Original Article

Resensitizing resistant *Escherichia coli* ST131 to Macrolide using Fluoroquinolones

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ABSTRACT

Despite the growing rate of worldwide antibiotic-resistant bacteria, introducing a new antibiotic to the market is significantly disappointing. A promising approach to control resistance is antibiotic combination. In the current study, a molecular network-based approach is developed to find activated/inactivated ATP-Binding Cassette (ABC) transporters in order to arrange a reasonable combination of antibiotics that is useful to inhibit the multidrug-resistant bacteria. To study the possibility of the antibiotic combination, RNA-seq analysis was performed on *Escherichia coli* ST131 treated by ciprofloxacin. Eight transporting systems were extracted from the differentially expressed genes including maltose, D/L methionine, spermidine, lipopolysaccharide, lipoprotein, macrolide, L-glutamine, and cystine ABC transporters (effluxes). Bacterial exposure to ciprofloxacin, as a typical fluoroquinolone, leads to the activation of the first four and inactivation of the last four effluxes. Among all inactivated effluxes, macAB-TolC ABC transporter is specifically responsible for expelling the macrolide antibiotics, e.g. erythromycin, and it seems that the combination of ciprofloxacin with erythromycin can overcome the resistance to the macrolide class of antibiotics.

Keywords: Resensitizing, ATP-Binding Cassette transporter, Antibiotic combination, Escherichia coli

Introduction

Antibiotic resistance crisis is described as a coincidence of two events, rapid worldwide emergence of resistant bacteria and decades of the inability of pharmaceutical sciences to discover a new class of antibiotics [1]. If this dilemma remains unresolved, deaths due to Antimicrobial Resistance (AMR) will reach more than 10 million cases per annum by 2050 [2]. This means, over the next 30 years, AMR higher than cancer will be the deadliest complication [3].

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Tabrizi AE, Tahmoorespur M, Ebrahimie E. Resensitizing resistant *Escherichia coli* ST131 to Macrolide using Fluoroquinolones. J Adv Pharm Educ Res. 2021;11(4):29-34. https://doi.org/10.51847/gVI1vrKJmO

Although the outbreak of resistance in bacteria is basically a natural phenomenon, inappropriate use/combination of antibiotics greatly has led to resistance evolution [4]. Though the drug combination has been relatively helpful in controlling antibiotic resistance [5], if the drugs are not combined in a proper mechanism, it can boost the resistance complications which is called Multidrug Resistance (MDR) [6-8]. Among all comprehensive mechanisms of antibiotic resistance including antibiotic modification/degradation, antibiotic sequestration, antibiotic target modification/bypass/protection, and antibiotic efflux pump activation **(Figure 1)**, because of the inherent characteristic of the bacterial cell, antibiotic efflux is an important mechanism in antibiotic resistance [9]. Efflux pumps are a class of multipass transmembrane multi-domain proteins called ATP-Binding Cassette (ABC) transporters [10, 11]. ABC transporters as an effective controller of bioavailability of (bio)chemical compounds are present in all three kingdoms of life [12]. ABC transporters not only cause antibiotic resistance, but also reduce the cancer cell's access to the drug during the pharmacotherapy phase, disrupting part or all of the

chemotherapy [13, 14]. Different chemotherapy agents including Doxorubicin, Vinblastine, and Taxol, and also different antibiotic agents including macrolides, β-lactams, fluoroquinolones, and tetracyclines have encountered the so-called resistant mechanism [15, 16].

Human genome as a highly intelligent primate contains only 49 ABC genes in seven families from ABCA to ABCG [17, 18]. The structure of the prokaryotic genome is a bit different because the total numbers of ABC systems have a direct relationship with the size of the genome, meaning the larger genome contains the more ABC genes [12]. On average, 62 ABC transporters are functionally and structurally characterized in prokaryotes and among them, surprisingly, 13 ABC transporters (~21%) are eukaryotic-like transporters [19]. Exactly the same as eukaryotes, the prokaryotic ABC transporters act as a semi-specified evacuator that can expel not all but a specific set of molecules [10]. Since one of the mechanisms of resistance development is the overexpression of efflux genes [20] and as mentioned above, because of the similarities in the development of resistance to cancer chemotherapeutic agents and antibiotics, it seems that the strategies for overcoming anticancer drug resistance can be used to cope with AMR [21, 22]. Maybe downregulating the ABC genes would be a useful way to resensitize bacteria to current antibiotics.

