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The Role of ABC Transporters in Ciprofloxacin Resistance of *E. coli* ST131

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ABSTRACT

Overexpression of efflux pumps is a common mechanism of multidrug resistance (MDR), and antibiotic resistance genes are found in bacteria that are generally considered benign, which poses a risk. The global spread of bacterial infections and the increase in antibiotic resistance are two major problems that medical science is currently facing. One type of efflux pump that plays an important role in bacterial MDR is the ATP-binding cassette (ABC) transporter. ABC transporters hydrolyze ATP to facilitate the removal of antibiotics from the bacterial cell. An *Escherichia coli* ST131 treated with ciprofloxacin was analyzed using RNA-seq to determine whether molecular/gene networks were operationalized or non-operational by each antibiotic and the antibiotic resistance caused by ABC transporters. Gene expression assessment revealed that 589 genes have differential expression (FDR p-value < 0.05). Three of these genes, lolCDE, glnHPQ, and malEFG, exhibited ABC transporters as an enrichment function. In total, 22 substantial networks were found from these genes (PPI < 0.05). MalG, lolE, and glnP are the genes that serve as the hubs of these networks. Because MalEFG possesses two distinct enhanced functions—ABC transporters and two-component systems—it is more likely to be actively involved in antibiotic resistance. The first network may be activated by ciprofloxacin, while the other two networks may be inactivated, as malEFG is upregulated and the other two networks are downregulated.

Keywords: Molecular network, ABC transporter, Two-component system, Efflux pump

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Introduction

The quick development of antibiotic resistance is a worldwide issue, but pathogenic microorganisms are also becoming more widespread [1]. Antibiotic resistance is not limited to dangerous bacteria; it can also occur in generally regarded as safe (GRAS) microorganisms, such as *Bacillus subtilis* [2, 3]. *Escherichia coli* ST131 was a worldwide pandemic clone that mostly caused microbiological illnesses that started in the community [4]. A specific family of medicines known as fluoroquinolones, including ciprofloxacin, is resistant to almost all isolates of *E. coli* ST131 [5]. The first quinolone to be identified in 1962 was nalidixic acid, which has a fully synthetic analog in ciprofloxacin (CIP). Bacterial mortality results from CIP's inhibition of topoisomerase II (DNA gyrase) and IV, which in turn prevents DNA synthesis [6]. One of the five kinds of resistant efflux pumps, ATP-Binding Cassette (ABC) transporters, mediate one of the many pathways of antibiotic resistance, including CIP [7]. Two integral membrane proteins, two peripheral proteins that bind to and hydrolyze ATP, and a periplasmic (or lipoprotein) substrate-binding protein are the usual components of ABC transporters in prokaryotes. The active transport process is used by ABC transporters, which are essential membrane proteins, to transport various chemicals across the cell membrane [8]. Data from the genomes of bacteria show that the majority of the genes involved in all three of these factors form operons [9]. Though some efflux pumps are selective for a particular substrate, many transporters possess unique characteristics that enable them to expel a class of structurally

unrelated medications [10]. These microbes are known as multidrug-resistant (MDR) bacteria because they may concurrently reduce or even suppress their sensitivity to a variety of antimicrobials due to the acquisition of ABC transporters [11]. ABC proteins are the biggest paralogous family of proteins in *E. coli*, with 79 proteins in the genome, according to an analysis of the coding genes for these proteins [12]. The bacterial MDR transporter LmrA, which is expressed in *E. coli* and is a multidrug-resistant ATP in Lactococcus, was discovered by researchers in 1996 to be able to hydrolyze ATP and use the free energy it contains to extrude medications from the cell. MDR1 is a human multidrug-resistant P-glycoprotein that shares structural and functional similarities with LmrA [13]. Not only can bacteria have multidrug resistance, but ABC transporters also make neoplastic cells resistant to certain medications [14].

Not much research has been done on antibiotic resistance, even though discussions about it from the standpoint of gene networks are crucial. Using Cytoscape stringApp and CentiScaPe, we have examined up and down-regulated genes to identify the important molecular networks that are activated or inactivated by ciprofloxacin. Lastly, the KEGG and GO databases are used to functionally analyze the genes that correspond to each network.

