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Novel Drug Targets for Food-Borne Pathogen *Campylobacter jejuni*: An Integrated Subtractive Genomics and Comparative Metabolic Pathway Study

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Abstract

Campylobacters are a major global health burden and a cause of food-borne diarrheal illness and economic loss worldwide. In developing countries, *Campylobacter* infections are frequent in children under age two and may be associated with mortality. In developed countries, they are a common cause of bacterial diarrhea in early adulthood. In the United States, antibiotic resistance against *Campylobacter* is notably increased from 13% in 1997 to nearly 25% in 2011. Novel drug targets are urgently needed but remain a daunting task to accomplish. We suggest that omics-guided drug discovery is timely and worth considering in this context. The present study employed an integrated subtractive genomics and comparative metabolic pathway analysis approach. We identified 16 unique pathways from *Campylobacter* when compared against *H. sapiens* with 326 non-redundant proteins; 115 of these were found to be essential in the Database of Essential Genes. Sixty-six proteins among these were non-homologous to the human proteome. Six membrane proteins, of which four are transporters, have been proposed as potential vaccine candidates. Screening of 66 essential non-homologous proteins against DrugBank resulted in identification of 34 proteins with drug-ability potential, many of which play critical roles in bacterial growth and survival. Out of these, eight proteins had approved drug targets available in DrugBank, the majority serving crucial roles in cell wall synthesis and energy metabolism and therefore having the potential to be utilized as drug targets. We conclude by underscoring that screening against these proteins with inhibitors may aid in future discovery of novel therapeutics against campylobacteriosis in ways that will be pathogen specific, and thus have minimal toxic effect on host. Omics-guided drug discovery and bioinformatics analyses offer the broad potential for veritable advances in global health relevant novel therapeutics.

Introduction

Campylobacters are a major cause of food-borne diarrheal illness and result in a high morbidity and mortality rate, and economic loss in every region of the world (WHO, 2011). In developing countries, *Campylobacter* infections are frequent in children under age 2, sometimes leading to death. In industrialized nations, they are most frequently identified cause of bacterial diarrhea in early adulthood (CDC, 2000). According to a report released by Centers for Disease Control and Prevention (CDC), there are 1.3 million incidences of campylobacteriosis and there is rapid escalation of antibiotic resistance in *Campylobacter* from 13% in 1997 to almost 25% in 2011 in United States (CDC, 2013). The growing body of literature has documented that resistance to antibiotics such as quinolones, macrolides, tetracyclines, chloramphenicol, cephalosporins, and aminoglycosides is increasing rapidly in most parts of the world due to common and indiscriminate use of these agents (Akhtar, 1988; Engberg et al., 2001; Hoge et al., 1998; Reina et al., 1994). *Campylobacters* are highly important from a socioeconomic perspective, which strongly indicates a need for novel therapeutic targets with a high potential to improve quality of life and survival rates.

Since the publication of pathogenic bacterial genome sequences *Haemophilus influenzae* (Fleischmann et al., 1995) and *Mycoplasma genetalium* (Fraser et al., 1995), in 1995, the number of completed genome sequence for various microbial species has increased rapidly. These data in the post-genomic era have provided researchers with the possibility to exploit it fully for identification of novel therapeutic targets and have opened up new avenues for genome-wide application of comparative and subtractive genomics approaches for therapeutic intervention.

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The subtractive genomics approach has been advertently used by many researchers (Chawley et al., 2014; Ghosh et al., 2014; Samal et al., 2015; Sarangi et al., 2009) in search of novel drug targets for various microbes such as *Vibrio cholera*e, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Neisseria meningitides,* respectively. Genome sequences of several C*ampylobacter* species have been published including *Campylobacter concisus*, *C. curvus*, *C. fetus, C. hominis, and six strains of C. jejuni* (http://gcid.jcvi.org/projects/msc/campylobacter/). In this study we report the subtractive genomics approach integrated with comparative metabolic pathway analysis aimed at identifying novel therapeutic target proteins of *C. jejuni* pathogenic strain NCTC11168.

Methods

Various databases and tools as described in the workflow (Fig. 1) were utilized for the identification of putative therapeutic targets against *C. jejuni,* integrating a subtractive

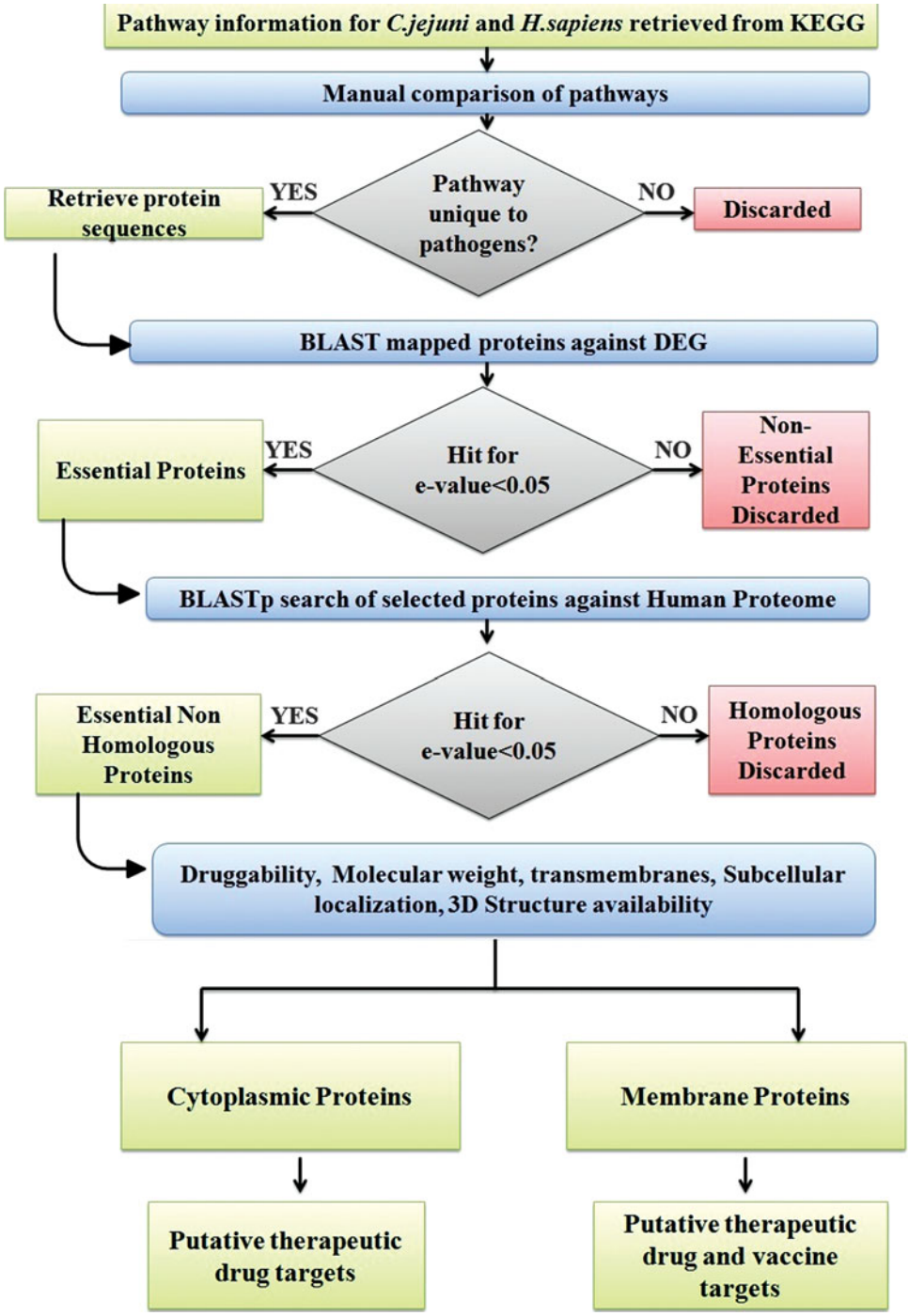

FIG. 1. Schematic workflow of comparative genomics approach to identify drug targets in pathogenic *C.jejuni* strain NCTC11168.

genomics approach with genome-wide comparative pathway analysis.