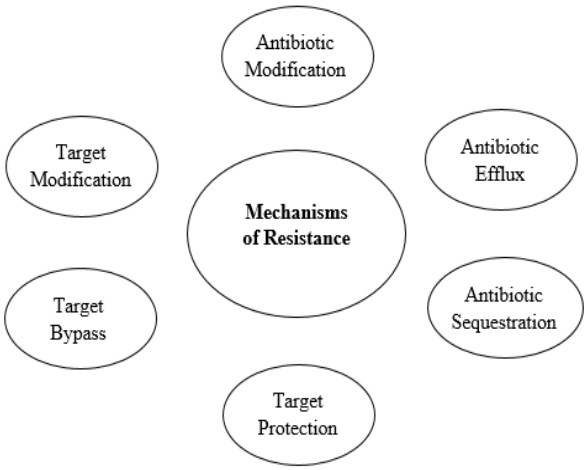


Figure 1. Mechanisms of Resistance to Antibiotics

Materials and Methods

The hierarchy of different stages of data preparation, data processing, and analysis are shown in **Figure 2**. RNA-seq NGS data was downloaded from the NCBI Gene Expression Omnibus (GEO). Datasets are GSM2374959 and GSM2374960, which are the control and ciprofloxacin-treated, respectively [23]. RNA-seq was performed on the MDR E. coli strain UR40 which is treated with a clinically relevant concentration of ciprofloxacin (2 μg/mL). In this study, two samples from the time point 30 min were analyzed. One sample is treated (CIP) and the other is not treated with ciprofloxacin (control). In order for exploring the gene expression pattern, differential gene expression analysis was run. To find the background molecular mechanism related to so-called sample groups, functional and network enrichment analysis was conducted. To facilitate the interpretation of results, each network was named as its relevant ABC transporter. A comprehensive Protein-protein Interaction (PPI) was constructed to identify significant molecular networks using Cytoscape stringApp [24], and all differentially expressed genes, down and up-regulated, were imported to extract the significant networks. To have a strong biological interpretation of the gene list, the functional analysis was run on differentially expressed genes using Cytoscape ClueGO. ClueGO uses KEGG pathway and KEGG compound database to make a comprehensive visualization and has the ability to load genes as clusters. This ability allows to run the analysis on separate gene sets or to merge them to have a combinatorial gene sets.

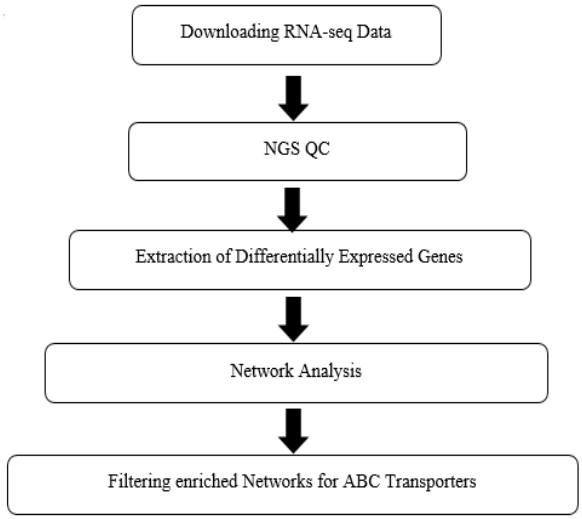
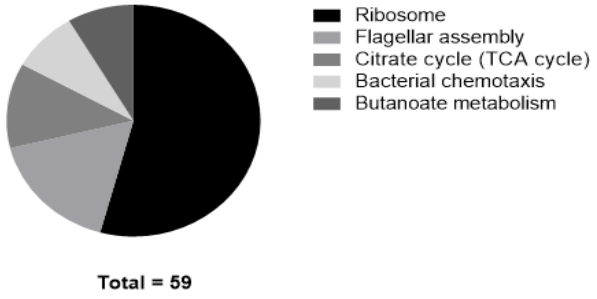