Materials and Methods

Study design and data collection

The research design, data preparation, data processing, and analysis are depicted in a flowchart *et al*. The RNA-seq data came from the NCBI Gene Expression Omnibus (GEO). The MDR *E. coli* strain ST131 was subjected to RNA-seq after receiving a therapeutically recommended dosage of ciprofloxacin (2 μg/mL). The datasets are GSM2374960 (ciprofloxacin-treated) and GSM2374959 (control) [15]. In the present research, two samples from the 30-minute time point were utilized. One sample receives ciprofloxacin treatment (CIP), whereas the second sample (control) does not. Network and functional enrichment analysis is required to investigate the biological relevance of the genes that are expressed differently in the treatment and control groups. Each network is termed according to its associated ABC transporter name to make the interpretations easier.

Network Analysis and Identification of Hub Genes

These strongly coupled nodes are known as hub genes. A protein-protein interaction (PPI) network was built in this study to find hub nodes using Cytoscape stringApp [16], and all genes that are down- and up-regulated are imported to extract the important networks. Cytoscape CentiScaPe is utilized to obtain a thorough examination of the connections among nodes [17]. In the network, nodes with a high level of connectedness are referred to as hubs [18].

Gene set enrichment analysis (GSEA)

Cytoscape stringApp was used to perform functional enrichment analysis on each network independently [16]. Two distinct databases, KEGG-pathway DB and Gene Ontology (GO) DB, are employed to perform enrichment analysis [19, 20]. Finding the associated pathways for every network is the first step, and categorizing the genes according to their molecular activities, cellular components, and biological processes is the second.

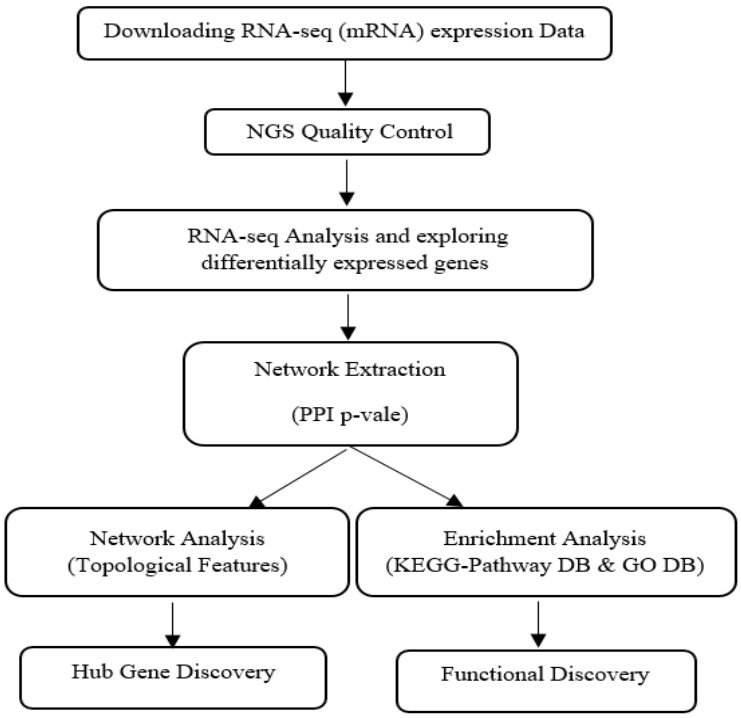
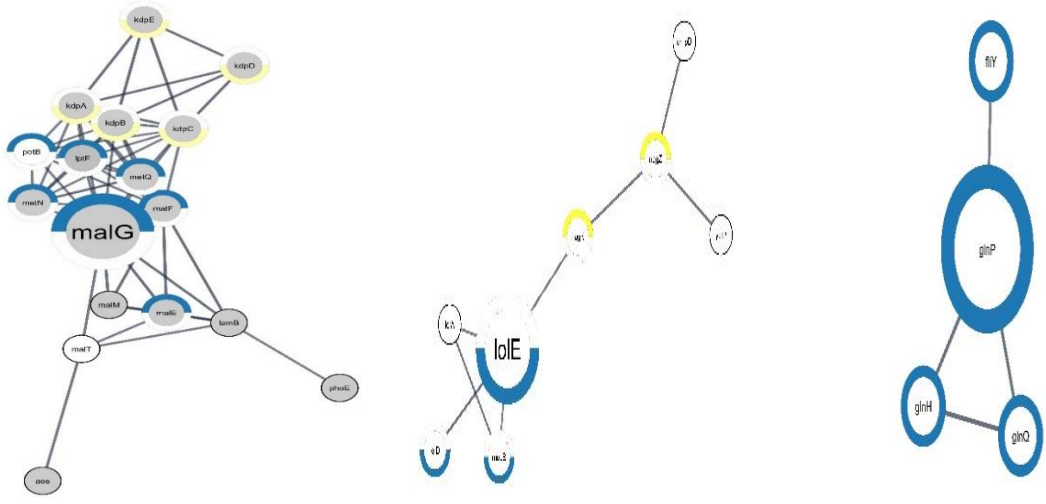


