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In-silico evaluation of druggability prioritization targets of *Escherichia coli* in *Gallus gallus*

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Abstract
An experimental study was conducted to evaluate the druggability prioritization targets of *E. coli* in *Gallus gallus*. Total proteome of pathogen Vs host comparison was done using comparative genomics followed by protein docking of the target proteins of the pathogen and drug target identification and then calculation of drug prioritization parameters for therapeutic targets. 4,288 proteins of *E. coli* were compared with 24068 proteins of *Gallus gallus* using BLASTP analysis. The docking results showed positive ligand poses for proteins 1s7d posed with ligands benzocaine, benzyl penicillin, brimonidine, clofibrate, and phenoxy methyl penicillin, 1sz2 posed with ligands benzyl penicillin, bezafibrate, brimonidine, chlorambucil, ibuprofen, phenoxy methyl penicillin, chlorambucil, tolbutamide and tolnaftate, protein 2azo posed with ligands amsacrine, benzocaine, benzyl penicillin, bezafibrate, brimonidine, chlorambucil, ibuprofen, phenoxymethylpenicillin, chlorambucil, sulfanilamide, tolbutamide, tolnaftate and acetaminophen, Protein 2f1f posed with ligand sulfanilamide, Protein 2glt posed with ligands benzocaine, brimonidine, sulfanilamide and acetaminophen, protein 2yva posed with ligands amsacrine, benzocaine, bezafibrate, brimonidine, chlorambucil and ibuprofen, protein 3hi2 posed with ligands benzocaine, brimonidine, sulfanilamide and tolnaftate. protein 1dkl posed with ligands amsacrine, bezafibrate, tolbutamide and tolnaftate, protein 1pho posed with ligands amsacrine, benzocaine, benzyl penicillin, bezafibrate, brimonidine, pirbuterol, ibuprofen, phenoxymethylpenicillin, chlorambucil, sulfanilamide, tolbutamide, tolnaftate and acetaminophen.
The results of the study revealed potential drug targets for developing novel molecules against *E. coli* viz *Gallus gallus*.

Keywords: *In-silico*, Durggability, *Escherichia coli, Gallus gallus*

1. Introduction
Colibacillosis is a broad term that refers to any infection or disease caused by the bacteria *Escherichia coli.* These infections include colisepticemia, coligranuloma, venereal colibacillosis, coliform cellulitis, peritonitis, salpingitis, orchitis and enteritis, among others [1, 2]. *E. coli* is the common inhabitant of the gastrointestinal tract and a few strains known as Avian Pathogenic *Escherichia coli* (APEC) are responsible for hazardous infection. These APEC along with other agents like *mycoplasma*, *Haemophilus influenzae,* Newcastle disease, infectious bronchitis [3], coccidiosis, aflatoxicosis and ascariais [4] scare the poultry farmers by causing high morbidity, mortality and production losses. The mortality rate due to colibacillosis is 5-50% in poultry flocks [5]. It was observed that 43% of broiler carcasses were condemned for disease at processing and the lesions exhibited were consistent with colisepticemia and the incidence of colibacillosis is increasing in layer flocks [6, 7].

A number of factors play a crucial role in the virulence and pathogenesis of infection. The fimbriae F1 and pili (P) are particularly important in establishing the infection at the level of the trachea and gut epithelium. Other predisposing factors are inadequate ventilation, overcrowding, high dust and high concentration of ammonia in air.

Experimental vaccines have been shown to protect against some colibacillosis causing serogroups [8], but this is still an active area of study. Growing concern about antibiotic resistance has also affected the way colibacillosis is being treated. Increasing rate of resistance poses a real threat to clinicians and famers around the world [9].

A variety of compounds, which are involved in the management of diseases of non-infectious aetiology have shown some antimicrobial activity *in vitro* against bacteria and other microorganisms [10]. However, further research must be done before a commercial product of any colibacillosis treatment is ready to be distributed. Thus, the present study is aimed to evaluate the druggability targets of pathogen.