Host and pathogen comparative metabolic pathway analysis

The Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa and Goto, 2000), the most comprehensive resource of pathway information, was used for comparative genome-wide pathway analysis of *C. jejuni* and *Homo sapiens.* Manual comparison was done to identify the pathways unique to *C. jejuni* as per KEGG database annotations. Protein sequences for the enzymes involved in the unique pathways were retrieved from the Uniprot protein database.

Subtractive genomics and identification of essential non-homologous pathogen proteins

Essential non-homologous proteins from pathogen proteome were selected by a two-step comparison. In the first comparison, a subtractive genomics approach was applied to pathogen proteins from unique pathways. The BLAST search (Altschul et al., 1990) based on essentiality criteria was performed against the Database of Essential Genes (DEG version 10.9) (Zhang et al., 2004), which hosts records of essential genomic elements critical for an organism's survival, such as protein-coding genes and non-coding RNAs, among bacteria, archaea, and eukaryotes. An expectation value (e-value) threshold of 0.005 was used as filtering criteria for BLAST hits; with *C. jejuni* and *Helicobacter pylori* as background organisms against which a similarity search for identification of essential genes was performed. In the second step comparison, essential pathogen proteins were further screened on the basis of homology with the host proteome at an e-value cut off of 0.05. In the BLASTP search, proteins that did not have any hits below e-value inclusion threshold were selected to be essential non-homologous proteins from *C. jejuni*.

Prioritization of essential non-homologous proteins for therapeutic targets

The molecular and structural properties of essential non-homologous pathogen proteins were calculated for aid in prioritization of the drug targets that will most likely lead to effective treatments. Subcellular localization of identified proteins was predicted by PSORTb (Yu et al., 2010), which uses a Bayesian network model to calculate associated probability value for five major localization sites viz. cytoplasmic, inner membrane, periplasmic, outer membrane, and extracellular with *p*-value criteria 7.5. TMHMM server, which is a Hidden Markov Model (HMM)-based tool for prediction of alpha helices in membrane proteins, was used for transmembrane predictions (Krogh et al., 2001).

A search was performed to identify the proteins for which experimentally or computationally solved structures are available in PDB (Berman et al., 2000), Modbase (Pieper et al., 2006), or Protein Model Portal (Haas et al., 2013). A search of identified proteins in Uniprot was performed for retrieving information pertaining to molecular weight and existence of proteins. The information on protein existence provides different types of evidence for experimental characterization at protein and/or transcript level, homology inference, or uncertainty.

The VaxiJen server (Doytchinova and Flower, 2007) was employed to check the antigenicity of the membrane-localized proteins with a threshold of 0.6. The antigenic sequences were further screened for their ability to bind to MHC Class I molecule using ProPred-I (Singh and Raghava, 2003). ProPred-I implements proteasomal processing with matrices for 47 MHC Class-I alleles to identify the regions in the antigenic sequences that can act as potential MHC binders.

Drug-ability of essential son-homologous proteins

To evaluate the drug-ability potential, which is the ability of a particular biological target to bind with high affinity to known drugs of each identified therapeutic target, we subjected each protein to a BLASTP search against DrugBank with an e-value 0.01. DrugBank is a unique comprehensive resource that integrates drug data with drug target information at sequence, structure, and pathway levels (Wishart et al., 2008). DrugBank version 4.2 currently has information about 7737 drug entries that include 1585 FDA-approved small molecule drugs, 158 FDA-approved biotech (protein/peptide) drugs, 89 nutraceuticals, and over 6000 experimental drugs, along with 4281 non-redundant protein sequences (drug target/enzyme/transporter/carrier) linked to these drugs.

Results

Identification of unique metabolic pathways

The KEGG database initiated in 1995 is a computational representation for biological systems, integrating genetic information of genes and proteins with chemical and systemic information of molecular interaction and reaction networks. It links genetic building block information with higher order functional information. Currently KEGG database houses 87 different pathways for *C. jejuni* 11168 strain and 292 pathways for *H. sapiens*. As described in the workflow (Fig. 1), a manual comparison of host and pathogen pathways resulted in identification of 16 unique pathways to the pathogen (Table 1), with 71 remaining pathways being shared by both humans and *Campylobacters.* Furthermore, the proteins involved in unique pathways were identified.

Identification of essential non-homologous pathogen proteins

A total of 446 proteins were identified to be involved in 16 unique pathways. Few proteins were involved in more than one pathway, which resulted in 326 protein sequences from unique pathways after removing redundant protein sequences. A BLASTP search of these 326 protein sequences against 551 essential genes from *C. jejuni* and *H. pylori* in DEG revealed a total of 115 essential protein sequences with hits below e-value 0.05.

These essential protein sequences were further filtered out by homology search against human proteome for identification of non-homologous protein sequences. This comparison detected 66 essential non-homologous proteins with no hits against *H. sapiens* below e-value 0.05. This comparison was performed to identify proteins unique to pathogen so as to

Table 1. Metabolic Pathways Unique to *C. jejuni* Against Human Host and Proteins Associated with Corresponding Pathway

No.	Pathway name	Pathway ID	No. of proteins
1	Carbapenem biosynthesis	cje0033	2
2	Novobiocin biosynthesis	cje00401	4
3	D-Alanine metabolism	cje00473	2
4	Streptomycin biosynthesis	cje00521	3
5	Lipopolysaccharide biosynthesis	cje00540	20
6	Peptidoglycan biosynthesis	cje00550	14
7	C5-Branched dibasic acid metabolism	cje00660	7
8	Methane metabolism	cje00680	15
9	Biosynthesis of secondary metabolites	cje01110	167
10	Microbial metabolism in diverse environments	cje01120	88
11	beta-Lactam resistance	cje01501	5
12	Vancomycin resistance	cje01502	5
13	Two-component system	cje02020	43
14	Bacterial chemotaxis	cje02030	20
15	Flagellar assembly	cje02040	33
16	Bacterial secretion system	cje03070	18

avoid adverse effects on the human host (Butt et al., 2012), as the potential drug may also target host enzymes. These 66 essential non-homologous protein sequences represent the potential to be further exploited for therapeutic drug design against *C. jejuni*.