Figure 1. The flowchart of data preparation and analysis was used in this study.

Results and Discussion

FDR p-values for 589 genes are less than 0.05 following RNA-seq and differential gene expression analysis. All of the genes that were expressed differently, such as those that were up- and down-regulated, were imported into Cytoscape stringApp (protein query) to get a confidence threshold score of 0.9. A total of 22 networks were identified, three of which functioned as ABC transporters **(Figure 2)**. In **Figure 3**, 29 genes related to the ABC transporter function are shown as heat maps.



a) b) c)

Figure 2. a) malEFG, PPI p-value: 1.0E-16, b) lolCDE, PPI p-value: 6.66E-16, and c) glnHPQ, PPI p-value: 1.09E-12, networks include 17, 8, and 4 genes, respectively. The gray and white circles indicate up and down-regulated genes, respectively. The hub gene is the major node in every network. Particularly, the ABC transporter route involves the nodes with a black band.

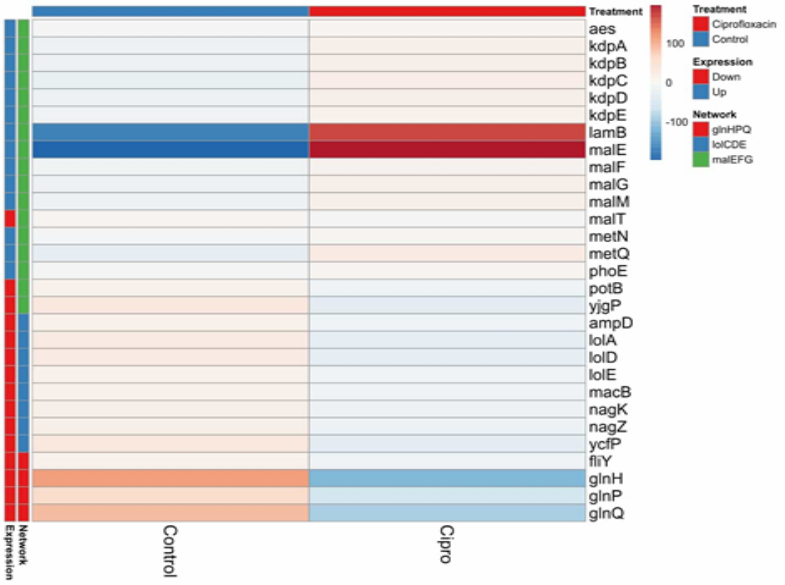
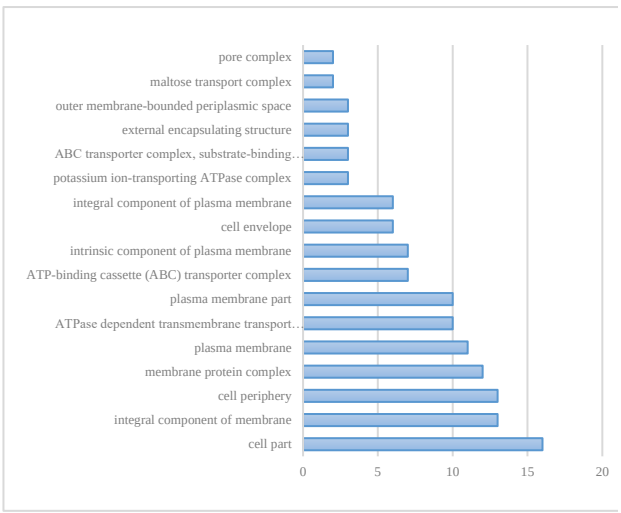


