Three drugs showed promising docking results that can be used for therapeutic purposes against *W. chondrophila* following the clinical validation of the study.

1. Introduction

Emerging Infectious Diseases (EID) and Re-Emerging Infectious Diseases can be a great threat to microbiological public health and can have a large impact on socio-economical aspects [1]. An emerging pathogen can be characterized as the causative agent of an infectious disease whose frequency is expanding following its appearance either in the current host or in another host population because of long-term changes in its fundamental epidemiology [1]. Environmental changes, natural disasters, large population size are some of the key factors that have a significant contribution to the emergence and spread of infectious diseases [2, 3, 4, 5].

Waddlia chondrophila, a member of the *Chlamydiales* order, is an emerging pathogen that has gained attention over the years for showing implications in human and ruminant diseases [6, 7]. According to genomic and growth studies, it shows many similarities with some other well-characterized zoonotic chlamydial abortifacients (pathogen cause abortion), such as *Chlamydia abortus* [8]. Association between *W. chondrophila* and adverse pregnancy outcomes on women who had experienced sporadic or recurrent miscarriage has been repeatedly observed [9, 10, 11, 12, 13]. Viability and mitochondrial membrane potential of human spermatozoa were reduced when challenged and infected with *W. chondrophila* [14]. Studies also showed a high seroprevalence of *W. chondrophila* in men of infertile couples although the

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organism was seemed to be absent in semen which suggested a possible hypothesis that the negative effect on male infertility may be observed only during an ongoing infection [15]. The organism was shown to replicate in several human cell lines including fibroblasts, A549 pneumocytes, peripheral blood mononuclear cells, and in Ishikawa endometrial cells [16, 17, 18]. A recent study showed genital infection in the murine model, demonstrating a systemic burden of bacteria that spreads to various organs e.g. vagina, spleen, and lumbar lymph nodes [19]. The infamy of the organism is not only limited to the diseases of reproductive systems as other studies implicated its role in respiratory infections [20, 21]. A possible mode of transmission zoonotic exposure [11, 22] which is unsurprising as the bacteria has also been implicated in its role in bovine abortion [16, 23]. Although it has been reported that *W. chondrophila* was susceptible to generic drugs e.g. doxycycline & azithromycin and resistant to β-lactams & fluoroquinolones in cell cultures [24], no official drug is present for this organism to this date. Unfortunately, it is common for pathogens to acquire resistance against antibiotics [25]. Taking into account the possibility of antibiotic resistance over time and considering the damage caused by the organism, rapid actions are required to develop a therapeutic strategy at an early stage to combat this pathogen.

Subtractive proteomics analysis is a well-devised approach used to detect novel drug targets in the pathogen [26, 27]. In recent years, subtractive proteomics is efficiently used to discover species-specific vaccine candidates and in determining potential drug targets against various pathogenic bacteria [28, 29, 30]. To bypass the cross-reactions of drugs and specifically targeting a pathogenic entity subtractive proteomics has been seen to be especially helpful [29]. Further using this data, *in-silico, in-vitro* and *in-vivo* experiments can be employed to discover novel information on the organism. Conventional approaches to drug exploration and advancements are strenuous, sluggish, and expensive [31]. To reduce these drawbacks, *in-silico* methods can be employed as an alternative approach thereby facilitating numerous discoveries [28, 29, 30, 32, 33, 34, 35]. These methods are dynamic, rapid, and cost-effective than traditional drug discovery and development methods [31]. *In-silico* drug discovery methods exploit 'omics' data (i.e. genomics, proteomics, and metabolomics) and have been efficiently used for determining appropriate therapeutic drug targets in diverse infectious microorganisms such as bacteria and fungi. Drug repositioning, a strategy for discovering unprecedented uses for approved or investigational drugs that are not within the scope of the original medical indication [36], can also potentially lower the time-frame and monetary investment for drug development and most importantly reduce the chances of failure [37]. An example of drug repositioning is Zidovudine, the first anti-HIV drug approved by the Food and Drug Administration (FDA), which was originally intended to use for cancer treatment [37].

The current study combined and implemented the state of the art techniques – subtractive proteomics, molecular docking, and drug repositioning and, in turn, identified druggable proteins of *W. chondrophila* along with prospective repositioned drug against one of the druggable protein candidates namely UDP -N-acetylenol pyruvoyl glucosamine. We hope that the findings of this study will pave the way for further research and can provide sufficient foundations for designing a specific therapeutic regime against this pernicious pathogen.

2. Method

2.1. Identification of druggable proteins

The whole proteome (excluding plasmid proteins) of *W. chondrophila* was retrieved from UNIPROT (proteome id: UP000001505). To remove the paralogous sequences, the proteins were subjected to CD-HIT server [38] (=http://weizhong-lab.ucsd.edu/cdhit-web-server/cgi-bin/index.cgi?cmdcd-hit) to identify proteins with 60% sequence identity cut-off using the global sequence identity algorithm and keeping the rest of the parameters as default. The server curated a protein subset (PSet1) that contained non-duplicate sequences under the aforementioned constraints.