Prioritization of essential non-homologous proteins for therapeutic drug targets

Although all 66 essential non-homologous identified proteins are potential drug targets, these can be further filtered using additional prioritization parameters. Subcellular localization prediction using PSORTb program identified 63 proteins to be of cytoplasmic origin and all the essential non-homologous proteins were found to be <110 kDa. A search for availability of 3D structure identified seven proteins for which there was no structure available, while for six of these proteins experimentally solved structures were available. The remaining 53 proteins had computationally solved structures available in either Modbase or ProteinModel Portal. TMHHM server predicted 16 proteins that had one or more helices traversing the membrane, six of which were found to be antigenic above the specified threshold. ProPred-I server predicted MHC binder regions in all of the six proteins (murF, frdC, ccoP, secD, Cj1094c, and tatC) for different MHC class I alleles, and these proteins represent potential vaccine candidates as most of them are transporters, surface exposed proteins. All these results are presented in Table 2.

Drug-ability of potential drug targets

To examine the drug-ability of each of the essential non-homologous proteins, they were subjected to a BLASTP search against all the drug-targeted proteins in DrugBank database, which resulted in the identification of 34 *C. jejuni* proteins that shared high similarity to the binding partners of these drug-targeted proteins from DrugBank. Nine of these 34 proteins were FDA-approved drugs or nutraceuticals, while the remaining 25 were under experimentation. In Table 3 we have summarized the identified target protein binding partners of the all the drugs of 34 *C. jejuni* target proteins.

Discussion

The advent of a huge array of omics technologies has revolutionized the identification of drug targets (Russell et al., 2013). Examples include proteome scale comparative modeling of *Corynebacterium pseudotuberculosis* responsible for ulcerative lymphangitis, mastitis in ruminants (Hassan et al., 2014); RNA-seq profiling based target identification for non-small cell lung cancer (Riccardo et al., 2014); identification of markers for early prediction of preeclampsia using metabolic profiling (Kuc et al., 2014); computational systems biology approach for drug prioritization in *Clostridium botulinum* (Muhammad et al., 2014); and using protein–protein interaction network for drug target identification (Li et al., 2015).

The subtractive genomics approach is very efficient and has greatly accelerated the identification of relevant drug targets against many pathogens such as *M. tuberculosis* and *Bacillus anthracis* (Hosen et al., 2014; Rahman et al., 2014). Large-scale genomic projects such as the 1000 Genome and Encode are a rich source of background information and provide a deeper understanding of the genomes in relation to diseases. These computational approaches, together with the availability of genomic sequences, make it feasible for us to perform subtractive genomics and comparative pathway analysis aimed at identifying putative therapeutic drug targets and potential vaccine candidate proteins of *C. jejuni.*

Selecting drug targets using subtractive genomics approaches essentially relies on looking for those proteins that are absent in host but present in the pathogen, as it will minimize any adverse effects on host biology (Butt et al., 2012). Gene essentiality is also thought to be an important criteria for identification of therapeutic drug targets (Agüero et al., 2008). But there is a limitation to this approach as it fails to identify some targets such as hypoxanthine phosphoribosyl transferase as essential in *Plasmodium falciparum* (false negative) (Winzeler et al., 1999), while sometimes it yields false positives as in the case of dihydrofolate reductase in *Leishmania major* (Titus et al., 1995).

Gene essentiality prediction via experimental methods such as single gene knockouts, RNA interference, and conditional knockouts is labor-intensive, expensive, and time-consuming (Butt et al., 2012). Furthermore only a few infectious agents are amenable to experimental approaches of gene essentiality, as the tools for identification of drug or vaccine targets are often limited or absent for many pathogens. In such scenarios, computational methods for gene essentiality prediction seem to streamline the gap between the amount of data generated from sequencing projects and whole genome approaches for prediction of essential genes (Agüero et al., 2008; Doyle et al., 2010; Volker and Brown, 2002).

A previous work also identified the drug targets in *C. jejuni* by utilizing the CAI (Codon Adaptation Index) criterion as a measure of gene essentiality (Tilton et al., 2014) Essential genes are highly conserved, highly expressed, and

preferentially positioned in the leading strand. But high gene expression rates do not correlate significantly with the gene strand biasness and nonessential genes also show high expression rates (Perrière and Thioulouse, 2002; Rocha and Danchin, 2003), in which case the definition of essentiality based on high expression could be erroneous. In our study, we have utilized a subtractive genomics approach to predict genes essential to *C. jejuni* through homology search against experimentally predicted essentiality data from *C. jejuni* and *H. pylori* in DEG, both of which belong to the epsilon class of proteobacteria.

Significant advancements in genome sequencing and bioinformatics coupled with experimental data have shown that factors which are determinant of structural and molecular properties of proteins, such as molecular weight, subcellular localization, transmembrane prediction, and availability of 3D structure, can aid in prioritization of drug targets (Agüero et al., 2008) and maximize the likelihood of landing to the best therapeutic target against pathogen, considerably reducing the time and resources for developing such an agent.

Molecular mass prioritization identified all the proteins with weight <110 kDa; suggesting the possibility of experimental verification of the identified targets. Smaller proteins are easy to purify, and localization information of proteins can yield insights into protein function (Duffield et al., 2010; Yu et al., 2010). There were only few experimentally solved structures available in PDB, which points to a gap in the structural characterization of pathogen proteins, albeit *C. jejuni* first whole genome was published in 2000 (Parkhill et al., 2000). Protein structural information can be used to a significant advantage in drug identification and validation, greatly reducing the cost of high throughput experimental assays (Grant, 2009). Sixteen proteins predicted as transmembrane represent potential vaccine candidate proteins, which were further filtered based on antigenicity and MHC-binding criteria.

The likelihood of developing a drug-like compound to modulate the target is an important consideration that can aid drug design and an important determinant for a non-homologous protein to be a potential therapeutic target. The proteins for which drugs are already available can be useful starting points for drug discovery. The distribution of the essential non-homologous proteins was checked before and after similarity search against DrugBank (Fig. 2). It was noticed that there was $\sim$49% reduction in the number of proteins and 20% reduction in the number of pathways after DrugBank analysis, which leads to a shift in the pathway priority. Before DrugBank analysis biosynthesis of secondary metabolites, two-component system, microbial metabolism in diverse environments, peptidoglycan biosynthesis, bacterial secretion system, flagellar assembly, and lipopolysaccharide biosynthesis pathways were having maximum drug-able targets, but after DrugBank analysis, biosynthesis of secondary metabolites, peptidoglycan biosynthesis, two-component systems were the major pathways of drug-able targets.

Finally, we have narrowed down the search of therapeutic targets to final 14 prioritized drug targets and vaccine candidates. 5′-Methylthioadenosine/S-adenosylhomocysteine nucleosidase (pfs) enzyme catalyzes the direct conversion of aminodeoxyfutalosine (AFL) into dehypoxanthine futalosine (DHFL) and adenine via the hydrolysis of the N-glycosidic bond that represents an essential step in the menaquinone biosynthesis pathway (Li et al., 2011). This enzyme is an attractive drug target as it is involved in many pathways, such as ubiquinone and other terpenoid-quinone biosynthesis, cysteine and methionine metabolism, and biosynthesis of amino acids.

