The use of the Heter-LP algorithm to reposition antibiotics for managing E. coli mastitis in dairy cattle

Somayeh Shari (ss.shari2015@gmail.com)
Isfahan University of Technology

Maryam Lotfi Shahreza
University of Shahreza

Abbas Pakdel
Isfahan University of Technology

James M. Reecy
Iowa State University

Nasser Ghadiri
Isfahan University of Technology

Hadi Atashi
Shiraz University

Mahmoud Motamedi
University of Tehran

Esmaeil Ebrahimie
The University of Adelaide Adelaide Nursing School

Research article

Keywords: Drug repositioning, Drug target, E. coli mastitis, Gene regulator, Heter-LP algorithm, semi-supervised learning

DOI: https://doi.org/10.21203/rs.3.rs-36172/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Mastitis, a disease with high incidence worldwide, is the most prevalent and costly disease in the dairy industry. Environmental mastitis pathogens, such as Escherichia coli (E. coli), are major etiological agents of bovine mastitis in well-managed dairy farms. However, there is still a need to develop more efficient, safe, and economical treatments for mastitis. In the current research, Heter-LP, a new system biology-based method of drug repositioning, was applied to potentially identify novel therapeutic avenues for the treatment of E. coli mastitis. On-line data repositories relevant to known diseases, drugs, and gene targets along with other specialized biological information for E. coli mastitis, including key genes with robust bio-signatures, drugs and related diseases were used as input data for analysis with the Heter-LP algorithm. Our analyses identified novel drugs such as Glibenclamide, Ipratropium, Salbutamol, and Carbidopa as possible therapeutics that could be used against E. coli mastitis. Predicted relationships can be used by pharmaceutical scientists or veterinarians to find commercially efficacious medicines or combination of two or more active compounds to treat mastitis.

1. Introduction

Clinical mastitis, an ongoing problem for dairy producers, results in considerable economic losses and has led to increased risk of culling and death in dairy cows [4–6]. Mastitis control programs with impact on prevalence of contagious mastitis pathogens, led to a reduction in the incidence of Staphylococcus aureus and Streptococcus agalactiae mastitis, and as a result the increase in the relative impact of environmental mastitis pathogens such as Escherichia coli (E. coli) [6–9]. E. coli infection can cause either subclinical infection of the mammary gland or a severe systemic disease. Although, intramammary E. coli infections with acute inflammation may be self-healing by spontaneously eradicated by host defenses, however in extreme case it can be fatal [6, 10–12]. Unfortunately, self-care is often associated with significant economic damage due to the longer duration of infection, lower milk yield, and the potential for pathological changes to the mammary gland [6, 13]. The rate of clearance of bacterial pathogens that may be governed by mammary gland responses within hours of initial infection, is a key determinant of the outcome of intra-mammary gland infection [6, 14].

The therapeutic success of bovine mastitis depends mainly on accurate diagnosis of kind of pathogen, it will contribute to improvement of clinical and microbiological efficacy and helps to prevent of emergence and spread of resistant microorganisms. Despite the development prospects for bovine mastitis diagnosis with a focus on specific pathogens at an early stage fast together with the efficient devices can offer a “cow-side” and “on-site” with high sensitivity and specificity [1, 6, 15–17]; the most efficient, safe, and economical treatments for mastitis are still topics of scientific debate [6, 18, 19]. Generally, narrow and/or broad spectrum antibiotics are generally used for the treatment of E. coli mastitis, but treatment studies have shown controversial results. Given the problems associated with antibiotic therapy, including emergence of antibiotic-resistant strains, and the concern about antibiotics entering the food chain, efforts are being made to substitute the alternative strategies for new antimicrobial agents including bacteriophage, vaccination, nanoparticles, cytokines, homeopathy, natural compounds from
plants, animals, and bacteria or the discovery of new drugs that are effective against mastitis pathogens [6, 20–22].

Novel computational systems biology tools, such as meta-analysis, the pathways analysis, data mining and machine learning have provided good opportunities to understand the molecular mechanisms associated with diseases [23–27]. Currently, the first step to drug development is the use of previously known drugs; this is known as drug repositioning. This approach has attracted a lot of interest in recent years because of the increased speed of this process, drug safety concerns, and its lower cost. The integration of drug, disease and gene target information, in addition to how they affect and function in the body, can have a significant impact on drug repositioning and the possible identification of disease treatments.

The primary goal of this research was to identify some drugs that might be used for the treatment of *E. coli* mastitis (most prevalent agent in clinical cases). We used a recently introduced computational approach to facilitate drug repositioning, called Heter-LP [3]. Heter-LP is a systems biology approaches that integrate different types of data at different levels. Its ability to detect drug-disease relation has been proven by various analyses [3]. Heter-LP was selected because of some more of its advantages such as accuracy, no need to negative samples, its ability to predict trivial and non-trivial relationships between drugs, diseases and protein targets, and also its ability to use heterogenous data. Data resources in the public repository relevant to diseases, drugs, and gene targets along with other specialize biological information for *E. coli* mastitis were used as input data network to Heter-LP algorithm. Key genes with robust bio-signatures achieved from our recent meta-analysis work, related disease constructed by Pathway Studio web tool and drugs extracted from the literature review were used as complementary biological information to add to dataset gathered from the public repository.