Important bacterial sequences were retrieved from different databases such as The Database of Essential Genes [39] (DEG) (http://tubic.tju.edu.cn/deg/), The Virulence Factor Database [40] (VFDB) (http://www.mgc.ac.cn/VFs/download.htm), and The Antibiotic Resistance Gene-ANNOTation [41] (ARG-ANNOT) database (AA V6 July 2019) (https://www.mediterranee-infection.com/acces-ressources/base-de-donnees/arg-annot-2/). PSet1 proteins were subjected to standalone BLAST [42] (v 2.6.0+) against the obtained protein sequences from DEG, ARG-ANNOT, VFDB with a cut-off of e-value $<$ 1e-4 and bit score $>$100 to determine the important proteins. The matched results against DEG, ARG-ANNOT, VFDB protein contained some overlap. Taking an entry once, the results collectively constructed a new protein set (PSet2) which contained all the unique entries.

NCBI BLASTp [43] (=https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGEProteins) was performed against the human proteome (taxid: 9606) with an e-value of 1e-10 using the PSet2. A new protein set (PSet3) was developed subtracting the BLAST matches with human proteins from PSet2. Afterward, PSet3 was provided to KEGG Automatic Annotation Server (KAAS) [44, 45] (https://www.genome.jp/kegg/kaas/) to perform functional annotation of proteins against the manually curated KEGG GENES of *W. chondrophila* using bi-directional best hit algorithm. The results generated from the server contained KEGG Orthology (KO) assignments as well as automatically generated KEGG pathway assignments (by assigning proteins to pathways with preset K-ids). Information regarding metabolic pathways of *W. chondrophila* and *Homo sapiens* were obtained from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Using the K-ids given in the database pathogen-specific pathways were identified. Thereafter, proteins unique to each pathogen-specific pathway were identified using KO-ids (assigned by KAAS) which collectively constructed a metabolic pathway-dependent protein subset (PSet-PD). Also, by comparing the K-ids of metabolic pathways of *W. chondrophila* from KEGG database against the KEGG BRITE results obtained from KAAS, metabolic pathway independent proteins (PSet-PI) were identified manually.

To evaluate the druggability of the proteins (PSet-PD & PSet-PI), a standalone BLAST search was done against FDA-approved drug targets obtained from the DrugBank [46] (https://www.drugbank.ca/) database (as of 2020-07-02) with a cut-off of e-value $<$ 0.005 and bit $>$100. An important thing to note is that there is no pre-established rule on how to set the BLAST cutoff criteria. Rather a general rule of thumb was followed where cut-offs were always kept in moderate range as a too stringent or too relaxed cutoff will generate higher false negative or false positive results respectively, a strategy seemed to be employed by other research groups as well [28, 29].

2.2. Subcellular localization & structure prediction of the druggable proteins

Subcellular localization using PSORTb [47] (https://www.psort.org/psortb/) and CELLO [48, 49] (http://cello.life.nctu.edu.tw/), Molecular weight using Compute pI/Mw tool [50] (https://web.expasy.org/compute_pi/), 3D structures using SWISS-MODEL [51] (https://swissmodel.expasy.org/) were determined for druggable proteins. Quality control for modeled structures was done with the aid of GMQE & QMEAN scores of SWISS-MODEL as well as the Ramachandran plots generated from PROCHECK server [52] (https://servicesn.mbi.ucla.edu/PROCHECK/).

2.3. Virtual screening against MurB and identification of repositionable drugs

The structure of UDP-N-acetylenol pyruvoyl glucosamine reductase (MurB) was based on the template PDB ID: 4PYT [53] (https://www.rcsb.org/structure/4PYT). The modeled protein was minimized in the GROMOS96 force field of Swiss PDB Viewer [54] (v4.1.0). The Auto Dock Tools [55] (ADT; v1.5.6) was used to generated a grid box (56 Å *

50 Å* 50 Å) with the center of 23.625, 50.019, 83.002 for X, Y, Z axes respectively. The ligand-docking site was guided by the bound flavin-adenine dinucleotide (FAD) in the crystal structure of the template. The FAD was extracted and docked to MurB to serve as the control for the virtual screening. The FDA approved drugs were fetched from BindingDB [56] (https://www.bindingdb.org/bind/ByFDAdrugs.jsp). 1279 drug molecules were selected for the virtual screening. They were minimized in the MMFF94 force field for 2000 steps with the convergence value of 1e-7 using the Open Babel Software [57] (v3.0.0). Molecular docking was performed using AutoDock Vina [58] (v1.1.2). The visualization of docking results was done in Discovery Studio (v20.1.0). The entire workflow in the methodology was provided as an illustration (Figure 1).

3. Results

3.1. Subtractive proteomics identified 8 druggable proteins

The proteome of *W. chondrophila* contained 1899 proteins. PSet1 was generated by removing 77 paralogous duplicate sequences using the CD-HIT [38] server. BLAST against databases of DEG [39], ARG-ANNOT [41], and VFDB [40] showed 653, 32, and 130 matches respectively totaling out 676 unique hits (PSet2) (see Table 1).