2. Materials and Methods

2.1 Identification of host and pathogen metabolic pathways

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database was used as a source of metabolic pathways information [11, 12]. A list of metabolic pathways and identification numbers of the host and the pathogen *E. coli* were extracted from the KEGG database and saved locally. Proteins from pathways were identified and the respective amino acid sequences were obtained from the Swiss-Prot database [13].

2.2 Screening of non-homologous and essential proteins

Two-step comparisons were performed between host and pathogen proteomes for the identification of non-homologous proteins of *E. coli* [14]. In each scenario, searching was restricted to proteins from *Gallus gallus* only through an option available under BLASTP parameters. Hits were filtered on the basis of expectation value (e-value) inclusion threshold being set to 0.005, and a minimum bit score of 100. Proteins, that did not have hits below the e-value inclusion threshold of 0.005, were picked out as non-homologous proteins.

2.3 Druggability of therapeutic targets

Druggability is another important target prioritization criterion, which is defined as the likelihood of being able to modulate the activity of the protein target with a small-molecule drug [15 16]. The druggability potential of each of the identified drug targets was calculated by mining DrugBank contents. The DrugBank database is a unique bioinformatics and cheminformatics resource that combines detailed drug (i.e. chemical, pharmacological and pharmaceutical) data with comprehensive drug target (i.e. sequence, structure and pathway) information. The database contains 6796 drug entries including 1437 FDA approved small molecule drugs, 134 FDA approved biotech (protein/peptide) drugs, 83 nutraceuticals and 5174 experimental drugs. Additionally, 4285 non-redundant proteins (i.e., drug target/enzyme/trans porter/carrier) sequences are linked to these drug entries [17]. BLASTP with default parameters were used to align the potential drug targets from E.coli against the list of protein targets of compounds found within Drug Bank. The selection criteria for filtering BLAST results were as described previously [18], that is, alignments with e-values less significant -25 than 1 x 10 were removed

3. Results

3.1 Identification of non-homologous proteins

4,288 proteins of *E.coli* were compared with 24068 proteins of *Gallus gallus* using BLASTP analysis. 389 non-homologous proteins of *E.coli* were found, while 3,899 proteins were found homologous. These 389 non-homologous proteins were analyzed using BLAST against PDB and all targets from Drug Bank. Number of proteins found hits against PDB (sequences of proteins with known structure): 35 (Table 4.1) and against Drug Bank were 17 (Table 3.2)

3.2 Druggability of therapeutic targets

Thirty five proteins found with solved PDB structures were analyzed using CLASTLW and having an average alignment of above or minimal 30% (Table 3.3). The thirty five proteins structures were also determined using PISA and the structures of the proteins were determined, of which 3 were monomers, 11 dimers, 2 trimers, 6 tetramers, 2 hexamers, 1 octamer, 1 nanome and 4 polymers,7 protein structures were not available with the database.

3.3 Molecular Docking

The 35 proteins were loaded into Discovery studio version 4.1 for receptor ligand interaction studies with 32 ligands. The docking results showed positive ligand poses for proteins 1s7d posed with ligands DB1086, 1053, 484, 636, 1080 and 417, 1sz2 posed with ligands 1053, 1393, 484, 291, 1050, 417, 1291, 1124 and 525, 2azo posed with ligands 276, 1086, 1053, 1393, 484, 291, 1050, 417, 291, 259, 1124, 525 and 316, 2f1f posed with ligand 259, 2glt posed with ligands 1086, 484, 259 and 316, 2yva posed with ligands 276, 1086, 1393, 484, 291 and 1050, 3hi2 posed with ligands 1086, 484, 1050, 259 and 525, 1dkl posed with ligands 276, 1393, 1124 and 525 and 1pho posed with ligands 276, 1086, 1053, 1393, 484, 291, 1050, 417, 291, 259, 1124, 525 and 316 (Fig. 3.1).

4. Discussion

The poultry industries are most vulnerable to attack by *Escherichia coli* that increases mortality of chickens. *E. coli* is one of the common microbial flora of gastrointestinal tract of poultry and other animals, but may become pathogenic to both [19, 20]. There is increasing evidence of resistance of these organisms to most of the currently available traditional anti-microbial agents. Hence there is a need to introduce novel molecules to treat *E. coli* infections. A computational and *in silico* system level framework was developed to identify and prioritize the antibacterial drug targets for *E.coli* in poultry so as to prevent heavy economic losses to farmers.