2. Material And Methods

Our approach consisted of two steps. The first step was to construct a heterogeneous network by using input data including different kinds of nodes and edges. There were three types of nodes in the proposed heterogeneous network: targets, drugs and diseases. There were six different kinds of edges with represented one type of similarity or association: target similarity, drug similarity, disease similarity, known drug–target interaction, known drug-disease association, and known disease-target interaction.

Then, at the second step, we tried to predict important links which were not identified in the input data by using of Heter-LP. As we used the Heter-LP for the second step, we had to arrange our network (step 1) according to its input structures, which consisted of six separate matrices: (1) drug similarities, (2) disease similarities, (3) targets similarities, (4) drug-target relations, (5) disease-target relations and (6) drug-disease relations [3].

We tried to find a specialized dataset for mastitis to integrate with the aforementioned data. Unfortunately, no dataset is publically available. It seems that all available data and information related to animal diseases, genes targets, and drugs are only embedding in the publication and there are no
comprehensive datasets or repositories for them. Lack of access to this data however did not negatively impact the current analysis because of the generality of the input datasets due to the similarity of this disease in humans and other animals. To specialize the results for \textit{E. coli} mastitis we added three parts of information to our generated datasets (cases 2 to 4 in the following).

Based on the above descriptions, data sets that were used to construct the input data network for Heter-LP algorithm included:

1. Previously known drugs information gathered by public repositories.
2. Key genes that had a robust bio-signature in response to mastitis specially in \textit{E. coli} infection. These genes are the results of our recent research findings based on meta-analyses to detect up/down regulated genes by utilizing machine learning algorithms [1] and network analysis [2, 28],
3. Functionally related diseases or biological processes related to bovine mastitis illustrated by Pathway Studio web tool.
4. Relevant drugs and antibiotics to \textit{E. coli} mastitis gathered by literature analysis.

Finally, by the integration of these data a heterogeneous network is constructed and used as input for the Heter-LP algorithm.

A brief description and the data preparation methods of each part are presented in next subsections (Fig. 1). Different parts of this workflow were described in its caption and in more details in sub-Sect. 2.1 to 2.5.

2.1 Previously known drugs information

As mentioned before six different kinds of data including (1) Drug similarity, (2) Disease similarity, (3) Target similarity, (4) Known drug-disease associations, (5) Known drug–target interactions, and (6) Known disease-target associations, were necessary as input data to develop the network for Heter-LP. The base of these data was gathered previously [3], from some important databases. The data resources are summarized in the Table 1. In order to have an updated version of these data, the last version of data resources to generate the six matrices are provided according to the methods described at GitHub (https://github.com/MLotfiSH/Heter-LP) and DKR site (http://dkr.iut.ac.ir/projects) and in detail in our previously published research [3].
Table 1
Resources of data related to each sub-network and the number of nodes in each one.

| Sub-network       | Using criterion                                      | Resource                          | Number of nodes |
|-------------------|------------------------------------------------------|-----------------------------------|-----------------|
|                   |                                                      | In each resource                  | In total        |
| Drugs             | Chemical substructures similarities                  | PubChem\(^1\)                     | 1103            | 5089            |
|                   | Side effect similarities                             | SIDER\(^2\)                       | 888             |
|                   | Anatomical Therapeutic Chemical (ATC) code similarities | KEGG\(^3\)                        | 4867            |
| Diseases          | Disease genes similarities                           | DisGeNET\(^4\)                    | 3295            | 9886            |
|                   | The similarity based on ICD-10 classification\(^5\)   | KEGG                              | 1366            |
|                   | Semantic similarity based on Disease Ontology (DO)\(^6\) | DOSE package in R                 | 6560            |
|                   | Semantic similarities based on GO\(^8\)             | GOSemSim\(^9\) package in R        | 1550            |
| Targets           | Semantic similarities based on HPO\(^10\)           | HPOSim\(^11\) package in R         | 979             | 2940            |
|                   | Semantic similarities based on DO                   | DOSE package in R                  | 1092            |
|                   | Similarities based on KEGG                          | KEGG                              | 1132            |
| Drug-disease      | Therapeutic Target Database (TTD)\(^12\)            | Drugs: 6931                       | Drugs: 7382     |
|                   |                                                      | Drugs: 1418                       |                 |
|                   |                                                      | KEGG                              | Drugs: 1052     | Diseases: 1970  |
|                   |                                                      |                                   | Diseases: 592   |
| Drug-target       | DrugBank\(^13\)                                     | Drugs: 1521                       | Drugs: 3350     |
|                   |                                                      | Targets: 1346                     |                 |
|                   |                                                      | KEGG                              | Drugs: 2440     | Targets: 1415   |
|                   |                                                      |                                   | Targets: 335    |
| Disease-target    | DiGeNet                                             | Diseases: 577                     | Diseases: 1838  |
|                   |                                                      |                                   | Targets: 2403   |


| KEGG | Diseases: 1271 | Targets: 4066 |
|------|---------------|--------------|
|      | Targets: 2563 |              |