Alanine racemase (alr) catalyzes the pyridoxal 5′-phosphate-dependent interconversion of L-alanine and D-alanine. D-alanyl-alanine synthetase A (ddl) is involved in cell wall formation by joining two of the D-alanine residues together, catalyzing the formation of the ATP-dependent D-alanine-D-alanine dipeptide bond between the resulting D-alanine molecules. Inhibition of these two enzymes leads to effective inhibition of peptidoglycan synthesis; ddl has been proposed to be an attractive drug target in *Mycobacterium tuberculosis* (Prosser and de Carvalho, 2013). Vancomycin binds to ddl, a peptidoglycan precursor, forming a stable complex under normal conditions and inhibits cell wall synthesis (Howden et al., 2010), ultimately leading to cell lysis. Thus ddl plays an important role in the vancomycin resistance pathway.

Penicillin Binding Proteins (PBPs) are of special interest, as these are target sites for beta-lactam antibiotics. They also play an important role in cell wall formation. pbpA is important for cell division and essential for growth (Wada and Watanabe, 1998). pbpB also is critical for bacterial growth and cell wall biosynthesis (Pinho et al., 2001). pbpC is a major protein of a cell division complex. PBPs have already been utilized as model drug target system (von Rechenberg et al., 2005). PBPs are highly similar to the binding partners of many FDA-approved and experimental drugs. Hence PBPs can be considered of high potential for experimental validation as vaccine candidates.

UDP-N-acetylglucosamine 1-carboxyvinyltransferase (murA) and UDP-N-acetylenolpyruvoylglucosamine reductase (murB) both catalyze important reactions in peptidoglycan precursor synthesis. murA catalyzes transfer of an enolpyruvate residue from phosphoenolpyruvate (PEP) to position 3 of UDP-N-acetylglucosamine, followed by a MurB-catalyzed NADPH dependent reduction of the UDP-N-acetylglucosamine enolpyruvate to UDP-N-acetylmuramic acid. Majority of antibiotics in clinical use today target later steps of peptidoglycan synthesis (El Zoeiby et al., 2003). murB is essential in *Escherichia coli* (Pucci et al., 1992). murA to murF genes are all essential and highly conserved among bacterial species, thus holding a great promise as future therapeutic drug targets.

Both frdC (fumarate reductase cytochrome B subunit) and ccoP are important constituents of oxidative phosphorylation pathway for ATP formation, often called a molecular unit of energy transfer. While frdC couples the reduction of fumarate to succinate with the oxidation of quinol to quinine, ccoP (Cbb3-type cytochrome c oxidase subunit) is required for transfer of electrons from donor cytochrome c via its heme groups to CcoO subunit. secD (protein translocase subunit SecD) a part of the Sec protein translocase complex, tatC (Sec-independent protein translocase protein TatC) an important part of the twin-arginine translocation (Tat) system and Cj1094c (Putative preprotein translocase protein) transports large proteins across membranes. The twin-arginine translocation (TAT) pathway is important to bacterial growth and virulence (Ding and Christie, 2003; Lavander et al.,

Table 2. Essential Non-Homologous Proteins of *C. jejuni* with Results of Prioritization Analysis (Transmembrane and Cellular Location Prediction)

No.	Gene name	Protein Id	Pathway involved	Protein existence	Subcellular location	TMHMM	3D Exp	3D Model	MW (Da)
1	alr	Q9PP26	D-Alanine metabolism; Vancomycin resistance	Inferred	Cytoplasmic	No	No	Yes	37,256
2	ddl	Q9PPC2	D-Alanine metabolism; Vancomycin resistance; Peptidoglycan biosynthesis	Inferred	Cytoplasmic	No	No	Yes	39,908
3	lpxA	Q9PIM1	Lipopolysaccharide biosynthesis	Predicted	Cytoplasmic	No	Yes	Yes	28,651
4	lpxD	Q9PHU0	Lipopolysaccharide biosynthesis	Inferred	Unknown	No	No	Yes	34,679
5	lpxC	Q9PIZ5	Lipopolysaccharide biosynthesis	Inferred	Cytoplasmic	No	No	Yes	32,883
6	murA	Q9PP65	Peptidoglycan biosynthesis	Inferred	Cytoplasmic	No	No	Yes	45,186
7	murB	Q9PM01	Peptidoglycan biosynthesis	Inferred	Cytoplasmic	No	No	Yes	28,994
8	murC	Q9PNN7	Peptidoglycan biosynthesis	Inferred	Cytoplasmic	No	No	Yes	48,372
9	murF	Q0PA95	Peptidoglycan biosynthesis; Vancomycin resistance	Predicted	Cytoplasmic Membrane	Yes	No	Yes	55,234
10	pbpA	Q0PB07	Peptidoglycan biosynthesis; Beta-Lactam resistance	Predicted	Cytoplasmic Membrane	Yes	No	Yes	72,628
11	fba	Q0PAS0	Methane metabolism; Biosynthesis of secondary metabolites; Microbial metabolism in diverse environments	Predicted	Cytoplasmic	No	Yes	Yes	38,730
12	aroQ	Q9PJ53	Biosynthesis of secondary metabolites	Predicted	Cytoplasmic	No	No	Yes	17,595
13	pfs	Q0PC20	Biosynthesis of secondary metabolites	Predicted	Cytoplasmic	No	No	Yes	25,225
14	panD	Q9PIK3	Biosynthesis of secondary metabolites	Predicted	Cytoplasmic	No	Yes	Yes	13,974
15	panC	Q9PIK2	Biosynthesis of secondary metabolites	Predicted	Cytoplasmic	No	Yes	Yes	32,087
16	panB	Q9PIK1	Biosynthesis of secondary metabolites	Inferred	Cytoplasmic	No	No	Yes	30,202
17	pheA	Q0PBJ3	Biosynthesis of secondary metabolites	Predicted	Cytoplasmic	No	No	Yes	40,477
18	aroE	Q0PBA5	Biosynthesis of secondary metabolites	Inferred	Cytoplasmic	No	No	Yes	29,978
19	trpC	Q9PI11	Biosynthesis of secondary metabolites	Inferred	Cytoplasmic	No	No	Yes	29,573
20	ispH	P0C632	Biosynthesis of secondary metabolites	Inferred	Cytoplasmic	No	No	No	31,551
21	aroA	P0C630	Biosynthesis of secondary metabolites	Inferred	Cytoplasmic	No	No	Yes	47,288
22	aroB	Q9PNT2	Biosynthesis of secondary metabolites	Inferred	Cytoplasmic	No	No	Yes	39,596
23	dxr	Q9PMV3	Biosynthesis of secondary metabolites	Inferred	Cytoplasmic	No	No	Yes	39,548
24	Cj1370	Q0P8N9	Biosynthesis of secondary metabolites	Predicted	Cytoplasmic	No	No	Yes	17,035
25	aroC	Q9PM41	Biosynthesis of secondary metabolites	Predicted	Cytoplasmic	No	Yes	Yes	39,252
26	dapB	Q9PIT2	Biosynthesis of secondary metabolites; Microbial metabolism in diverse environments	Inferred	Cytoplasmic	No	No	Yes	26,696
27	rpiB	Q0P9X4	Biosynthesis of secondary metabolites; Microbial metabolism in diverse environments	Predicted	Cytoplasmic	No	No	Yes	16,293
28	pbpB	Q0PAZ1	Peptidoglycan biosynthesis; Beta-Lactam resistance	Predicted	Cytoplasmic Membrane	Yes	No	Yes	68,378
29	pbpC	Q0PAL6	Peptidoglycan biosynthesis; Beta-Lactam resistance	Predicted	Cytoplasmic Membrane	Yes	No	Yes	67,971