NCBI BLAST [43] search against the human proteome revealed 453 hits, which were excluded from further analysis, constituting PSet3 with 223 proteins. Comparing the metabolic pathways (KEGG database) of *W. chondrophila* and *Homo sapiens*, 22 pathways were found to be the *Waddlia* specific (Supplementary Table 1). KAAS [45] annotation assigned 65 pathways to the proteins provided to the server. Using bacteria-specific pathway data (Supplementary Table 1) from KEGG database, 14 were found unique to the bacteria among the 65 assigned pathways by KAAS annotation. Using the assigned K-ids of pathways and KO-ids of proteins, forty-eight proteins (PSet-PD) were selected (Table 1, Supplementary Table 2) for further analysis that were absent in multiple pathways among the 14.

Waddlia specific pathways. Also, KEGG BRITE data (from KAAS server) contained 61 K-id assignments to 70 proteins (PSet-PI), which were metabolic pathway independent (Table 1, Supplementary Table 3). These proteins were additionally kept for further analysis.

Search against the DrugBank [46] database using PSet-PD and PSet-PI showed eight matches (Table 2). The resultant druggable proteins were as follows – Riboflavin synthase, alpha subunit (D6YVD7), Putative cytochrome d ubiquinol oxidase subunit 2 (D6YVJ2), Chorismate synthase (D6YWA2), Glycerol-3-phosphate dehydrogenase [NAD(P)+] (D6YST7), Putative D-alanyl-D-alanine carboxypeptidase (D6YS23), UDP-N-acetylenol pyruvoyl glucosamine reductase (D6YVJ9), DNA topoisomerase (D6YVX0), Putative multidrug resistance protein MdtC (D6YSV9). Among the eight proteins, DNA topoisomerase and Putative multidrug resistance protein MdtC were metabolic pathways independent and the rest were metabolic pathways dependent. DrugBank database targets and their corresponding drugs were also provided along with the matched *Waddlia* proteins (Tables 1 and 2).

3.2. Analysis of 3D structure and subcellular localization of the druggable proteins

Additional information for the eight druggable proteins PSORTb [47], CELLO [48, 49], and Compute pI/Mw tool [51] summarized and provided (Supplementary Table 4). Employing homology modeling, the 3D structures of the eight proteins were constituted on SWISS-MODEL. GMQE and QMEAN4 values of SWISS-MODEL [51] were considered to develop the model and Ramachandran plot from PROCHECK [52] server results were taken into consideration for the validation of the predicted models (Figure 2; Supplementary Table 5). Models with below 90% of residues under acceptable regions were excluded from the further studies.

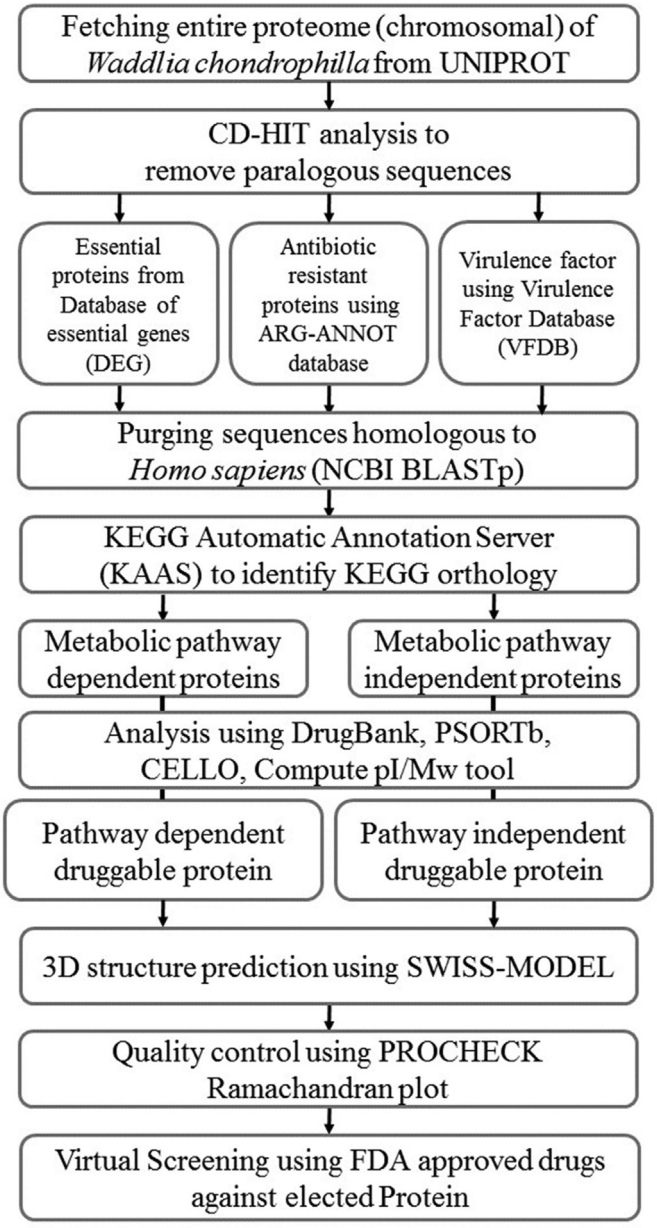

Figure 1. Illustration of the workflow of the entire research project.