1. https://pubchem.ncbi.nlm.nih.gov/score_matrix/score_matrix.cgi
2. http://sideeffects.embl.de/
3. Kyoto Encyclopedia of Genes and Genomes (http://www.kegg.jp)
4. http://www.disgenet.org
5. International Statistical Classification of Diseases and Related Health Problems-10
6. http://disease-ontology.org/
7. Disease Ontology Semantic and Enrichment analysis (https://bioconductor.org/packages/release/bioc/html/DOSE.html)
8. Gene Ontology (http://www.geneontology.org/)
9. https://bioconductor.org/packages/release/bioc/html/GOSemSim.html
10. Human Phenotype Ontology, https://hpo.jax.org/ (http://human-phenotype-ontology.github.io/)
11. https://mran.microsoft.com/snapshot/2014-10-20/web/packages/HPOSim/index.html
12. http://bidd.nus.edu.sg/group/cjttd/
13. http://drugbank.ca

### 2.2 Disease genes

Previously we identify differentially expressed genes in response to *E. coli* mastitis [1, 2, 28]. These genes have a robust bio-signature and thereby may be useful biomarker or therapeutic target candidates in mastitis [1, 2]. These genes/proteins are listed in Table 2 and added to the disease-gene relation part of the dataset shown in Table 1.
The key genes or regulators with robust bio-signature in response to *E. coli* mastitis reported to our previous meta-analysis based microarray studies [1, 2]

| Row | Gene symbol | Functional group | Gene name (alias) |
|-----|-------------|------------------|------------------|
| 1   | CXCL2       | Ligand           | *Chemokine (C-X-C motif) ligand 2 (GRO3)* |
| 2   | CXCL8       | Ligand           | *C-X-C motif chemokine ligand 8 (IL-8, IL8)* |
| 3   | GRO1        | Ligand           | *Chemokine (C-X-C motif) ligand 1 (CXCL1, MGSA)* |
| 4   | CFB         |                  | *Complement factor B (BF)* |
| 5   | ZC3H12A     |                  | *Zinc finger CCCH-type containing 12A* |
| 6   | CCL20       | Ligand           | *C-C motif chemokine ligand 20* |
| 7   | NFKB1Z      |                  | *NFkB inhibitor zeta (MAIL)* |
| 8   | S100A9      | Ligand           | *S100 calcium binding protein A9* |
| 9   | S100A8      | Ligand           | *S100 calcium binding protein A8* |
| 10  | PDE4B       |                  | *Phosphodiesterase 4B* |
| 11  | CASP4       |                  | *Caspase 4, apoptosis-related cysteine peptidase (CASP13)* |
| 12  | HP          | Ligand           | *Haptoglobin* |
| 13  | MAPK1       | Protein kinase   | *Mitogen-activated protein kinase* |
| 14  | TP53 (p53)  | Transcription factor | *Tumor protein p53* |
| 15  | SP1         | Transcription factor | *Sp1 transcription factor* |
| 16  | MAPK14      | Protein kinase   | *Mitogen-activated protein kinase 14* |
| 17  | INS         | Ligand           | *Insulin* |
| 18  | EGF         | Ligand           | *Epidermal growth factor* |
| 19  | AKT1        | Protein kinase   | *AKT serine/threonine kinase 1* |
| 20  | IFNG        | Ligand           | *Interferon gamma* |
| 21  | MAPK3       | Protein kinase   | *Mitogen-activated protein kinase 3* |
| 22  | MAPK8       | Protein kinase   | *Mitogen-activated protein kinase 8* |
| 23  | VEGFA       | Ligand           | *Vascular endothelial growth factor A* |
| 24  | MMP2        |                  | *Matrix metalloproteinase 2* |
| Row | Gene symbol | Functional group | Gene name (alias) |
|-----|-------------|------------------|------------------|
| 25  | BCL2        |                  | BCL2, apoptosis regulator |
| 26  | IL10        | Ligand           | Interleukin 10   |

### 2.3 Disease similarity data

One of the problems that arises when examining diseases is the use of different names or identifiers for the same disease. In the case of the disease in the current research, bovine mastitis was used for dairy cattle, and mastitis was used for human and other mammals in the literature. The Pathway Studio web tool 12.0.1.5 was used to construct a network of disease or cell processes that were functionally associated with mastitis or bovine mastitis. Pathway Studio as a pathway analysis tool incorporates some commercial and public databases such as BIND [29], KEGG [30], and GO [30] that utilizes the ResNet Mammal database. Moreover, it also uses the powerful text-mining tool MedScan to seek the latest information from PubMed and other public sources (Elsevier-Ariadne Genomics, Rockville, MD) [31]. For more confidence, all relationships which were reported by more than two references were selected. All relations between mastitis or bovine mastitis with other diseases or cell process are indicated in Table 3. Additional details and references are provided in Additional file 1. As shown, most of the cases related to mastitis or bovine mastitis are the same and demonstrated the similarity of this disease in all mammals. This information has been added to disease similarity part of dataset shown in Table 1.
Table 3
Known disease or cell process related to mastitis or bovine mastitis by using Pathway Studio web tool (based on at list two references)