Figure 3. Heat map for 29 genes related to ABC transporter pathway

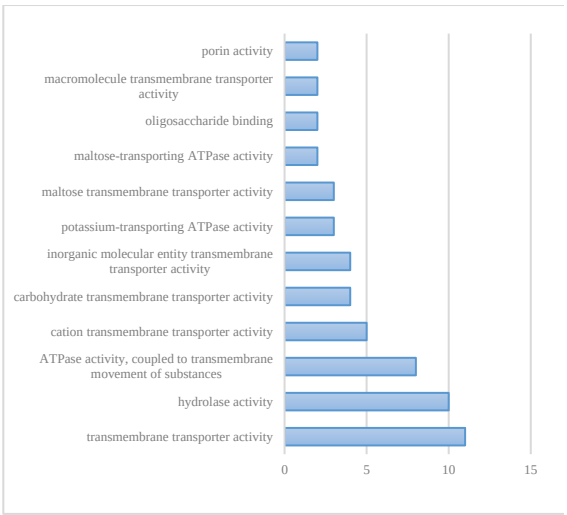
The nodes malG, lolE, and glnP in the malEFG, lolCDE, and glnHPQ networks, respectively, have the greatest degree, according to network analysis on node parameters; hence, they may be regarded as hub genes. With 12, 4, and 4 direct connections, respectively, the hub genes malG, lolE, and glnP can be regarded as important genes [21]. The gene malG produces a 296 amino acid residue protein. Because it has six hydrophobic domains, this protein is thought to be particularly hydrophobic. This protein is an essential inner membrane protein that is found in all integral membrane proteins of binding protein-dependent transport systems because of the highly conserved sequence of the malG gene [22]. An important membrane protein of the same name is produced by the lolE gene in conjunction with lolC [23]. The presence of the genes glnP, glnH, and glnQ allows *E. coli* to use glutamine as its only carbon source, and they are the main players in the glutamine transport system [22].

GO analysis yields useful results in a variety of classifications, such as biological processes, cellular components, and molecular functions. **Figures 4-6** display the enhanced GO keywords along with their charts. The number of genes in each term determines its length. To address this issue, we may state that each network can perform a certain biochemical function and that each cell can carry out a particular biological activity in the following ways:

- *malEFG* mostly as a transporter at the cell membrane transports ions and organic substrates **(Figure 4)**.
- *lolCDE* as a transporter moves lipoproteins to the outer membrane **(Figure 5)**.
- *glnHPQ* as a transmembrane transporter translocate amino acids **(Figure 6)**.



a)