3.3. Virtual screening identified 3 repositionable drugs against MurB

Virtual screening against UDP-N-acetylenol pyruvoyl glucosamine reductase (MurB; UNIPROT: D6YVJ9), showed various compound which showed higher affinity than the control FAD (-9.5 kcal/mol). Bupivacaine hydrochloride (L1; CHEMBL3288678; -12.0 kcal/mol), Nilotinib hydrochloride monohydrate (L2; CHEMBL255863; -11.5 kcal/mol), Regorafenib (L3; CHEMBL1946170; -11.4 kcal/mol) are the top three ligands which were finally selected for a more comprehensive analysis. The resulting interactions of these ligands and corresponding graphical representations were provided (Figures 3 and 4; Supplementary Table 6).

The control molecule (FAD) interacted with LYS56, SER94, SER96, ARG104, SER209, GLY115, THR18, ARG97, PRO117, ALA100 residues of the protein. L1 showed interactions with GLY129, ILE116, ALA210, GLY211, MET126, ALA128, and PRO117 among which GLY129 was the only one forming hydrogen bond and the rest of them are various hydrophobic interactions. There was only a single unfavorable bond with THR119 at a distance of 2.05218 Å. Although L1 shows fewer interactions compared to control, it had the highest binding affinity with a

Table 1. Subtractive proteomic and metabolic pathway analysis result for *Waddlia chondrophila*.

Features of *W. chondrophila*	Counts
Total number of proteins	1899
Paralogous in CD-HIT (>60% identity)	77
Non-paralogous	1822
Essential proteins from Database of essential genes (DEG) (e-value < 1e-4 & bit >100)	653
Antibiotic resistant proteins using ARG-ANNOT database (e-value < 1e-4 & bit >100)	32
Virulence factor using Virulence Factor Database (VFDB) (e-value < 1e-4 & bit >100)	130
Nonhuman Homologous Protein (E-value 1e-10)	223
Pathways unique to *W. chondrophila* (Using KEGG database)	22
Pathways unique to *W. chondrophila* (Matched in KAAS)	14
Metabolic pathway-dependent proteins	48
Metabolic pathway independent proteins	70
DrugBank (e-value < 0.005 & bit >100)	8

value of -12.0 kcal/mol. L2 showed binding energy of -11.5 kcal/mol taking the next to the top position. It formed hydrogen bond with residue ALA128, GLY129, GLN203, GLY122, ARG199 and halogen bond with ALA128, GLY129, ARG164. L2 also showed hydrophobic interactions with ALA128, PHE248, MET126, ILE116, PRO117, and ARG199 residues of MurB protein. L3 engaged in non-covalent interactions with SER58, ASN59. ARG199, GLY122, GLY115, GLY129, GLN203, PRO204, ARG164, PHE248, ALA123, ILE116, ALA128, PRO117, MET126 residues. The ligand had a binding affinity of -11.4 kcal/mol. PRO117 is present in all the interaction complexes constituting a hydrophobic Pi-Alkyl with the ligands.

4. Discussion

W. chondrophila is an emerging pathogen associated with both human and bovine disease [6, 7]. An study conducted by Baud et al. on 69 women with sporadic miscarriages, 200 with recurrent miscarriages and 169 control women with uneventful pregnancies, comprising a total of 438 women, the seroprevalence of anti-*Waddlia* IgG for women with sporadic miscarriages, recurrent miscarriages and control women 31.9%, 33%, and 7% respectively [11]. Overtime other studies documented the association of this bacteria in unpropitious pregnancy events [9, 10, 12, 13]. Due to the absence of commercially available standardized methods for the detection *W. chondrophila* in patients, the actual epidemiology is still not available. The possible modes of transmission can be zoonotic exposure, ingestion of contaminated meat or milk [11]. The water networks were also proposed to as the presence of *W. chondrophila* was detected in well water samples [59]. Considering the mode of transmissions mass order abortions can be possibility with women working in the farm and dairy industry being at the highest risk. The *a priori* clinical preparations are necessary in order to fight off such pathogens an attempt to which is the overarching goal of the current study.

In the study, the chromosomal proteins were first retrieved from UNIPROT. As it can abandoned by the organism under selection pressure, the utility of plasmid proteins for the purpose of this study was questionable and therefore excluded beforehand. Generally, a large protein dataset contains sequences that are highly similar (paralogous), and removing them reduces the volume of search space thereby saving time and computational resources. CD-HIT was used to remove the 77 duplicate sequences within the proteome that had >60% similar or related structure and function with other proteins in the proteome. This reduced the redundancy of the data constructing the protein subset PSet1.