|   | Related disease to mastitis or Bovine mastitis | Related disease to mastitis (continue) | Related disease to mastitis (continue) |
|---|-----------------------------------------------|----------------------------------------|----------------------------------------|
| 1 | Bovine mastitis                               | bacterial infection                     | 34 injury                              | 67 innate immune response |
| 2 | cellular immune response                      | 35 injury                               | 68 ketosis                             |
| 3 | cryptococcosis                                | 36 injury                               | 69 lactation                           |
| 4 | Escherichia coli infection                    | 37 pain                                 | 70 life span                           |
| 5 | fever                                        | 38 sepsis                               | 71 lipid degradation                   |
| 6 | fibrosis                                     | 39 skin disease                         | 72 lupus erythematosus profundus       |
| 7 | infection                                    | 40 swelling                             | 73 maedi                               |
| 8 | inflammation                                 | 41 systemic lupus erythematosus         | 74 milk production                     |
| 9 | inflammatory disease                         | 42 tuberculosis                         | 75 MRSA infection                      |
|10 | protothecosis                                 | 43 type 1 diabetes                      | 76 mumps                               |
|11 | staphylococcal infection                     | 44 apoptosis                             | 77 neoplasm                            |
|12 | milk production                              | 45 breast cancer                        | 78 neutrophil recruitment              |
|13 | Mastitis                                     | 46 breast pain                          | 79 nipple discharge                     |
|14 | angiogenesis                                 | 47 breast-feeding                       | 80 ovarian follicle development        |
|15 | autoimmune disease                           | 48 contagious agalactia                 | 81 ovary function                      |
|16 | bacterial infection                           | 49 diabetes mellitus                    | 82 ovulation                           |
|17 | benign breast disease                        | 50 endemic disease                      | 83 parity                              |
|18 | breast abscess                               | 51 energy homeostasis                   | 84 parturient paresis                  |
| Related disease to mastitis or Bovine mastitis | Related disease to mastitis (continue) | Related disease to mastitis (continue) |
|---------------------------------------------|---------------------------------------|---------------------------------------|
| 19 cancer                                   | 52 fat necrosis                       | 85 parturition                        |
| 20 candidiasis                              | 53 fatty liver                        | 86 pregnancy                          |
| 21 cattle disease                           | 54 fertilization                      | 87 proteolysis                        |
| 22 cell count                               | 55 fibrosis                           | 88 pseudotuberculosis                 |
| 23 death                                    | 56 galactorrhea                       | 89 respiratory tract infection        |
| 24 edema                                    | 57 Gram-negative bacterial infection  | 90 retained placenta                  |
| 25 endotoxemia                              | 58 granulomatous mastitis            | 91 smoking                            |
| 26 Escherichia coli infection               | 59 hypocalcemia                       | 92 staphylococcal infection           |
| 27 fever                                    | 60 IgG4-related disease               | 93 subfertility                       |
| 28 hyperemia                                | 61 immune response                    | 94 virulence                          |
| 29 immunity                                 | 62 immune system activation           | 95 virus infection                    |
| 30 immunopathology                         | 63 immune system function             | 96 weaning                            |
| 31 infection                                | 64 inbreeding                         | 97 wounds and injuries                |
| 32 inflammation                             | 65 infectious disease                 |                                       |
| 33 inflammatory disease                     | 66 inflammatory response              |                                       |

### 2.4 Drugs disease

With a review of the literature, we were able to develop a comprehensive list of drugs or antibiotics that have been used to treat *E. coli* mastitis (see Table 4). This information has been added to drug-disease relation part of dataset shown in Table 1.
### Table 4
List of known drugs or antibiotics reported in literature to treat *E. coli* mastitis

| Drug or Antibiotic                                      | Reference |
|--------------------------------------------------------|-----------|
| 1. Ampicillin                                           | [32]      |
| 2. Aspirin                                              | [33]      |
| 3. Ceftazidime                                          | [32]      |
| 4. Cephalexin                                           | [32]      |
| 5. Cephapirin ( Cefoperazone, Ceftiofur, Cefquinome )   | [34]      |
| 6. Chloramphenicol                                      | [35]      |
| 7. Cinoxacin                                            | [36]      |
| 8. Ciprofloxacin                                        | [32, 36] |
| 9. Dexamethasone                                        | [37]      |
| 10. DHS (dihy-drostreptomycin sesquisulfate sa)         | [32]      |
| 11. Flunixin meglumine                                  | [38]      |
| 12. Fluoroquinolones (enrofloxacin, danofloxacin, marbofloxacin ) | [34] |
| 13. Gentamicin                                          | [32, 35] |
| 14. Isoflupredone acetate                               | [33]      |
| 15. Ketoprofen                                          | [32]      |
| 16. Meloxicam                                           | [39]      |
| 17. Oxytetracycline                                     | [40]      |
| 18. Penethamate hydriodide                              | [39]      |
| 19. Polymixin                                           | [41]      |
| 20. Prednisolone                                        | [42]      |
| 21. Tetracycline                                        | [32]      |
| 22. Trimethoprim                                        | [32]      |
| 23. Sulfadoxine                                         | [40]      |
| 24. Sulfamethoxazole                                    | [35]      |
| 25. Sulfadiazine                                        | [32]      |

### 2.5 Integration of data
The final heterogeneous network model was constructed by integration of these four mentioned data which were discussed in previous sections. It is necessary to mention that Heter-LP input data could be incoincident in different parts of the heterogeneous network. This means that a complete list of drugs, diseases and proteins/genes (as targets) will be achieved by union of similar typed items in each sub-network.