A variety of compounds which are involved in the management of diseases of non-infectious aetiology have shown some antimicrobial activity *in vitro*, against bacteria and other microorganisms [10]. Such compounds are called “non-antibiotics”. By the end of the nineteenth century, the dyes were known to possess antimicrobial activity, for instance Paul Ehrlich used methylene blue (one of phenothiazine compounds) as an antimicrobial agent [21]. So far, a lot of attention has been focused on thioxanthenes, phenothiazines and other agents with affinities to cellular transport systems which influence the structure of cellular membrane or ions transport etc. [22]. In one study [23], it was indicated that some of preparations inhibited growth of at least one of the four examined standard microbial strains. The drugs with the following active substances showed significant antimicrobial activity: amlodipine, acepromazine, butorphanol, cisapride, cisplatine, clomipramine, diltiazem, emedastine, fluvastatin, ketamine, levocabastine, metipranolol. Methotrexate, nicergoline, perphenazine, proxymetacaine, sertraline, tegaserod, tetrahydrozoline, ticlopidine and tropicamide.

Chen *et al*. [24] confirmed that non- antibiotic compounds enhance the *in vitro* activity of certain antibiotics against specific bacteria. Moreover, the antimicrobial activity of such non-traditional anti-microbial drugs emphasises a necessity of the neutralization of their activity during the microbial purity tests of pharmaceutical products [25].

In the present study, paracetamol, benzocaine, clofibrate, ibuprofen, amsacrine, bezafibrate, brimonidine, chlorambucil, pirbuterol, tolbutamide, sulfanilamide and tolnaftate were identified as the lead molecules based on *in silico* virtual screening (computational technique). Efficacy of drugs against microbes depends on their entry and accumulation in

the microbe in the active form in therapeutic concentrations. Further *in vitro* studies are warranted by using these drugs in combination with traditional anti-microbial agents for any synergistic effect.

Table 3.1: List of non-homologous proteins of *E.coli* after BLASTP with *Gallus gallus* with their functional characters

S. No.	*E.coli* protein	PDB	Function of *E.coli* protein
1	sp|P76344|ZINT_ECOLI	1s7d	May function as a periplasmic zinc chaperone or mediate direct transport of zinc from the periplasm to the cytoplasm.
2.	sp|P0A6V8|GLK_ECOLI	1sz2	Not highly important in *E.coli* as glucose is transported into the cell by the PTS system.
3.	sp|P06722|MUTH_ECOLI	2azo	Sequence-specific endonuclease that cleaves unmethylated GATC sequences. It is involved in DNA mismatch repair.
4.	sp|P00894|ILVH_ECOLI	2f1f	Acetolactate synthase isozyme 3 small subunit; (catalytic activity)
5.	sp|P04425|GSHB_ECOLI	2glt	Glutathione synthetase; (catalytic activity)
6.	sp|P66817|DIAA_ECOLI	2yva	DnaA initiator-associating protein required for the timely initiation of chromosomal replication via direct interactions with the DnaA initiator
7.	sp|Q46865|MQSR_ECOLI	3hi2	Toxic component of a type II toxin-antitoxin (TA) module. Plays a significant role in the control of biofilm formation and induction of persister cells in the presence of antibiotics.
8.	sp|P0AGL7|RSME_ECOLI	4e8b	Specifically methylates the N3 position of the uracil ring of uridine 1498(m3U1498) in 16S rRNA. Acts on the fully assembled 30S ribosomal subunit.
9.	sp|P07102|PPA_ECOLI	1dkl	Periplasmic AppA protein; Includes: Phosphoanhydride phosphohydrolase (catalytic activity)
10.	sp|P02932|PHOE_ECOLI	1pho	Outer membrane pore protein. Uptake of inorganic phosphate, phosphorylated compounds, and some other negatively charged solutes

Table 3.2: List of drug targets matching with non-homologus proteins of *E.coli* after BLAST with *Gallus gallus* proteome