To identify the important proteins of *W. chondrophila*, the aid of various databases was taken. The Database of Essential Genes (DEG) contains experimentally identified protein sequences that are indispensable for the organism. The Antibiotic Resistance Gene-ANNOTation (ARG-ANNOT) contains database consists of a single file covering nucleotide/protein sequences in FASTA format from all antibiotic classes. This is used to identify extant and putative new antibiotic resistance (AR) genes in bacterial genomes. The virulence factor database (VFDB) is a combined and extensive online resource for curating information about virulence factors of bacterial pathogens. Virulence factors are molecular entities that ameliorate bacterial colonization, immunosuppression, immunoevasion, etc. within the host, thereby facilitating the process of disease. Standalone BLAST searches against these databases helped to construct the PSet2 protein dataset containing 676 important proteins of the bacteria.

Even though PSet2 is important to bacteria, not all of them can be used as a drug target as some of the protein can potentially be associated with the metabolic pathway of the host. Targeting those proteins may eventually cause host toxicity & cross-reactions. KEGG Automatic

Table 2. Result summary of DrugBank database matched proteins of *Waddlia chondrophila*.

	Sl.	Protein Name	UNIPROT ID	DrugBank Target UNIPROT ID	DrugBank ID	DrugName	E-value	Bit Score
Metabolic Pathway Dependent	1	Riboflavin synthase, alpha subunit	D6YVD7	P0AFU8	DB00140	Riboflavin	1.12E-60	186
	2	Putative cytochrome dubiquinol oxidase subunit 2	D6YVJ2	A0A045ISQ8	DB05154	Pretomanid	1.28E-50	169
	3	Chorismate synthase	D6YWA2	P56122	DB03247	Flavin mononucleotide	1.06E-91	276
	4	Glycerol-3-phosphate dehydrogenase [NAD(P)+]	D6YST7	P21695	DB00157, DB00331	NADH, Metformin	9.54E-27	105
	5	Putative D-alanyl-D-alanine carboxypeptidase	D6YS23	P08506	DB00578, DB01000, DB01602, DB09050, DB09319, DB00274, DB00303, DB00430, DB01329, DB01331, DB01332	Carbenicillin, Cyclacillin, Bacampicillin, Ceftolozane, Carindacillin, Cefmetazole, Ertapenem, Cefpiramide, Cefoperazone, Cefoxitin, Ceftizoxime	1.24E-29	116
	6	UDP-N-acetylenol pyruvoyl glucosamine reductase	D6YVJ9	P61432	DB03147	Flavin adenine dinucleotide	3.22E-48	160
Metabolic Pathway Independent	7	DNA topoisomerase	D6YVX0	Q06AK7	DB00487, DB01051	Pefloxacin, Novobiocin	3.63E-36	142
	8	Putative multidrug resistance protein MdtC	D6YSV9	P31224	DB03619, DB04209	Deoxycholic acid, Dequalinium	1.39E-105	352