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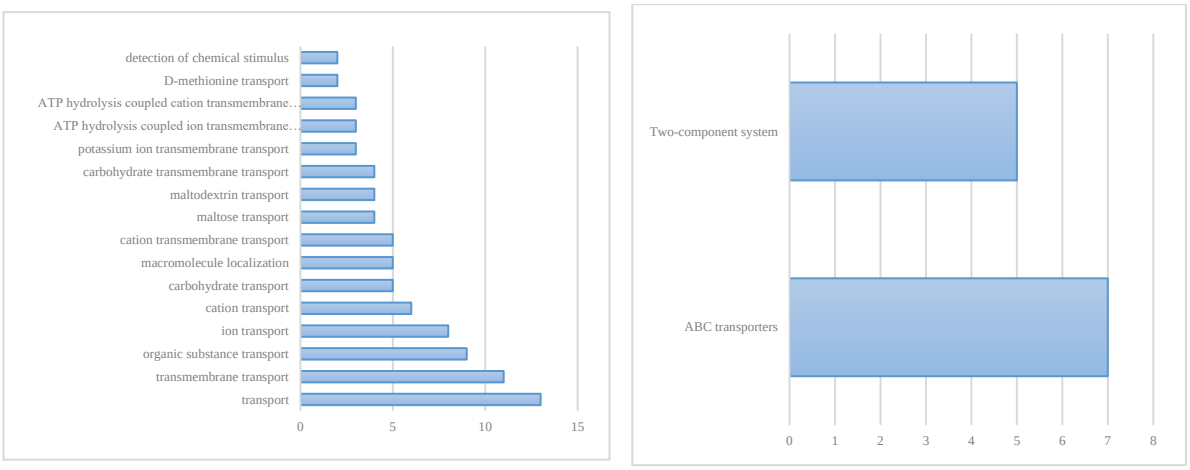
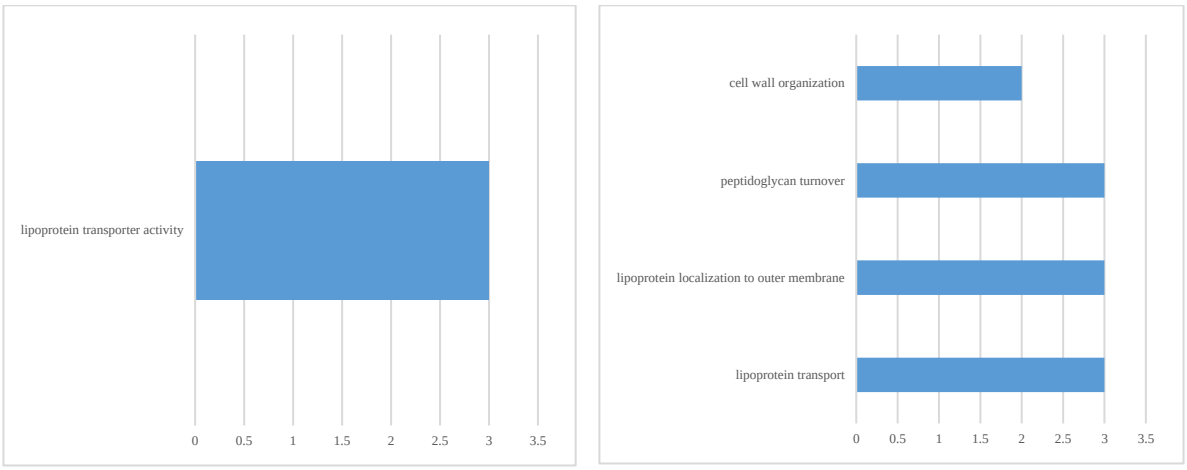


c) d)

Figure 4. Functional analysis of genes in the *malEFG* network; a) Cellular component, b) Molecular function, c) Biological process, and d) KEGG pathways



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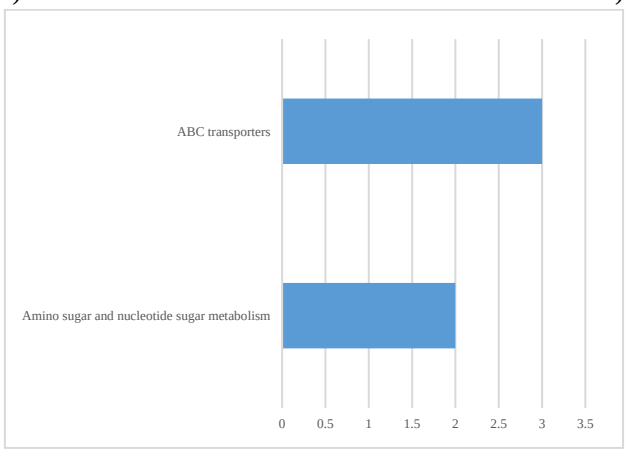

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Figure 5. Functional analysis of genes in *lolCDE* network; a) Molecular function, b) Biological process, and c) KEGG pathways.