3. Results

The repositioning of antibiotics for managing *E. coli* mastitis in dairy cattle is the main findings of this study. Based on Heter-LP categorization, there are two kinds of predictions, known and novel [3]. The 30 top predicted drugs and antibiotics associated with *E. coli* mastitis are presented in Table 5. Most of the drugs listed in Table 4 have been reported in literature as treatments for *E. coli* mastitis. These results demonstrate that Heter-LP could identify known relations correctly, which indicates that the novel compounds may be realistic predictions. All predicted results of Heter-LP are presented in Additional file 2.
30 top predicted drugs associated with *E. coli* mastitis by the Heter-LP algorithm

| Drug          | Ranking Score | Verification     |
|---------------|---------------|-------------------|
| Cefoperazone  | 0.005000691   | known drug       |
| Meloxicam     | 0.004998696   | known drug       |
| Cephapirin    | 0.003363298   | known drug       |
| Cephalexin    | 0.003362269   | known drug       |
| Oxytetracycline | 0.003352667  | known drug       |
| Cinoxacin     | 0.003351841   | known drug       |
| Ketoprofen    | 0.003350183   | known drug       |
| Aspirin       | 0.002526886   | known drug       |
| Ampicillin    | 0.001301824   | known drug       |
| Ceftazidime   | 0.001164398   | known drug       |
| Tetracycline  | 0.001162658   | known drug       |
| Chloramphenicol | 0.000958009 | known drug       |
| Gentamicin    | 0.000937666   | known drug       |
| Ciprofloxacin | 0.000680685   | known drug       |
| Dexamethasone | 0.000618516   | known drug       |
| Prednisolone  | 0.000513524   | known drug       |
| Penicillin G  | 8.63E-05      | New drug         |
| Leucovorin    | 8.19E-05      | New drug         |
| Rifampicin    | 7.91E-05      | New drug         |
| Cefprozil     | 7.87E-05      | New drug         |
| Ipratropium   | 7.81E-05      | New drug         |
| Cefadroxil    | 7.77E-05      | New drug         |
| Clidinium     | 7.66E-05      | New drug         |
| Lopinavir     | 7.64E-05      | New drug         |
| Glibenclamide | 7.61E-05      | New drug         |
| Thyroxine     | 7.57E-05      | New drug         |
### 4. Discussion

The efficacy of antibiotic and/or anti-inflammatory drugs/compounds in the treatment of mastitis disease is not fully specified. Given the problems associated with antibiotic therapy, including emergence of antibiotic-resistant strains, and the concern about antibiotics entering the food chain, efforts are being made to substitute the alternative strategies for new antimicrobial agents including bacteriophage, vaccination, nanoparticles, cytokines, homeopathy and natural compounds from plants and animals, and bacteria or the discovery of new drugs that are effective against mastitis pathogens [6, 20–22].

While the pharmaceutical industry has explored the use of drug repositioning to identify novel treatments for diseases, this work has been hampered by a lack of a fundamental and systematic approach. In the current research, the biological algorithm Heter-LP was used to reposition antibiotics for managing *E. coli* mastitis in dairy cattle. The utility of Heter-LP, to discover new drug repositioning to rare diseases in human have been explored previously [43]. Data that was available in the public repositories along with other specialize biological information for *E. coli* mastitis including crucial genes, antibiotic or drugs used for treatment of *E. coli* mastitis, and its association with other disease or cell processes were used as input data for the Heter-LP algorithm. By using Heter-LP, we were able to introduce a list of most likely candidate drugs that could be used as therapeutic strategies against the *E. coli* infection. It is noteworthy that these drugs have been suggested among more than 11000 different drugs, which could help to accelerate and facilitate the drug identification process. Certainly, this list of suggested drugs is valubale for pharmaceutical scientists or veterinarians in order to find a commercial and efficacious medicine or combinations of two or more active compounds. In the following, we have tried to validate and confirm most of these new predictions by review of available scientific literature.

Penicillin G (also known as Benzylpenicillin), Rifampicin, Cefprozil and Cefadroxil are antibiotic drugs. Recent research has shown that Rifampicin could be used as a solo medical therapy in humans for chronic mastitis [44]. Cefprozil, a second-generation cephalosporins antibiotic, is approved worldwide strictly for the treatment of mastitis disease in dairy cattle. Cefadroxil, a broad-spectrum cephalosporins, is a first-generation cephalosporin antibacterial drug that is effective against gram-positive and gram-negative bacterial infections. It is the para-hydroxy derivative of cefalexin that is a bactericidal antibiotic and is used in the treatment of mild to moderate susceptible infections. Lipopoly saccharides on the the outer membrane of the gram-negative bacteria such as *E. coli* are an important barrier that provides protection against toxic compounds, which include antibiotics and host innate immune molecules such

| Drug               | Ranking Score | Verification |
|--------------------|---------------|--------------|
| 27 Salbutamol      | 7.55E-05      | New drug     |
| 28 Carbidopa       | 7.51E-05      | New drug     |
| 29 Benzquinamide   | 7.50E-05      | New drug     |
| 30 Diethylpropion  | 7.49E-05      | New drug     |
as cationic antimicrobial peptides. These bacteria use a wide variety of mechanisms to resist antimicrobials [45, 46].

Glibenclamide is an antidiabetic drug in a class of medications known as sulfonylureas, closely related to sulfonamide antibiotics. Sulfonamides are also occasionally used to treat septicemia caused by coliform mastitis in dairy cattle [47]. It has been investigated that, effects of inflammation markers (TNFα and NFκB), and activation of cell injury or cell death markers (IgG endocytosis and caspase-3), significantly reducing with glibenclamide [48].