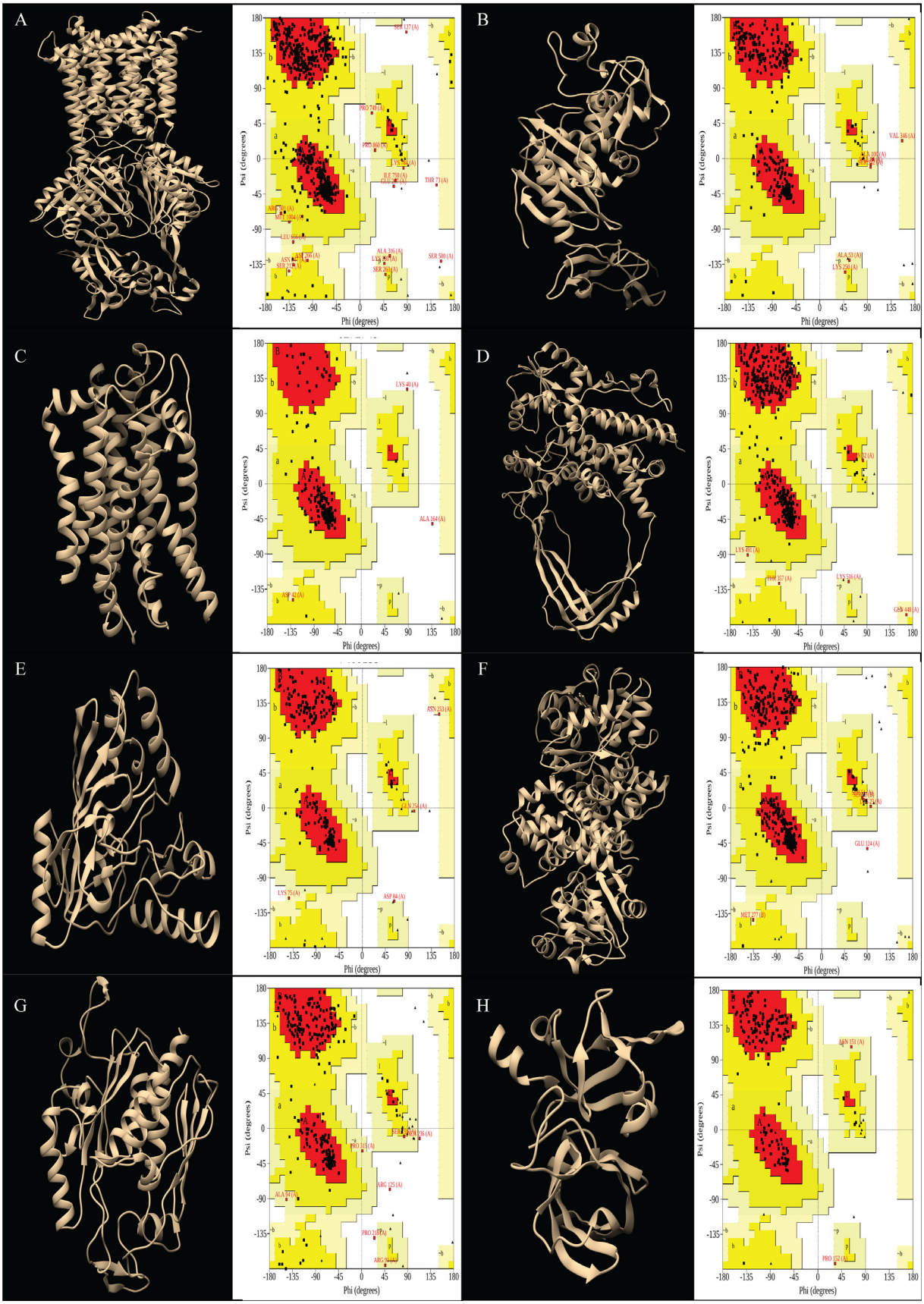

Figure 2. 3D structure (SWISS-MODEL) and corresponding Ramachandran plot (PROCHEK) for A. Putative multidrug resistance protein MdtC (D6YSV9), B. Putative D-alanyl-D-alanine carboxypeptidase (D6YS23), C. Putative cytochrome d ubiquinol oxidase subunit 2 (D6YVJ2), D. DNA topoisomerase III (D6YVX0), E. UDP-N-acetylenol pyruvoyl glucosamine reductase (D6YVJ9), F. Glycerol-3-phosphate dehydrogenase [NAD(P)+] (D6YST7), G. Chorismate synthase (D6YWA2), and H. Riboflavin synthase, alpha subunit (D6YVD7).

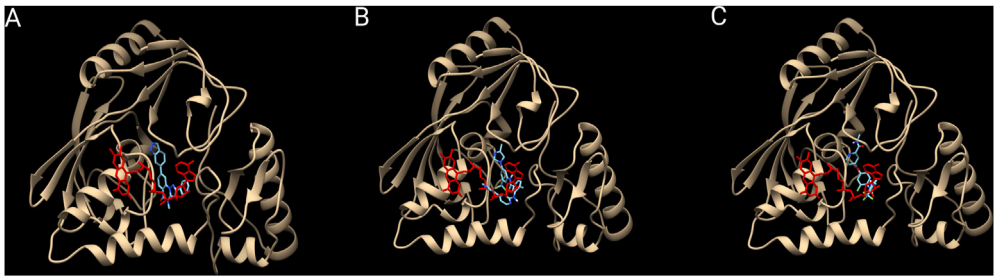

Figure 3. Image of UDP-N-acetylenol pyruvoyl glucosamine reductase docked with flavin-adenine dinucleotide (Red) and A. Bupivacaine hydrochloride (Blue), B. Nilotinib hydrochloride monohydrate (Blue), and C. Regorafenib (Blue).