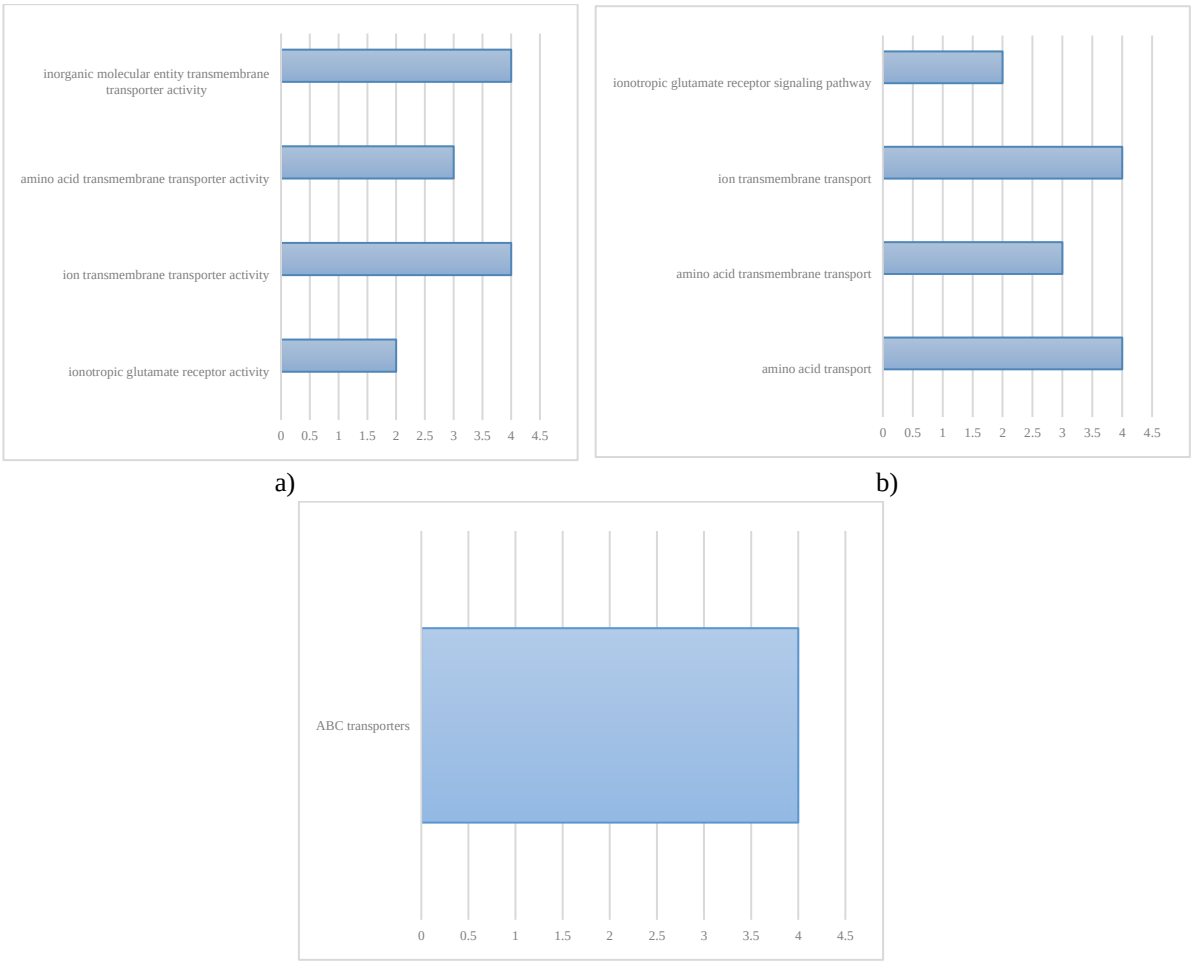

Figure 6. Functional analysis of genes in the *glnHPQ* network. GO DB: a) Molecular function, b) Biological process, and c) KEGG pathway.

Figure 1 shows 2 ways that are enriched for the malEFG network according to KEGG-pathway enrichment analysis. There are five genes (yellow belt circles) that are engaged in two-component systems (TCSs) and seven genes (black belt circles) that are enriched for ABC transporters. Even though the hub gene is not directly involved in TCS, all of the genes in the so-called ways have a link with the hub, according to the hubs' initial neighbors. Since there are no genes shared by these 2 ways, the hub gene serves as their primary driver. All genes are up-regulated in both ways, except potB and lptF.