Figure 2. The Hierarchy of Different Stages of Data Preparation, Processing, and Analysis

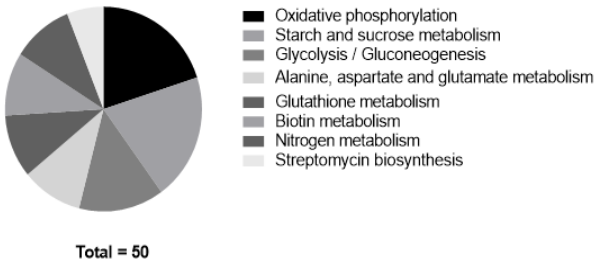
Results and Discussion

Based on RNA-seq and gene expression analysis, 589 genes had an FDR p-value of less than 0.05. Out of these differentially expressed genes, 354 and 235 genes are up- and down-regulated, respectively, and were imported to Cytoscape ClueGO simultaneously but in two separated clusters. 209 upregulated genes (~89%) and 340 downregulated genes (~96%) were recognized by ClueGO. 54 upregulated and 50 downregulated genes had significant functional annotations ($p\_value \leq 0.05$). For upregulated, downregulated, and a mixture of all differentially expressed genes, 5, 8, and 6 annotation terms were enriched, which are shown in **Figure 3** (a, b, and c, respectively). The enriched terms for upregulated genes were ribosome-related biosynthesis, flagellar assembly, TCA cycle, bacterial chemotaxis, and butanoate metabolism. The enriched annotations for downregulated genes were oxidative phosphorylation, carbohydrate metabolism (glycolysis/gluconeogenesis, starch, and sucrose) metabolism, amino acid (alanine, aspartate, and glutamate) metabolism, glutathione metabolism, biotin metabolism, nitrogen metabolism, streptomycin biosynthesis. Functional analysis on all annotated genes without any separation (up and down) leads to

having 6 enriched terms, which are pyrimidine metabolism, nicotinate and nicotinamide metabolism, lipopolysaccharide biosynthesis, RNA degradation, ABC transporters, and two-component system. The genes corresponding to each enriched function are listed in **Table 1**.



a)



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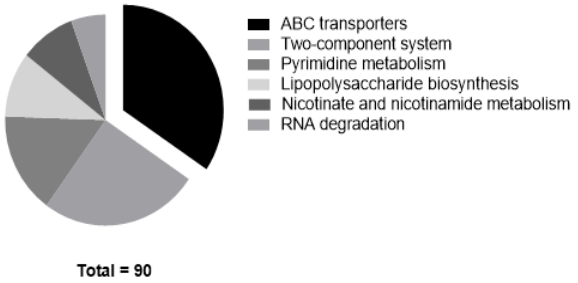


c)

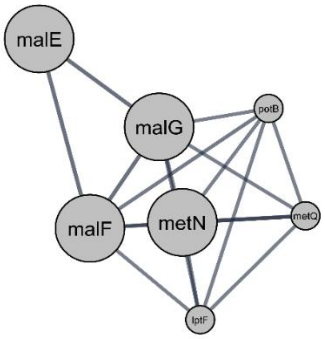
Figure 3. Enriched Annotation Terms for Upregulated (a) Downregulated (b) and All Genes (c)