S. No.	Query protein ID	Drug target	%id	E value
1.	sp|P00864|CAPP_ECOLI	drugbank_target|P41789	32.43	5.00E-91
2.	sp|P60723|RL4_ECOLI	drugbank_target|P60723	100	3.00E-86
3.	sp|P02932|PHOE_ECOLI	drugbank_target|P02931	60.16	0
4.	sp|P02943|LAMB_ECOL	drugbank_target|P02943	100	2.00E-08
5.	sp|P03007|DPO3E_ECOLI	drugbank_target|P03007	100	0
6.	sp|P06996|OMPC_ECOLI	drugbank_target|Q9K597	89.53	7.00E-171
7.	sp|P07102|PPA_ECOLI	drugbank_target|P19926	32.05	9.00E-22
8.	sp|P0A6B4|ALR1_ECOLI	drugbank_target|Q9HTQ2	47.43	7.00E-139
9.	sp|P0A6R3|FIS_ECOLI	drugbank_target|P41789	41.46	6.00E-33
10.	sp|P0A6V8|GLK_ECOLI	drugbank_target|P0A6V8	100	3.00E-11
11.	sp|P0A780|NUSB_ECOLI	drugbank_target|Q9X286	35.66	0
12.	sp|P0AAJ3|FDNH_ECOLI	drugbank_target|P0AAJ3	100	5.00E-80
13.	sp|P0ABD3|BFR_ECOLI	drugbank_target|Q93PP9	30.2	5.00E-08
14.	sp|P11350|NARI_ECOLI	drugbank_target|P11350	100	4.00E-59
15.	sp|P32056|GMM_ECOLI	drugbank_target|P32056	100	1.00E-25
16.	sp|P66817|DIAA_ECOLI	drugbank_target|Q9HVZ0	56.92	6.00E-04
17.	sp|P77173|ZIPA_ECOLI	drugbank_target|P77173	100	9.00E-29

Table 3.3: List of PDB structures matching with non-homologous proteins of *E.coli* after BLAST with *Gallus gallus* proteome

S. No.	Query protein ID	Subject protein ID	%id	E value	bit score	Clustalw	PISA
1	sp|P00864|CAPP_ECOLI	1qb4_A	100	0	1181	100	Dimer
2	sp|P00894|ILVH_ECOLI	2f1f_B	100	0	1753	100	Octamer
3	sp|P60723|RL4_ECOLI	5aka_E	100	0	832	100	Polymer
4	sp|P02932|PHOE_ECOLI	1pho_A	100	0	953	100	Trimer
5	sp|P02943|LAMB_ECOLI	1mpq_C	100	0	720	100	Trimer
6	sp|P03007|DPO3E_ECOLI	2xy8_A	100	0	669	100	Dimer
7	sp|P04425|GSHB_ECOLI	2glt_A	100	0	837	100	Tetramer
8	sp|P06609|BTUC_ECOLI	1l7v_B	100	0	652	100	Tetramer
9	sp|P06722|MUTH_ECOLI	2azo_B	100	0	707	100	Dimer
10	sp|P06996|OMPC_ECOLI	4a8d_M	100	0	989	100	?
11	sp|P07026|SDIA_ECOLI	4lgw_A	100	0	1424	100	Dimer
12	sp|P07102|PPA_ECOLI	1dkl_B	100	0	1148	100	Monomer
13	sp|P09152|NARG_ECOLI	1q16_A	100	0	682	100	Hexamer
14	sp|P0A6B4|ALR1_ECOLI	2rjh_D	100	0	814	100	Dimer
15	sp|P0A6C1|END4_ECOLI	1qum_A	100	0	1494	100	Tetramer
16	sp|P0A6R3|FIS_ECOLI	4ihy_B	100	0	750	100	Tetramer
17	sp|P0A6V8|GLK_ECOLI	1sz2_B	100	0	1127	100	Dimer
18	sp|P0A6Y1|IHFB_ECOLI	2ht0_B	100	0	2608	100	Dimer
19	sp|P0A780|NUSB_ECOLI	1ey1_A	100	0	1363	100	Monomer