In the case of Ipratropium, it has been shown that partially protect the lungs against inflammation by reducing neutrophilic infiltration. This protective effect is associated with a reduction in the MMP-9 activity, which is known to play an important pro-inflammatory role in the acute inflammatory process [49].

It has been demonstrated that hypothyroidism is associated with signs of low-grade inflammation (raised C-reactive protein levels) which may be elicited by the raised level of triglyceride or be an independent effect of an intracellular hypometabolic state or of a combination of them [50]. Also, other research has shown that l-thyroxine treatment of patients with subclinical hypothyroidism can reduce inflammation [51]

Salbutamol, the other predicted drug listed in Table 5, has been shown to decrease acute and chronic inflammation, decrease myeloperoxidase (MPO) activity and lipid peroxidation (LPO) level and increased the activity of superoxide dismutase (SOD) and level of glutathione (GSH) during the acute phase of inflammation possibly through the stimulation of β-2 adrenergic receptors [52].

Carbidopa has been used as a treatment for Parkinson's disease. New research has demonstrated that it inhibits early events in T cell activation and promotes the development of anti-inflammatory effects. Thus, it has been suggested as apotential therapeutic for the management and/or treatment inflammatory and autoimmune disorders in humans [53].

Based on these results, it can be concluded that the Heter-LP has successfully predicted drugs/compounds that can be used as suitable alternatives for the treatment of E. coli mastitis.

5. Conclusions

In the current study, has been shown that the system biology-based algorithm, Heter-LP, can be used to identify repositioned drugs that may be useful for the treatment of important disease. Integration of the biological data and using the Heter-LP algorithm enabled us to introduce novel drugs relevant to E. coli mastitis. Our results provide valuable information for pharmaceutical scientists or veterinarians in the dairy industry to find a commercial and efficacious medicine or a combination of two or more active compounds.
Declarations

Ethics approval and consent to participate:
Not applicable

Consent for publication:
Not applicable

Competing interests:
The authors declare that they have no competing interests

Funding:
The authors received no specific funding for this work.

Authors' contributions:
Somayeh Sharifi and Maryam Lotfi Shahreza wrote the main manuscript text, Abbas Pakdel and Esmaeil Ebrahimie are supervisor, James M. Reecy and Nasser Ghadiri are advisors of this manuscript. All authors reviewed the manuscript.

I can confirm I have included a statement regarding data and material availability in the declaration section of my manuscript.

Acknowledgement

We are grateful to Dr. Peng Liu from Iowa State University and Dr. Mahmoud Arabi for their generous help.

References
1. Sharifi S, Pakdel A, Ebrahimie M, Reecy JM, Fazeli Farsani S, Ebrahimie E. Integration of machine learning and meta-analysis identifies the transcriptomic bio-signature of mastitis disease in cattle. PLoS One. 2018;13(2):e0191227.
2. Sharifi S, Pakdel A, Ebrahimie E, Aryan Y, Reecy JM: Prediction of key regulators and downstream targets of E. coli induced mastitis. Journal of applied genetics 2019, DOI 10.1007/s13353-019-00499-7.
3. Lotfi Shahreza M, Ghadiri N, Mousavi SR, Varshosaz J, Green JR. Heter-LP: A heterogeneous label propagation algorithm and its application in drug repositioning. J Biomed Inform. 2017;68:167–83.

4. Rollin E, Dhuyvetter KC, Overton MW. The cost of clinical mastitis in the first 30 days of lactation: An economic modeling tool. Prev Vet Med. 2015;122(3):257–64.

5. Bar D, Tauer LW, Bennett G, Gonzalez RN, Hertl JA, Schukken YH, Schulte HF, Welcome FL, Grohn YT. The cost of generic clinical mastitis in dairy cows as estimated by using dynamic programming. J Dairy Sci. 2008;91(6):2205–14.

6. Sharifi S, Pakdel A. Bovine Mastitis: Etiology and Epidemiology, challenges, current trends and future perspectives in monitoring, detection and treatment. In: Mastitis symptoms, triggers and treatment. edn. New York: NOVA; 2019.

7. Bradley A. Bovine mastitis: an evolving disease. Vet J. 2002;164(2):116–28.

8. Hogan J, Larry Smith K. Coliform mastitis. Vet Res. 2003;34(5):507–19.

9. Zadoks R, Fitzpatrick J. Changing trends in mastitis. Ir Vet J. 2009;62(Suppl 4):59–70.

10. Bannerman DD, Jai-Wei JPM, Xin L, Hope Z. JC, P. R: Escherichia coli and Staphylococcus aureus Elicit Differential Innate Immune Responses following Intramammary Infection. Clin Diagn Lab Immunol. 2004;11(3):463–72.

11. Burvenich C, Bannerman DD, Lippolis JD, Peelman L, Nonnecke BJ, Kehrli ME Jr, Paape MJ. Cumulative physiological events influence the inflammatory response of the bovine udder to Escherichia coli infections during the transition period. J Dairy Sci. 2007;90(Suppl 1):E39–54.

12. Hagiwara S, Mori K, Nagahata H. Predictors of fatal outcomes resulting from acute Escherichia coli mastitis in dairy cows. J Vet Med Sci. 2016;78(5):905–8.