The malB region contains two operons, including malEFG. The maltose system involves the malA and malB regions of the *E. coli* major genome. The malT gene and the malPQ operon make up the malA region. The malEFG operon and the malK-lamB operon are adjacent in the malB region, and both are transcribed differently from promoters situated between the first gene in each operon, malE, and malK [24]. MalT, the maltose system's transcription factor (TF), has a close relationship with other components of the system [25]. Three genes—malE, malF, and malG—are engaged in the maltose system out of the seven genes in the malEFG network that are enriched as ABC transporters. Furthermore, the methionine ABC transporter system is encoded by the metD locus, which includes metN (abc) and metQ (yaeC) [26]. The lptFG and potABCD operons include the remaining genes, lptF, and potB. Lipopolysaccharide-transporting proteins are produced by the lptFG operon. The primary components of the ABC transporter protein complex lptB2FG are lptF and lptG [27]. A filamenting phenotype, higher susceptibility to hydrophobic antibiotics, and a changed shape of lipopolysaccharides are the outcomes of lptF depletion [28]. A polyamine (putrescine/spermidine) transport mechanism is encoded by the potABCD operon [29]. Polyamines are essential for appropriate growth and proliferation in both prokaryotes and eukaryotes [30]. Another augmented function in the malEFG network is the two-component system (TCS). TCS is the

primary mechanism by which bacteria adapt to shifting environmental conditions and is essential for bacterial adaptation to a variety of niches [31, 32].

LolaCDE produces an ABC transporter in the inner membrane that discharges mature lipoproteins from the inner membrane to lolA. LolaA then transports the lipoproteins to lolB, an exterior membrane lipoprotein, via the periplasm [33]. The lolCDE complex in *E. coli* is a component of the larger lolABCDE machinery, which transports lipoproteins to the outer membrane. One of the three primary mechanisms aimed at exterior membrane construction is the lipoprotein (lol) way. Lipopolysaccharide transport proteins (lpt) and β-barrel assembly machine (bam) are the other two routes. Because of how crucial they are, if any of them are inhibited, *E. coli* dies and lyses. [34].

In *E. coli*, a specific transport mechanism for the polar amino acid glutamine is encoded by the glutamine permease operon, glnHPQ. The three genes in this operon are glnH, glnP, and glnQ. The periplasmic glutamine-binding protein (glnH) is encoded by glnH, whereas the two genes downstream of glnH, glnP and glnQ, share a single promoter [35, 36].

The expression of three ABC transporters—malEFG, lolCDE, and glnNPQ—was altered by ciprofloxacin in a way that upregulates malEFG and downregulates the other two networks. If ABC transporters are the mechanism behind an MDR bacterium, this does not imply that all ABC transporters are capable of pumping all drugs. Out of all the ABC transporters in the current investigation, we only discovered one that ciprofloxacin activates. Cancer cells exhibit the same characteristic. When common drugs are administered alongside them, the genes linked to ABCB1/P-pg, ABCC1/MRP1, and BCRP are overexpressed in breast, colon, and blood cancer, respectively [37].

Apart from coordinating a TCS, the malEFG is engaged in four distinct ABC transporters. Most ABC transporters are capable of detecting the presence of antibiotics in the external environment, according to Ahmad *et al*. results. The ABC transport system and TCSs in a malEFG network did not coincide by accident, however, because TCSs tightly regulate this detection ability and are important in triggering a quick and targeted reaction to antibiotics [38].

Techniques such as drug/antibiotic combination are used to improve efficacy and reduce resistance [39, 40]. As previously stated, ciprofloxacin downregulates lolCDE. MacB is one of the lolCDE network's downregulated genes. Even though this gene is not enriched in a distinct ABC transporter, it is genetically positioned inside the macAB operon, which encodes two proteins in the macAB ABC transporter that contribute to resistance to macrolide antibiotics [41, 42]. According to the findings of the current investigation, ciprofloxacin would promote susceptibility to other antibiotics by downregulating the lolCDE network, which is the primary framework of the antibiotic resistance-breaking (ARB) procedure [43-45].

Conclusion

As per the findings of this investigation, ciprofloxacin has inactivated the lolCDE and glnHPQ ABC transporters and activated malEFG. Since the activation of TCS and malEFG occurred in the same network, it is plausible that malEFG may be a contributing factor to ciprofloxacin resistance. The lolCDE ABC transporter also causes resistance to macrolide antibiotics, and its inactivation implies the possibility of controlling its resistance, which means that antibiotic resistance can be managed by a combination of drugs. Of course, more wet lab validations are required before final decisions are made.

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Conflict of Interest: None

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Ethics Statement: None

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