Table 1. The Distribution of Genes in each Enriched Function

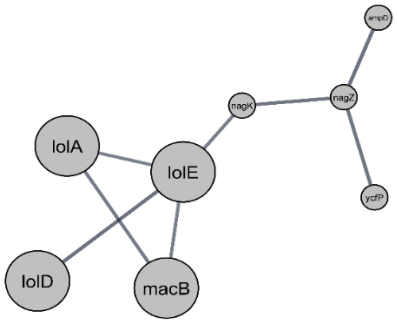
Type of Regulation (Gene No.)	GO Term	Gene No.	Genes
Both (90 Genes)	ABC transporters	31	fecC, malE, malF, malG, metN, metQ, modF, pstS, sapF, yehW, yejF,cydD, cysP, cysU, fliY, ftsE, glnH, glnP, glnQ, lolA, lolD, lolE, lptF, macB, mlaE, mlaF, potB, nagK, nagZ, ampD, ycfP
	Two-component system	23	basR, cheR, glnD, kdpA, kdpB, kdpC, kdpD, kdpE, maeA, pstS, rcsF, tar ,creB, creC, dcuB, evgA, fdnG, frdA, glnA, glnG, glnL, narG, zraS
	Pyrimidine metabolism	14	deoD, dut, ndk, nrdA, nrdE, rpoA,carA, carB, cdd, nrdD, nrdF, pyrE, udp, ushA
	Lipopolysaccharide biosynthesis	9	eptC, waaA, waaR, waaU, waaZ,gmhB, hldE, kdsB, waaC
	Nicotinate and nicotinamide metabolism	8	deoD, nadK, sthA,nadE, nadR, pntA, pntB, ushA
	RNA degradation	5	pcnB, rho,dnaK, groL, hfq
Downregulated (50 Genes)	Oxidative phosphorylation	10	cyoD, cyoE, frdA, ndh, nuoE, nuoH, nuoI, nuoJ, nuoK, nuoL
	Starch and sucrose metabolism	10	bglA, glgB, glgC, glgP, glgX, glk, mak, malP, malQ, malZ
	Glycolysis / Gluconeogenesis	7	adhE, bglA, glk, gpmM, pgk, yeaD, yihX
	Alanine, aspartate and glutamate metabolism	5	argH, aspA, carA, carB, glnA
	Glutathione metabolism	5	gshB, gss, gstB, pepB, pepN
	Biotin metabolism	5	bioB, bioF, bisC, fabB, ynfK
	Nitrogen metabolism	5	glnA, napA, narG, narK, nirB
	Streptomycin biosynthesis	3	glk, rfbA, rfbD
Upregulated (59 Genes)	Ribosome	32	rplB, rplD, rplL, rplM, rplP, rplQ, rplT, rplV, rplW, rplY, rpmB, rpmC, rpmE, rpmF, rpmG, rpmH, rpsA, rpsB, rpsG, rpsI, rpsQ, rpsS, rrlA, rrlC, rrlD, rrlE, rrlG, rrlH, rrsA, rrsB, rrsE, rrsH
	Flagellar assembly	10	flgB, flgK, fliF, fliI, fliM, fliN, fliO, fliP, fliS, fliT
	TCA cycle	7	acnB, fumA, gltA, sdhA, sdhB, sdhC, sdhD
	Butanoate metabolism	5	fadB, sdhA, sdhB, sdhC, sdhD
	Bacterial chemotaxis	5	cheR, fliM, fliN, malE, tar

In general, **Table 1** shows that when the bacteria exposed to antibiotics, the pattern of gene expression changes in such a way that the level of some metabolic pathways including carbohydrate, amino acid, and oxidative phosphorylation decrease and by activating chemotaxis and flagellar assembly pathways, the bacteria is forced to escape from the unfavorable (antibiotic-rich) environment [25]. Simultaneously, triggering the two-component signal transduction system activates the ABC

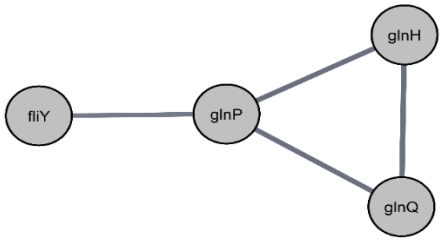
transporters to empty the bacterial cell from the entered antibiotic molecules [26]. These interrelated mechanisms make a bacteria resistant to a given antibiotic, e.g. ciprofloxacin. As some researchers mentioned, the so-called ABC transporter activation is one of the major self-resistance mechanisms in bacteria [9]. If one looks more closely at the 31 genes involved in the ABC transporter as enriched function, three distinct gene networks can be extracted **(Figure 4)**.



a) Maltose ABC Transporter ($FDR\ Value = 5.09E-10$)



b) Lipoprotein-Macrolide ABC transporter ($FDR\ Value = 0.01$)



c) Glutamine ABC transporter ($FDR\ Value = 3.19E-6$)

Figure 4. Enriched Network for ABC Transporters
The main genes corresponding to each transporter are shown in large circles and small circles are the genes with an indirect relationship to each transporter.