(*continued*)

TABLE 2. (CONTINUED)

No.	Gene name	Protein Id	Pathway involved	Protein existence	Subcellular location	TMHMM	3D Exp	3D Model	MW (Da)
30	frdC	Q0PBA2	Biosynthesis of secondary metabolites; Microbial metabolism in diverse environments; Two-component system	Predicted	Cytoplasmic Membrane	Yes	No	Yes	30,028
31	ccoP	Q0P8D0	Two-component system	Inferred	Periplasmic	Yes	No	Yes	31,162
32	cheA	Q0PBM2	Two-component system; Bacterial chemotaxis	Predicted	Cytoplasmic	No	No	Yes	85,300
33	cheV	Q0PBM1	Two-component system; Bacterial chemotaxis	Predicted	Cytoplasmic	No	No	Yes	35,858
34	cheY	P0C635	Two-component system; Bacterial chemotaxis	Inferred	Cytoplasmic	No	No	Yes	14,437
35	kdtA	Q0PAH2	Lipopolysaccharide biosynthesis	Predicted	Cytoplasmic	Yes	No	Yes	45,217
36	gmhB	Q6TG07	Lipopolysaccharide biosynthesis	Inferred	Cytoplasmic	No	No	Yes	20,246
37	waaC	Q0P9C1	Lipopolysaccharide biosynthesis	Predicted	Cytoplasmic	No	No	Yes	39,350
38	Cj0462	Q0PB51	Biosynthesis of secondary metabolites	Predicted	Cytoplasmic	No	No	Yes	39,759
39	ubiD	Q0PAX0	Biosynthesis of secondary metabolites	Predicted	Cytoplasmic	No	No	Yes	69,160
40	ispG	Q9PPM1	Biosynthesis of secondary metabolites	Inferred	Cytoplasmic	No	No	Yes	39,366
41	Cj0837c	Q0PA53	Biosynthesis of secondary metabolites	Predicted	Cytoplasmic	No	No	No	36,994
42	hisH1	Q0P8U2	Biosynthesis of secondary metabolites	Predicted	Cytoplasmic	No	No	Yes	22,699
43	Cj1368	Q0P8P1	Biosynthesis of secondary metabolites	Predicted	Cytoplasmic	No	No	Yes	41,278
44	hisB	Q9PM76	Biosynthesis of secondary metabolites	Inferred	Cytoplasmic	No	No	Yes	39,580
45	oorD	Q0PAY1	Microbial metabolism in diverse environments	Predicted	Unknown	No	No	Yes	11,443
46	oorA	Q0PAY0	Microbial metabolism in diverse environments	Predicted	Cytoplasmic	No	No	Yes	41,052
47	oorB	Q0PAX9	Microbial metabolism in diverse environments	Predicted	Cytoplasmic	No	No	Yes	31,208
48	oorC	Q0PAX8	Microbial metabolism in diverse environments	Predicted	Cytoplasmic	No	Yes	Yes	20,097
49	lysC	Q0PAT5	Biosynthesis of secondary metabolites; Microbial metabolism in diverse environments	Inferred	Unknown	No	No	Yes	42,676
50	csrA	Q0P9F1	Two-component system	Predicted	Unknown	No	No	Yes	8,441
51	Cj1153	Q0P9A3	Microbial metabolism in diverse environments; Two-component system	Predicted	Periplasmic	Yes	No	Yes	10,838
52	ccoN	Q0P8C7	Two-component system	Inferred	Cytoplasmic Membrane	Yes	No	Yes	55,888
53	ccoQ	Q0P8C9	Two-component system	Predicted	Unknown	Yes	No	No	10,376
54	fliE	Q9PHY8	Flagellar assembly	Inferred	Unknown	No	No	No	10,830
55	flgA	Q0PAC1	Flagellar assembly	Predicted	Cytoplasmic	No	No	Yes	25,114
56	flaD	Q0PA11	Flagellar assembly	Predicted	Extracellular	No	No	Yes	82,051
57	flaB	P56964	Two-component system; Flagellar assembly	Inferred	Extracellular	No	No	Yes	59,186
58	flaA	P56963	Two-component system; Flagellar assembly	Inferred	Extracellular	No	No	Yes	59,039
59	flaC	P96747	Two-component system; Flagellar assembly	Predicted	Periplasmic	No	No	Yes	26,606
60	secD	Q0P9G1	Bacterial secretion system	Inferred	Cytoplasmic Membrane	Yes	No	Yes	57,251
61	secF	Q0P9G2	Bacterial secretion system	Inferred	Cytoplasmic Membrane	Yes	No	Yes	36,112
62	secE	Q0PB41	Bacterial secretion system	Inferred	Cytoplasmic Membrane	Yes	No	No	6,745
63	secY	Q0P7U3	Bacterial secretion system	Inferred	Cytoplasmic Membrane	Yes	No	Yes	46,050
64	Cj1094c	Q0P9G0	Bacterial secretion system	Predicted	Cytoplasmic Membrane	Yes	No	Yes	10,172
65	tatB	Q9PHT7	Bacterial secretion system	Inferred	Cytoplasmic	No	No	No	15,746
66	tatC	Q9PHT8	Bacterial secretion system	Inferred	Cytoplasmic Membrane	Yes	No	No	27,808

Table 3. Essential Non-Homologous Proteins of *C.jejuni* Similar to Binding Partners of Drugs Available in Drug Bank by Homology Search