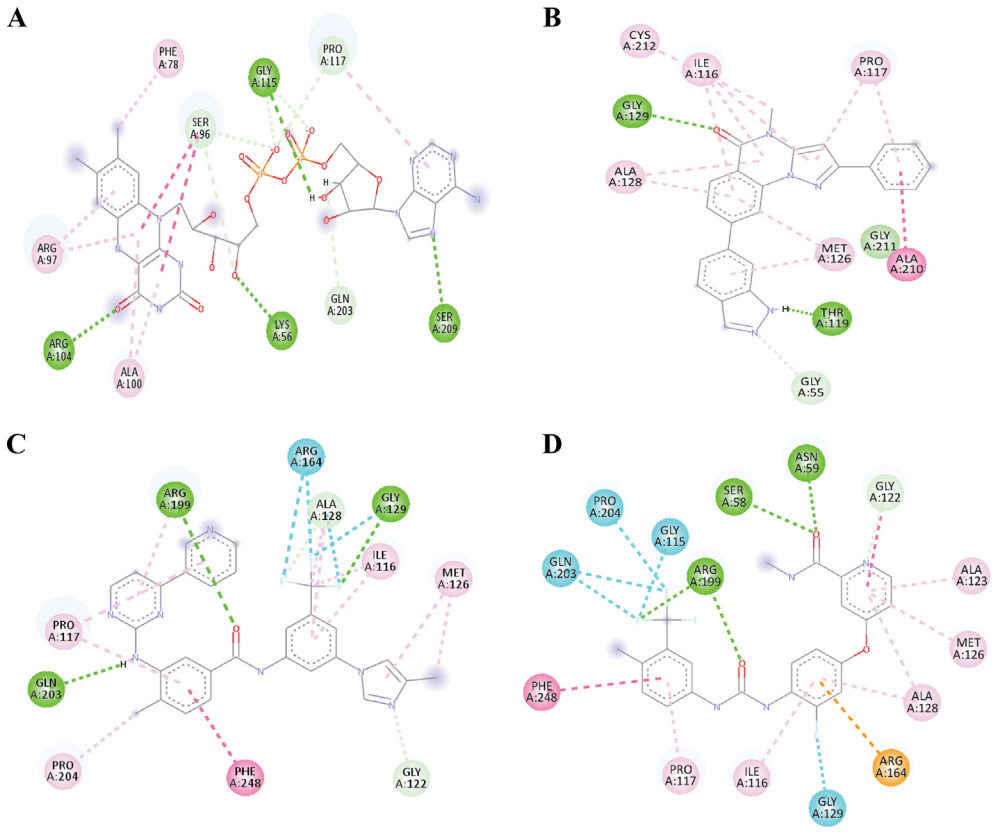

Figure 4. 2D interaction plots for UDP-N-acetylenol pyruvoyl glucosamine reductase docked with A. Flavin-adenine dinucleotide, B. Bupivacaine hydrochloride, C. Nilotinib hydrochloride monohydrate, and D. Regorafenib.

Annotation Server or KAAS annotations were used to circumvent this issue. KEGG (Kyoto Encyclopedia of Genes and Genomes) is a database resource for understanding high-level functionalities of biological systems with the aid of molecular-level information like high throughput sequencing or other experimental datasets. Metabolic pathway information of *W. chondrophila* and its host *Homo sapiens* are enlisted in the KEGG database with their respective K-ids. The pathways were retrieved from the database and compared manually to identify pathogen-specific pathways. Afterward, KO-ids were used to identify proteins that are present in only one pathway. This resulted in the PSet-PD dataset with 48 protein entries. KAAS annotation also gives information about KEGG BRITE, which is a collection of hierarchical classification systems encapsulating functional hierarchies of various biological objects. This contains additional information on proteins that are not metabolic pathway-dependent yet they perform key roles in biological systems. Using this information 70 metabolic pathway independent proteins (PSet-PI) were identified through manual search. Considering both the pathway dependent and independent proteins allow for addressing a broader range of targets within the microorganism hence enhancing the chances of identifying substantial druggable candidates.

Sequence similarity with a previously known drug target would facilitate the drug searching process immensely, aiding the rational drug discovery & repurposing/repositioning process. Therefore, PSet-PI & PSet-PD proteins were subjected to the standalone BLAST search against the DrugBank database. The DrugBank (https://www.drugbank.ca/) is a comprehensive online database, which accommodates a massive amount of data on drugs and drug targets. High similarity with FDA approved drug targets present in the database allows for a much safer and more specific therapeutic regime. Only eight proteins matched the cut-off criteria strictly (Table 2). Riboflavin synthase, alpha subunit (D6YVD7), Putative cytochrome d ubiquinol oxidase subunit 2 (D6YVJ2), Chorismate synthase (D6YWA2), Glycerol-3-phosphate dehydrogenase [NAD(P)+] (D6YST7), Putative D-alanyl-D-alanine carboxypeptidase (D6YS23), UDP-N-acetylenol pyruvoyl glucosamine reductase (D6YVJ9) were metabolic pathway-dependent and DNA topoisomerase (D6YVX0) & Putative multidrug resistance protein MdtC (D6YSV9) were metabolic pathway

independent. PSORTb and CELLO servers were used to predict the subcellular localization and the Compute pI/Mw tool was used to obtain the molecular weight (Supplementary Table 4) of the eight proteins that will help in formulating the drug delivery strategy. These druggable proteins play a myriad of crucial roles in the organism and the following are some of the highlights – Riboflavin synthase catalyzes riboflavin biosynthesis in bacteria [60]. Riboflavin is an indispensable micronutrient which acts as cofactor for numerous flavoproteins either directly or through derivatized form [61]. Cytochrome d ubiquinol oxidase participates in the reactions of the respiratory chain in many human-pathogenic bacteria. It is not structurally associated with mitochondrial cytochrome c oxidases and found exclusively on prokaryotes [62]. Biosynthesis of various aromatic compounds depends on the shikimate pathway which are responsible for electron transport, signaling, communication etc. Chorismate synthase is the seventh enzyme of the shikimate pathway which catalyzes the synthesis of chorismate from 5-enolpyruvylshikimate 3-phosphate. The enzyme has an absolute prerequisite of reduced flavin adenine mononucleotide as co-factor for function [63]. Glycerol-3-phosphate dehydrogenase breaks down glycerol-3-phosphate and produces energy [64]. D-alanyl-D-alanine carboxypeptidases are members of penicillin-binding proteins (PBPs) which are generally involved in peptidoglycan biosysnthesis. PBPs are sensitive to β-lactam antibiotics and constitute a family of acyltransferases with a unified evolutionary origin [65]. DNA topoisomerases are involved in controlling the topology of the DNA in the cellular environment. They exert their enzymatic prowess by causing transient single/double strand breakage in DNA which stabilizes DNA-protein covalent binding [66]. Multidrug resistant proteins belongs to the major facilitator superfamily [67, 68] which confers antibiotic resistance to bacteria upon overexpression [69]. These proteins also provide protection against a myriad environmental toxins through active extrusion of the pernicious compounds [70]. Finally, UDP-N-acetylenol pyruvoyl glucosamine reductase (MurB) is the product of murB gene which catalyzes the terminal steps of the formation of UDP-N-acetylmuramic acid in the peptidoglycan biosynthesis pathway [71].