The genes enriched in the maltose ABC transporter network are all upregulated **(Figure 4a)**. This network contains 7 genes including malE, malF, malG, metN, metQ, potB, and lptF, which are located in four different operons. The genes malE, malF, and malG belong to malEFG, the genes metN and metQ belong to metNIQ, the gene lptF belongs to lptFG and finally, the potB belongs to potABCD operons [27]. The malEFG, metNIQ, and potABCD operons are responsible for the biosynthesis of maltose, L/D methionine, and spermidine ABC transporters, respectively [28-31]. The lptFG operon alongside with lptCAB operon involves in the biosynthesis of the lipopolysaccharide ABC transporter system [32, 33].

The genes enriched in the lipoprotein-macrolide ABC transporter network are all downregulated **(Figure 4b)**. This network contains 8 genes including lolA, lolD, lolE, macB, nagZ, nagK, ompD, ycfP, and consists of two separated ABC transporter systems including lipoprotein and macrolide ABC transporters [27]. Lipoprotein ABC transporter is synthesized by lolA and lolCDE operons [34, 35]. The gene macB is one of two genes in macAB operon and takes part in encoding the macrolide (macAB-TolC) ABC transport system [36, 37].

The genes enriched in glutamine ABC transporter network are all downregulated **(Figure 4c)**. This network contains 4 genes, which are glnH, glnP, glnQ, and fliY. The first three genes involve in encoding L-glutamine ABC transporting system [27, 38]. The last gene, fliY, as a part of fliAZY operon involves in cystine ABC transporting system [39, 40].

As mentioned above, based on the in silico study was conducted on RNA-seq data earned from MDR *Escherichia coli*, eight ABC transporting systems have been affected by ciprofloxacin. Among all, maltose, D/L methionine, spermidine, and lipopolysaccharide ABC transporting systems are activated and the rest including lipoprotein, macrolide, L-glutamine, and cystine ABC transporting systems are inactivated. Now it is known that by exposure to antibiotics, e.g. ciprofloxacin, the bacteria have activated and inactivated sets of transporting systems, simultaneously. There is experimental evidence that the macrolide ABC transporter as one of inactivated transporting systems captured in the current study, specified to expel macrolide class of antibiotics, e.g. erythromycin, from the bacterial cell [41, 42].

Conclusion

Escherichia coli ST131 in exposure to ciprofloxacin inactivates the macAB-TolC ABC transporter, which can be a promising mechanism to surmount the macrolide resistance. Although the results of this study have opened a window for the antibiotic combination with the aim of counteracting antibiotic resistance, for these results to be applicable, laboratory and clinical research are needed.

Acknowledgments: The authors would like to thank Klitgaard et al. for sharing the valuable data used in this article.

Conflict of interest: None

Financial support: This research was fund by Ferdowsi University of Mashhad.