No.	Uniprot Id	Gene name	Protein name	Pathway involved	Drug Bank Id	Drug name	Drug group
1	Q9PP26	alr	Alanine racemase	D-Alanine metabolism, Vancomycin resistance	DB01993, DB02142, DB03097, DB03327, DB03579, DB03766, DB03801, DB04467, DB03252, DB03801, DB00260	N-(5′-Phosphopyridoxyl)-D-Alanine, Pyridoxamine-5′-Phosphate, Pmp-Hydroxyisoxazole, Pyridoxamine-5-Phosphate-Hydroxyisoxazole, {1-[(3-Hydroxy-Methyl-5-Phosphonooxy-Methyl-Pyridin-4-Ylmethyl)-Amino]-Ethyl}-Phosphonic Acid, Pyridoxyl-N,O-Cycloserylamide-5-Monophosphate, Propanoic Acid, Lysine Nz-Carboxylic Acid, Pyridoxyl-Alanine-5-Phosphate, D-Lysine, Lysine Nz-Carboxylic Acid, Cycloserine	Experimental (11); Approved
2	Q9PPC2	ddl	D-Alanine–D-alanine ligase	D-Alanine metabolism, Vancomycin resistance, Peptidoglycan biosynthesis	DB00260, DB07805	Cycloserine, 3-chloro-2,2-dimethyl-n-[4-(trifluoromethyl)phenyl]propanamide	Approved; Experimental
3	Q9PIM1	lpxA	Acyl-[acyl-carrier-protein]–UDP-N-acetylglucosamine O-acyltransferase	Lipopolysaccharide biosynthesis	DB01694, DB08558	D-tartaric acid, 2-hydroxymethyl-6-octylsulfanyl-tetrahydro-pyran-3,4,5-triol	Experimental (2)
4	Q9PHU0	lpxD	UDP-3-O-acylglucosamine N-acyltransferase	Lipopolysaccharide biosynthesis	DB01694, DB08558	D-tartaric acid, 2-hydroxymethyl-6-octylsulfanyl-tetrahydro-pyran-3,4,5-triol	Experimental (2)
5	Q9PIZ5	lpxC	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase	Lipopolysaccharide biosynthesis	DB07861, DBo1991, DB04257, DB07355, DB07536, DB08231	(2R)-N-hydroxy-3-naphthalen-2-yl-2-[(naphthalen-2-ylsulfonyl)amino] propanamide, Tu-514, Palmitoleic Acid, 3-(heptyloxy)benzoic acid, N-{(1S,2R)-2-hydroxy-1-[(hydroxyamino)carbonyl]propyl}-4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl} benzamide, myristic acid	Experimental (6)
6	Q9PP65	murA	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	Peptidoglycan biosynthesis	DB01879, DB02435, DB02995, DB03089, DB04174, DB04474, DB03397, DB00828,	(S)-2-{Methyl-[2-(Naphthalene-2-Sulfonylamino)-5-(Naphthalene-2-Sulfonyloxy)-Benzoyl]-Amino}-Succinicacid, Aminomethylcyclohexane, Cyclohexylammonium Ion, L-Iso-Aspartate, 3′-1-Carboxy-1-Phosphonooxy-Ethoxy-Uridine-Diphosphate-N-Acetylglucosamine, 1-Anilino-8-Naphthalene Sulfonate, Uridine-Diphosphate-N-Acetylglucosamine, Fosfomycin	Experimental (7); Approved
7	Q9PM01	murB	UDP-N-acetylenolpyruvoyl glucosamine reductase	Peptidoglycan biosynthesis	DB03147	Flavin adenine dinucleotide	Approved
8	Q9PNN7	murC	UDP-N-acetylmuramate–L-alanine ligase	Peptidoglycan biosynthesis	DB01673, DB03909, DB04395	Uridine-5′-Diphosphate-N-Acetylmuramoyl-L-Alanine, Adenosine-5′-[Beta, Gamma-Methylene]Triphosphate, Phosphoaminophosphonic Acid-Adenylate Ester	Experimental (3)
9	Q0PA95	murF	UDP-N-acetylmuramoyl-tripeptide D-alanyl-D-alanine ligase	Peptidoglycan biosynthesis, Vancomycin resistance	DB06970	2-chloro-n-(3-cyano-5,6-dihydro-4h-cyclopenta[b]thiophen-2-yl)-5-diethylsulfamoyl-benzamide	Experimental

(*continued*)

TABLE 3. (CONTINUED)

No.	Uniprot Id	Gene name	Protein name	Pathway involved	Drug Bank Id	Drug name	Drug group
10	Q0PB07	pbpA	Penicillin-binding protein	Peptidoglycan biosynthesis, beta-Lactam resistance	DB00229, DB00267, DB00301, DB00417, DB00456, DB00713, DB00833, DB00948, DB01000, DB01060, DB01061, DB01066, DB01112, DB01139, DB01147, DB01602, DB01604, DB01605, DB01150, DB00415, DB00438, DB00485, DB00493, DB00567, DB00607, DB01331, DB01603, DB08795, DB00274, DB00430, DB01327, DB01328, DB01329, DB01332, DB01333, DB01414 DB01415, DB01598, DB04570, DB04133, DB03313, DB08375 DB00447, DB01163, DB05659, DB00739, DB01140, DB00303	Cefotiam, Cefmenoxime, Flucloxacillin, Penicillin V, Cefalotin, Oxacillin, Cefaclor, Mezlocillin, Cyclacillin, Amoxicillin, Azlocillin, Cefditoren, Cefuroxime, Cefapirin, Cloxacillin, Bacampicillin, Pivampicillin, Pivmecillinam, Cefprozil, Ampicillin, Ceftazidime, Dicloxacillin, Cefotaxime, Cephalexin, Nafcillin, Cefoxitin, Meticillin, Azidocillin, Cefmetazole, Cefpiramide, Cefazolin, Cefonicid, Cefoperazone, Ceftizoxime, Cefradine, Cefacetrile, Ceftibuten, Imipenem, Latamoxef, Degraded Cephaloridine, Cephalosporin C, Pcnotaxime group, Loracarbef, Amdinocillin, faropenem medoxomil, Hetacillin, Cefadroxil, Ertapenem	Approved (39); Experimental (3); Withdrawn (2); Investigational; Approved, withdrawn (2); Approved, investigational
11	Q0PAS0	fba	Fructose-bisphosphate aldolase	Methane metabolism, Biosynthesis of secondary metabolites, Microbial metabolism in diverse environments	DB03026	Phosphoglycolohydroxamic Acid	Experimental
12	Q9PJ53	aroQ	3-Dehydroquinate dehydratase	Biosynthesis of secondary metabolites	DB03868, DB04698, DB02801, DB03739, DB02786, DB04347, DB04656, DB08485	3-Dehydroquinic Acid, N-(1,4-dihydro-5h-tetrazol-5-ylidene)-9-oxo-9h-xanthene-2-sulfonamide, 2,3 -Anhydro-Quinic Acid, 3-Hydroxyimino Quinic Acid, 2-Anhydro-3-Fluoro-Quinic Acid, 3-Dehydroshikimate, 1,3,4-trihydroxy-5-(3-phenoxypropyl)-cyclohexane-1-carboxylic acid, (1S,4S,5S)-1,4,5-trihydroxy-3-[3-(phenylthio) phenyl]cyclohex-2-ene-1-carboxylic acid	Experimental (8)
13	Q0PC20	pfs	Aminodeoxyfutalosine nucleosidase	Biosynthesis of secondary metabolites	DB07463, DB02158, DB02281, DB02933, DB08606, DB07649, DB02857, DB00173	(3R,4S)-1-[(4-amino-5H-pyrrolo[3,2-d]pyrimidin-7-yl)methyl]-4-[(butylsulfanyl)methyl]pyrrolidin-3-ol, (1s)-1-(9-Deazaadenin-9-Yl)-1,4,5-Trideoxy-1,4-Imino-5-Methylthio-D-Ribitol, Formycin, 5′-Deoxy-5′-(Methylthio)-Tubercidin, 3R,4S)-1-[(4-amino-5h-pyrrolo[3,2-d]pyrimidin-7-yl)methyl]-4-[(methylsulfanyl)methyl]pyrrolidin-3-ol, (3R,4S)-1-[(4-amino-5H-pyrrolo[3,2-d]pyrimidin-7-yl)methyl]-4-[(benzylsulfanyl) methyl]pyrrolidin-3-ol, Guanosine, Adenine	Experimental (7); Approved, Nutraceutical
14	Q9PIK3	panD	Aspartate 1-decarboxylase	Biosynthesis of secondary metabolites	DB02175, DB03382,	Malonic acid, S-Oxy Cysteine	Experimental (2)
15	Q9PIK2	panC	Pantothenate synthetase	Biosynthesis of secondary metabolites	DB01930, DB02596, DB02694, DB03107	2,4-Dihydroxy-3,3-Dimethyl-Butyrate, Alpha, Beta-Methyleneadenosine-5′-Triphosphate, Pantoyl Adenylate, Beta-Alanine	Experimental (4)
16	Q9PIK1	panB	3-Methyl-2-oxobutanoate hydroxymethyltransferase	Biosynthesis of secondary metabolites	DB03795, DB04074	2-Dehydropantoate, Alpha-ketoisovalerate	Experimental (2)
17	Q0PBJ3	pheA	Chorismate mutase/ prephenate dehydratase	Biosynthesis of secondary metabolites	DB08648, DB03884	8-hydroxy-2-oxa-bicyclo[3.3.1]non-6-ene-3, 5-dicarboxylic acid, 3-Phenylpyruvic Acid	Experimental (2)