20	sp|P0A7B8|HSLV_ECOLI	1ht2_L	100	0	707	99	Polymer
21	sp|P0A7Q1|RL35_ECOLI	5afi_3	100	0	634	100	?
22	sp|P0AAJ3|FDNH_ECOLI	1kqg_B	100	0	663	100	Nanomer
23	sp|P0ABD3|BFR_ECOLI	3e1p_L	100	0	794	96	Polymer
24	sp|P0AE72|MAZE_ECOLI	1mvf_E	100	0	754	100	Tetramer
25	sp|P0AE85|CPXP_ECOLI	3itf_B	100	0	671	100	Dimer
26	sp|P0AG99|SECG_ECOLI	2aki_A	100	0	682	100	?
27	sp|P0AGL7|RSME_ECOLI	4e8b_A	100	0	635	100	Dimer
28	sp|P11350|NARI_ECOLI	3ir7_C	100	0	1217	99.7	Hexamer
29	sp|P32056|GMM_ECOLI	2gt2_D	100	0	1289	100	Dimer
30	sp|P66817|DIAA_ECOLI	2yva_B	100	0	773	100	Tetraer
31	sp|P76344|ZINT_ECOLI	1s7d_A	100	0	1852	100	Dimer
32	sp|P76632|CSE2_ECOLI	4u7u_O	100	0	1162	100	Polmer
33	sp|P77173|ZIPA_ECOLI	1f7x_A	100	0	1074	97	Mnomer
34	sp|Q46865|MQSR_ECOLI	3hi2_D	100	0	637	100	Dimer
35	sp|Q47150|DINJ_ECOLI	4q2u_O	100	0	864	100	Polymer

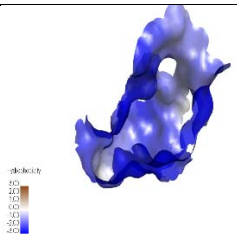
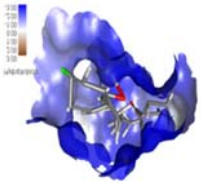
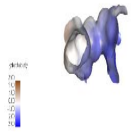
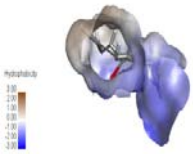
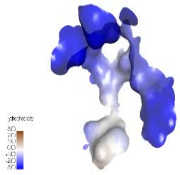
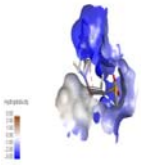
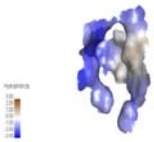
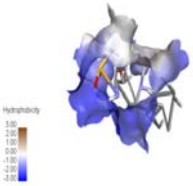
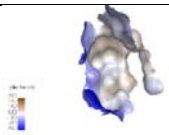
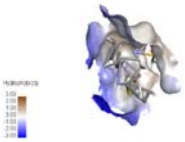
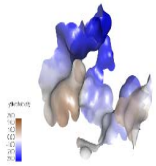
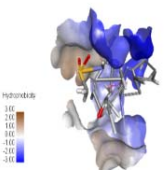
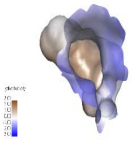
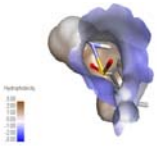
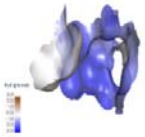
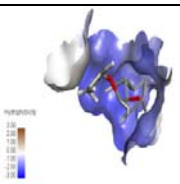
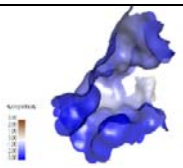
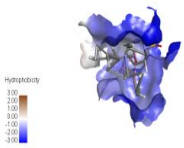
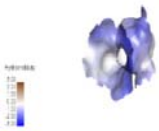
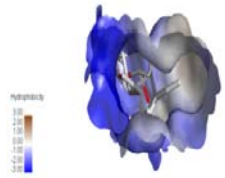
S. No.	Receptor	Receptor-Ligand Interaction
1.	 **1dkl**	
2.	 **4e8b**	
3.	 **1pho**	
4.	 **1s7d**	
5.	 **1sz2**	
6.	 **2azo**	
7.	 **2f1f**	
8.	 **2glt**	
9.	 **2yva**	
10.	 **3hi2**	

Fig 3.1: Receptor Ligand Interaction

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