The experimental structures of the eight selected proteins were absent in UNIPROT. Hence, the three-dimensional structures of the eight proteins were modeled using SWISS-MODEL, which is a fully automated service that uses homology-modeling to predict reliable protein models. For each protein, a template search was done followed by model construction. Predicted models that possessed the highest GMQE & QMEAN were taken for further analysis (Supplementary Table 5). To validate the reliability of the predicted models, Ramachandran plots were generated for all the models using the PROCHECK server. Two models were not found reliable as they did not have the minimum 90% residues inside the acceptable region (Supplementary Table 5). Subsequently, the study was geared towards identifying drug molecules that can effectively inhibit the critical proteins of *W. chondrophila*. Even though from a functional perspective all six protein candidates were prominent drug targets, the rest of the study only focused on MurB for the following reasons – Due to its involvement in peptidoglycan biosynthesis, MurB a prominent target against bacteria especially against its cell wall, and various researches had taken place on this protein to discover the inhibition strategy [72, 73, 74, 75]. Although putative D-alanyl-D-alanine carboxypeptidase was related to peptidoglycan biosynthesis pathway, the unsuccessful prediction of the structure led to exclusion of the protein from virtual docking study. Also, 4PYT was the only template structure that contained co-enzyme bound in the crystal structure which was eventually used to guide the ligand-binding site for virtual screening.

To implement the drug repositioning strategy, the FDA approved drugs obtained from BindingDB [56] were used in virtual screening. Using FDA-approved drugs has several advantages over using a random pool of drugs. For example, FDA approved drugs already have known indications and contraindications, which facilitates the administration process. For a large chunk of these drugs, their interaction network is known which also helps in using the drug field trials. This can allow for a more precise target search thereby accelerating the overall novel target discovery workflow. As FAD is co-factor of MurB, it served as the control in the study for molecular docking. Virtual screening using the FDA approved drug candidates identified various drugs that have a higher binding affinity than its co-factor FAD. Bupivacaine hydrochloride (L1), Nilotinib hydrochloride monohydrate (L2), and Regorafenib (L3) were the top three identified ligands with binding affinity -12.0 kcal/mol, -11.5 kcal/mol, and -11.4 kcal/mol respectively. L1 is a local anesthetic [76] that causes absence of pain sensation in a specific location of the body. It can be cardiotoxic [77], and able to shows dose dependent cytotoxicity [78] and arrhythmia [79]. L2 is a potent selective inhibitor of the BCR-ABL tyrosine kinase [80] used in the treatment of chronic myeloid leukemia that is resistant to imatinib. Some of side effects associate with L2 includes cardiotoxicity [81], elevated lipase and/or amylase levels, pancreatitis, hyperglycemia [82] etc. L3 is a diphenylurea multikinase inhibitor acting on VEGFR1-3, c-KIT, TIE-2, PDGFR-β, FGFR-1, RET, RAF-1, BRAF, and p38 MAP kinase [83]. L3 can cause cardiotoxicity [84], rash, alopecia, desquamation, fatigue, mucositis, hypertension, diarrhea, thyroid dysfunction [83]. L2 & L3 are used as anticancer agents that generally have higher adverse effects [81, 82, 85]. On the other hand, L1 has a favorable potency to toxicity ratio [86]. Therefore, we propose that primarily L1, followed by L2 and L3 as repositionable drugs for the MurB protein. Also, the structure of L1, L2 and L3 can be taken as a scaffold and newer drugs can be developed which has lower toxicity but higher inhibitory property.

In conclusion, an extensive computational approach had been taken in this study to narrow down the most 8 druggable proteins of *W. chondrophila* appropriate and three inhibitory compounds were proposed for one of those druggable proteins, MurB, using drug repositioning and virtual screening. Finally, further experimental validations are necessary to establish these findings and use proposed drugs and drug targets for clinical purposes.

Declarations

Author contribution statement

Umar Faruq Chowdhury: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Abdullah Al Saba: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Abu Sufian Sufi: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Akib Mahmud Khan, Ishrat Sharmin, Aziza Sultana, Md Ohedul Islam: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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