(*continued*)

TABLE 3. (CONTINUED)

No.	Uniprot Id	Gene name	Protein name	Pathway involved	Drug Bank Id	Drug name	Drug group
18	Q0PBA5	aroE	Shikimate dehydrogenase	Biosynthesis of secondary metabolites	DB02363, DB03461, DB04447	2′-Monophosphoadenosine-5′-Diphosphate, 2′-Monophosphoadenosine 5′-Diphosphoribose, 1,4-Dithiothreitol	Experimental (3)
19	Q9PI11	trpC	Indole-3-glycerol phosphate synthase	Biosynthesis of secondary metabolites	DB03543	1-(O-Carboxy-Phenylamino)-1-Deoxy-D-Ribulose-5-Phosphate	Experimental
20	P0C632	ispH	4-Hydroxy-3-methylbut-2-enyl diphosphate reductase	Biosynthesis of secondary metabolites	DB01785, DB04714	Dimethylallyl Diphosphate, Isopentenyl Pyrophosphate	Experimental (2)
21	P0C630	aroA	3-Phosphoshikimate 1-carboxyvinyltransferase	Biosynthesis of secondary metabolites	DB04328, DB04539, DB01942, DB03116	Shikimate-3-Phosphate, Glyphosate, Formic Acid, 5-(1-Carboxy-1-Phosphonooxy-Ethoxyl)-Shikimate-3-Phosphate	Experimental (4)
22	Q9PNT2	aroB	3-Dehydroquinate synthase	Biosynthesis of secondary metabolites	DB02592	Carbaphosphonate	Experimental
23	Q9PMV3	dxr	1-Deoxy-D-xylulose 5-phosphate reductoisomerase	Biosynthesis of secondary metabolites	DB02496, DB02948, DB03649, DB04272	1-Deoxy-D-xylulose 5-phosphate, Fosmidomycin, [{(5-Chloro-2-Pyridinyl)Amino} Methylene]-1, 1-Bisphosphonate, Citric Acid	Experimental (3); Nutraceutical
24	Q0P8N9	Cj1370		Biosynthesis of secondary metabolites	DB02075	(1s)-1(9-Deazahypoxanthin-9yl)1, 4-Dideoxy-1,4-Imino-D-Ribitol-5-Phosphate	Experimental
25	Q9PM41	aroC	Chorismate synthase	Biosynthesis of secondary metabolites	DB03247, DB03350, DB03969, DB04267	Riboflavin Monophosphate, Cobalt Hexammine Ion, 3-Acetyl Pyridine Adenine Dinucleotide, Dipicolinic Acid	Experimental (4)
26	Q9PIT2	dapB	4-Hydroxy-tetrahydrodipicolinate reductase	Biosynthesis of secondary metabolites, Microbial metabolism in diverse environments	DB03969, DB04267	3-Acetyl Pyridine Adenine Dinucleotide, Dipicolinic Acid	Experimental (2)
27	Q0P9X4	rpiB	Ribose 5-phosphate isomerase	Biosynthesis of secondary metabolites, Microbial metabolism in diverse environments	DB03661, DB03108, DB04496	Cysteinesulfonic Acid, 4-Phospho-D-Erythronate, 4-Phospho-D-Erythronohydroxamic Acid	Experimental (3)

(*continued*)

TABLE 3. (CONTINUED)

No.	Uniprot Id	Gene name	Protein name	Pathway involved	Drug Bank Id	Drug name	Drug group
28	Q0PAZ1	pbpB	Putative penicillin-binding protein	Peptidoglycan biosynthesis, beta-Lactam resistance	DB00274, DB00430, DB00438, DB01327, DB01328, DB01329, DB01331, DB01332, DB01414, DB01415, DB01598, DB04570, DB00319, DB00415, DB00456, DB00485, DB00493, DB00567, DB00607, DB00713, DB01603, DB08795, DB00303, DB08401, DB03313, DB08508, DB00739, DB01140, DB01163	Cefmetazole, Cefpiramide, Ceftazidime, Cefazolin, Cefonicid, Cefoperazone, Cefoxitin, Ceftizoxime, Cefacetrile, Ceftibuten, Imipenem, Latamoxef, Piperacillin, Ampicillin, Cefalotin, Dicloxacillin, Cefotaxime, Cephalexin, Nafcillin, Oxacillin, Meticillin, Azidocillin, Ertapenem, (2e)-2-({(2s)-2-carboxy-2-[(phenoxyacetyl)amino]ethoxy}imino) pentanedioic acid, Cephalosporin C, N-benzoyl-D-alanine, Hetacillin, Cefadroxil, Amdinocillin	Approved (22); Approved, Investigational; Experimental (3); Approved, withdrawn (2); Withdrawn;
29	Q0PAL6	pbpC	Penicillin-binding protein	Peptidoglycan biosynthesis, beta-Lactam resistance	DB01327	Cefazolin	Approved
30	Q0PBA2	frdC	Fumarate reductase cytochrome B subunit	Biosynthesis of secondary metabolites, Microbial metabolism in diverse environments, Two-component system	DB07669	2,3-dimethyl-1,4-naphthoquinone	Experimental
31	Q0P8D0	ccoP	Cbb3-type cytochrome c oxidase subunit	Two-component system	DB02949, DB03224	2-Acetyl-Protoporphyrin Ix, 2-Formyl-Protoporphryn Ix	Experimental (2)
32	Q0PBM2	cheA	Chemotaxis histidine kinase	Two-component system, Bacterial chemotaxis	DB02524, DB03909, DB04395	Spiro(2,4,6-Trinitrobenzene[1,2a]-2o',3o'-Methylene-Adenine-Triphosphate, Adenosine-5′-[Beta, Gamma-Methylene]Triphosphate, Phosphoaminophosphonic Acid-Adenylate Ester	Experimental (3)
33	Q0PBM1	cheV	Chemotaxis protein	Two-component system, Bacterial chemotaxis	DB02355, DB02596, DB07706, DB02461, DB03487, DB04156	Adenosine-5′-Rp-Alpha-Thio-Triphosphate, Alpha,Beta-Methyleneadenosine-5′-Triphosphate, 2,3,17beta-trihydroxy-1,3,5(10)-estratriene, S-Methyl Phosphocysteine, 3-Aminosuccinimide, Aspartate Beryllium Trifluoride	Experimental (6)
34	P0C635	cheY	Chemotaxis protein	Two-component system, Bacterial chemotaxis	DB02461, DB03487, DB04156,	S-Methyl Phosphocysteine, 3-Aminosuccinimide, Aspartate Beryllium Trifluoride,	Experimental (3)