13. Bramley AJ, Dodd FH. Reviews of the progress of dairy science: mastitis control–progress and prospects. J Dairy Res. 1984;51(3):481–512.

14. Bannerman DD. Pathogen-dependent induction of cytokines and other soluble inflammatory mediators during intramammary infection of dairy cows. J Anim Sci. 2009;87(13 Suppl):10–25.

15. Mestorino N, Errecalejo: Pharmacokinetic – Pharmacodynamic Considerations for Bovine Mastitis Treatment. In. Edited by Medicine ABs-EVoV: IntechOpen; 2012.

16. Viguier C, Arora S, Gilmartin N, Welbeck K, O’Kennedy R. Mastitis detection: current trends and future perspectives. Trends Biotechnol. 2009;27(8):486–93.

17. Lewis JD. The utility of biomarkers in the diagnosis and therapy of inflammatory bowel disease. Gastroenterology. 2011;140(6):1817–26 e1812.

18. Sipka A, Klaessig S, Duhamel GE, Swinkels J, Rainard P, Schukken Y. Impact of intramammary treatment on gene expression profiles in bovine Escherichia coli mastitis. PLoS One. 2014;9(1):e85579.

19. Suojala L, Simojoki H, Mustonen K, Kaartinen L, Pyorala S. Efficacy of enrofloxacin in the treatment of naturally occurring acute clinical Escherichia coli mastitis. J Dairy Sci. 2010;93(5):1960–9.
20. Tiwari JG, Babra C, Tiwari HK, Williams V, Wet SD, Gibson J, Paxman A, Morgan E, Costantino P, Sunagar R, et al: **Trends In Therapeutic and Prevention Strategies for Management of Bovine Mastitis: An Overview** *J Vaccines Vaccin* 2013, 4(2).

21. Gomes F, Henriques M. Control of Bovine Mastitis: Old and Recent Therapeutic Approaches. *Curr Microbiol.* 2016;72(4):377–82.

22. Camerlink I, Ellinger L, Bakker EJ, Lantinga EA. Homeopathy as replacement to antibiotics in the case of Escherichia coli diarrhoea in neonatal piglets. *Homeopathy.* 2010;99(1):57–62.

23. Alanazi IO, Ebrahimie E. Computational Systems Biology Approach Predicts Regulators and Targets of microRNAs and Their Genomic Hotspots in Apoptosis Process. *Mol Biotechnol.* 2016;58(7):460–79.

24. Kargarfard F, Sami A, Ebrahimie E. Knowledge discovery and sequence-based prediction of pandemic influenza using an integrated classification and association rule mining (CBA) algorithm. *J Biomed Inform.* 2015;57:181–8.

25. Sharifi S, Pakdel A, Jahanbakhsh J, Aryan Y, Mahdavi AH: *Molecular mechanisms of resistance to bovine mastitis.* Anim product sci 2019, Inpress.

26. Ebrahimie E, Ebrahimie F, Ebrahimie M, Tomlinson S, Petrovski KR. A large-scale study of indicators of sub-clinical mastitis in dairy cattle by attribute weighting analysis of milk composition features: highlighting the predictive power of lactose and electrical conductivity. *J Dairy Res.* 2018;85(2):193–200.

27. Ebrahimie M, Mohammadi-Dehcheshmeh M, Ebrahimie E, Petrovski KR. Comprehensive analysis of machine learning models for prediction of sub-clinical mastitis: Deep Learning and Gradient-Boosted Trees outperform other models. *Comput Biol Med.* 2019;114:103456.

28. Sharifi S, Pakdel A, Ebrahimie E. Meta-analysis of transcriptomic data of mammary gland infected by Escherichia coli Bacteria in dairy cows. *Iranian Journal of Animal Science.* 2017;Vol 48((3):343–52.

29. Kanehisa M. **KEGG.** In.: Metabolic database; 2008.

30. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, et al: **Gene ontology.** In.: Tool for the unification of biology. The Gene Ontology Consortium; 2000.

31. Nikitin A, Egorov S, Daraselia N, Mazo I. Pathway studio—the analysis and navigation of molecular networks. *Bioinformatics.* 2003;19(16):2155–7.

32. Lehtolainen T, Shwimmer A, Shpigel NY, Honkanen-Buzalski T, Pyorala S. In vitro antimicrobial susceptibility of Escherichia coli isolates from clinical bovine mastitis in Finland and Israel. *J Dairy Sci.* 2003;86(12):3927–32.

33. Wagner SA: **Thee effects of anti-inflammatory drugs on clinical signs, milk production, and mammary epithelial cells in cows with endotoxin-induced mastitis.** Iowa State University; 2003.

34. Suojala L, Kaartinen L, Pyorala S. Treatment for bovine Escherichia coli mastitis — an evidence-based approach. *J vet Pharmacol Therap.* 2013;36:521–31.
35. Oliver SP, Murinda SE, Jayarao BM. Impact of antibiotic use in adult dairy cows on antimicrobial resistance of veterinary and human pathogens: a comprehensive review. Foodborne Pathog Dis. 2011;8(3):337–55.

36. Srinivasan V, Gillespie BE, Lewis MJ, Nguyen LT, Headrick SI, Schukken YH, Oliver SP. Phenotypic and genotypic antimicrobial resistance patterns of Escherichia coli isolated from dairy cows with mastitis. Vet Microbiol. 2007;124(3–4):319–28.

37. Lohuis JA, Van Leeuwen W, Verheijden JH, Van Miert AS, Brand A. Effect of dexamethasone on experimental Escherichia coli mastitis in the cow. J Dairy Sci. 1988;71(10):2782–9.

38. Wagner SA, Apley MD. Effects of two anti-inflammatory drugs on physiologic variables and milk production in cows with endotoxin-induced mastitis. Am J Vet Res. 2004;65(1):64–8.

39. McDougall S, Bryan MA, Tiddy RM. Effect of treatment with the nonsteroidal antiinflammatory meloxicam on milk production, somatic cell count, probability of re-treatment, and culling of dairy cows with mild clinical mastitis. J Dairy Sci. 2009;92(9):4421–31.

40. Olson ME, Ceri H, Morck DW, Buret AG, Read RR. Biofilm bacteria: formation and comparative susceptibility to antibiotics. Can J Vet Res. 2002;66(2):86–92.

41. Ziv G, Shem-Tov M, Ascher F. Combined effect of ampicillin, colistin and dexamethasone administered intramuscularly to dairy cows on the clinico-pathological course of E. coli-endotoxin mastitis. Vet Res. 1998;29(1):89–98.

42. Barlow J. Mastitis therapy and antimicrobial susceptibility: a multispecies review with a focus on antibiotic treatment of mastitis in dairy cattle. J Mammary Gland Biol Neoplasia. 2011;16(4):383–407.

43. Lotfi Shahreza M, Ghadiri N, Green JR. A computational drug repositioning method applied to rare diseases: Adrenocortical carcinoma. Sci Rep. 2020;10(1):8846.

44. Farouk O, Abdelkhalek M, Abdallah A, Shata A, Senbel A, Attia E, Elghaffar MA, Mesbah M, Soliman N, Amin M, et al. Rifampicin for Idiopathic Granulomatous Lobular Mastitis: A Promising Alternative for Treatment. World J Surg. 2017;41(5):1313–21.

45. Blair JM, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol. 2015;13(1):42–51.

46. Miller SI. Antibiotic Resistance and Regulation of the Gram-Negative Bacterial Outer Membrane Barrier by Host Innate Immune Molecules. MBio 2016, 7(5).

47. Erskine RJ, Walker RD, Bolin CA, Bartlett PC, White DG. Trends in antibacterial susceptibility of mastitis pathogens during a seven-year period. J Dairy Sci. 2002;85(5):1111–8.

48. Simard JM, Geng Z, Woo SK, Ivanova S, Tosun C, Melnichenko L, Gerzanich V. Glibenclamide reduces inflammation, vasogenic edema, and caspase-3 activation after subarachnoid hemorrhage. J Cereb Blood Flow Metab. 2009;29(2):317–30.

49. Zhang W, Fievez L, Cheu E, Bureau F, Rong W, Zhang F, Zhang Y, Advenier C, Gustin P. Anti-inflammatory effects of formoterol and ipratropium bromide against acute cadmium-induced pulmonary inflammation in rats. Eur J Pharmacol. 2010;628(1–3):171–8.
50. Kvetny J, Heldgaard PE, Bladbjerg EM, Gram J. Subclinical hypothyroidism is associated with a low-grade inflammation, increased triglyceride levels and predicts cardiovascular disease in males below 50 years. Clin Endocrinol (Oxf). 2004;61(2):232–8.

51. Abbas AM, Sakr HF. Effect of magnesium sulfate and thyroxine on inflammatory markers in a rat model of hypothyroidism. Can J Physiol Pharmacol. 2016;94(4):426–32.

52. Uzkeser H, Cadirci E, Halici Z, Odabasoglu F, Polat B, Yuksel TN, Ozaltin S, Atalay F. Anti-inflammatory and antinociceptive effects of salbutamol on acute and chronic models of inflammation in rats: involvement of an antioxidant mechanism. Mediators Inflamm. 2012;2012:438912.

53. Zhu H, Lemos H, Bhatt B, Islam BN, Singh A, Gurav A, Huang L, Browning DD, Mellor A, Fulzele S, et al. Carbidopa, a drug in use for management of Parkinson disease inhibits T cell activation and autoimmunity. PLoS One. 2017;12(9):e0183484.

Figures
Figure 1

The workflow of this research. (a) Data related to diseases, drugs and their targets are gathered from different data sources (described in sub-section 2.1). (b) Key genes with robust biosignatures and key regulatory effects in response to E. coli mastitis were identified by meta-analysis to identify up-/down-regulated genes followed by the application of machine learning algorithms [1] and conduction of network analyses [2] (described in sub-section 2.2). (c) Functionally related diseases or biological processes to mastitis by using the Pathway Studio web tool. (described in sub-section 2.3). (d) Relevant drugs and antibiotics to E. coli mastitis gathered by literature mining (described in sub-section 2.4) (e) A suitable heterogeneous network model is constructed by integration of achieved data from parts A, B, C, D (described in sub-section 2.5). (f) Running the Heter-LP algorithm [3] on constructed network to predict some more important relations of mastitis. (g) Predicted drugs according to their score computed by Heter-LP.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile2.xlsx
- Additionalfile1.xlsx