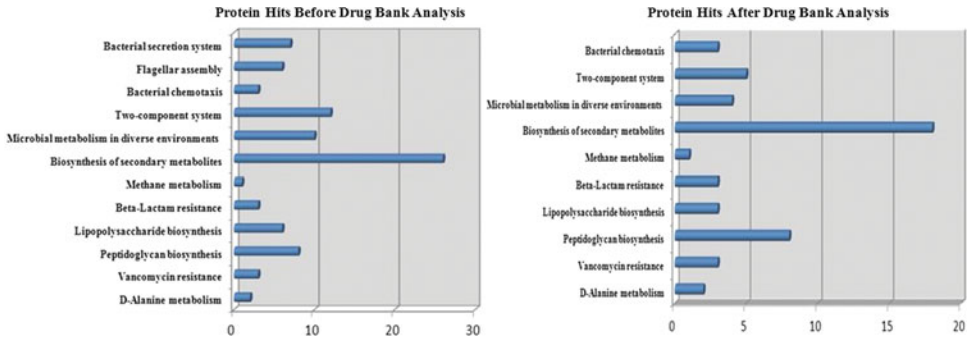

FIG. 2. Distribution of essential non-homologous proteins of *C.jejuni* in different pathways before and after drug bank search for drug-ability potential evaluation.

2007). secD and Cj1094c help in secretion across the inner membrane via preprotein translocase pathway. Transport proteins are associated with pathogenesis and virulence and have been identified as potential vaccine candidates in several previous studies as well (Garmory and Titball, 2004; Harris et al., 2011).

Peptidoglycan is an important component of bacterial cell wall, responsible for maintaining a definite cell shape and primarily conferring mechanical resistance to higher osmotic pressure (Vollmer et al., 2008). Any interference with peptidoglycan biosynthesis will result in cell lysis. Peptidoglycan biosynthesis, the pathway with largest distribution of final identified drug targets, can be exploited for therapeutic drug targets owing to its multiple target enzymes whose inhibition could lead to disruption of cell well and in turn attenuate bacterial cell growth.

In summary, the computational subtractive genomics approach integrated with comparative pathway analysis resulted in a significant reduction in the number of protein targets (Fig. 3) at each step. Thus we were able to identify several essential proteins critical for bacterial growth and survival and with minimum toxicity to host. Further studies are warranted to validate these findings by *in vitro* and *in vivo* experiments for effective drug design against *Campylobacter* infections.

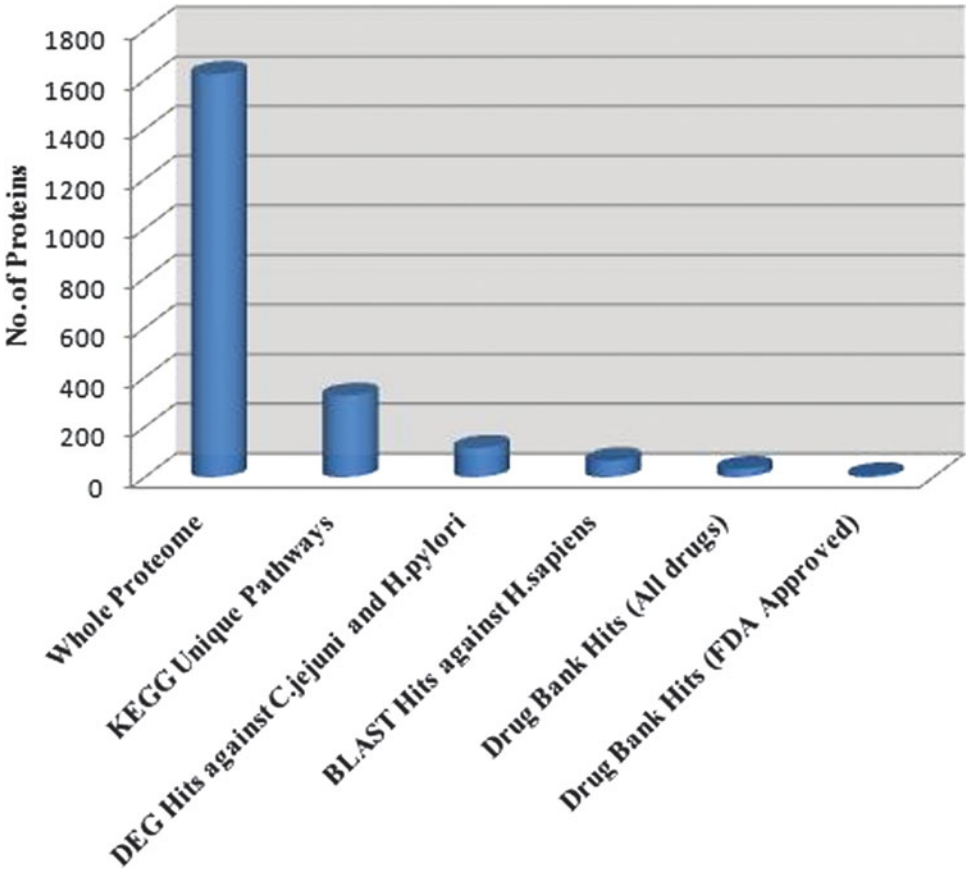

FIG. 3. Step-wise reduction in the total number of proteins in the subtractive analysis of *C.jejuni* proteome for drug target identification.

Conclusion

We have performed subtractive genomics analyses of the *C. jejuni* pathogenic strain NCTC11168, and have identified several proteins in the genome that can prove to be potential targets for effective drug design. As many of the identified drug targets have already been reviewed to play critical role in the metabolic pathways that regulate bacterial growth and survival, a systematic approach to develop antibiotics against the identified targets would likely be very promising for the treatment of *Campylobacter* infections. Information about these targets can also lead to significant progress in testing the efficacy of already existing drugs, which is as equally important as development of new drugs. It is believed that the drugs developed against these identified targets will be pathogen specific and with minimal toxic effects on the host. Omics-guided drug discovery and bioinformatics analyses offer a broad and veritable potential for advances in global health relevant novel therapeutics (Cuadrat et al. 2014; Preidis and Hotez, 2015).

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Author Disclosure Statement

The authors declare that there are no conflicting financial interests.

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