Ethics statement: None

References

1. Årdal C, Balasegaram M, Laxminarayan R, McAdams D, Outterson K, Rex JH, et al. Antibiotic development—economic, regulatory and societal challenges. Nat Rev Microbiol. 2020;18(5):267-74.
2. de Kraker ME, Stewardson AJ, Harbarth S. Will 10 million people die a year due to antimicrobial resistance by 2050? PLoS Med. 2016;13(11):e1002184.
3. Bassetti M, Poulakou G, Ruppe E, Bouza E, Van Hal SJ, Brink A. Antimicrobial resistance in the next 30 years, humankind, bugs and drugs: a visionary approach. Intensive Care Med. 2017;43(10):1464-75.
4. Burnham CA, Leeds J, Nordmann P, O'Grady J, Patel J. Diagnosing antimicrobial resistance. Nat Rev Microbiol. 2017;15(11):697-703.
5. Tyers M, Wright GD. Drug combinations: a strategy to extend the life of antibiotics in the 21st century. Nat Rev Microbiol. 2019;17(3):141-55.
6. Karaiskos I, Lagou S, Pontikis K, Rapti V, Poulakou G. The "old" and the "new" antibiotics for MDR gram-negative pathogens: for whom, when, and how. Front Public Health. 2019;7:151.
7. Tohamy ST, Aboshanab KM, El-Mahallawy HA, El-Ansary MR, Afifi SS. Prevalence of multidrug-resistant Gram-negative pathogens isolated from febrile neutropenic cancer patients with bloodstream infections in Egypt and new synergistic antibiotic combinations. Infect Drug Resist. 2018;11:791-803.
8. Exner M, Bhattacharya S, Christiansen B, Gebel J, Goroncy-Bermes P, Hartemann P, et al. Antibiotic resistance: What is so special about multidrug-resistant Gram-negative bacteria? GMS Hyg Infect Control. 2017;12:Doc05.
9. Peterson E, Kaur P. Antibiotic resistance mechanisms in bacteria: relationships between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. Front Microbiol. 2018;9:2928.
10. El-Awady R, Saleh E, Hashim A, Soliman N, Dallah A, Elrasheed A, et al. The role of eukaryotic and prokaryotic ABC transporter family in failure of chemotherapy. Front Pharmacol. 2017;7:535.
11. Auda IG, Salman IM, Odah JG. Efflux pumps of Gram-negative bacteria in brief. Gene Rep. 2020;20:100666.
12. Davidson AL, Dassa E, Orelle C, Chen J. Structure, function, and evolution of bacterial ATP-binding cassette systems. Microbiol Mol Biol Rev. 2008;72(2):317-64.
13. Robey RW, Pluchino KM, Hall MD, Fojo AT, Bates SE, Gottesman MM. Revisiting the role of ABC transporters in multidrug-resistant cancer. Nat Rev Cancer. 2018;18(7):452-64.
14. Domenichini A, Adamska A, Falasca M. ABC transporters as cancer drivers: Potential functions in cancer development. Biochim Biophys Acta Gen Subj. 2019;1863(1):52-60.
15. Mansoori B, Mohammadi A, Davudian S, Shirjang S, Baradaran B. The different mechanisms of cancer drug resistance: a brief review. Adv Pharm Bull. 2017;7(3):339.
16. Blanco P, Hernando-Amado S, Reales-Calderon JA, Corona F, Lira F, Alcalde-Rico M, et al. Bacterial multidrug efflux pumps: much more than antibiotic resistance determinants. Microorganisms. 2016;4(1):14.
17. Vasiliou V, Vasiliou K, Nebert DW. Human ATP-binding cassette (ABC) transporter family. Hum Genomics. 2009;3(3):281-90.
18. Vendelbo J, Olesen RH, Lauridsen JK, Rungby J, Kleinman JE, Hyde TM, et al. Increasing BMI is associated with reduced expression of P-glycoprotein (ABCB1 gene) in the human brain with a stronger association in African Americans than Caucasians. Pharmacogenomics J. 2018;18(1):121-6.
19. Moussatova A, Kandt C, O'Mara ML, Tieleman DP. ATP-binding cassette transporters in Escherichia coli. Biochim Biophys Acta Biomembr. 2008;1778(9):1757-71.
20. Du D, Wang-Kan X, Neuberger A, van Veen HW, Pos KM, Piddock LJ, et al. Multidrug efflux pumps: structure, function and regulation. Nat Rev Microbiol. 2018;16(9):523-39.
21. Borowski E, Bontemps-Gracz MM, Piwkowska A. Strategies for overcoming ABC-transporters-mediated multidrug resistance (MDR) of tumor cells. Acta Biochimica Polonica. 2005;52(3):609-27.
22. Beretta GL, Cassinelli G, Pennati M, Zuco V, Gatti L. Overcoming ABC transporter-mediated multidrug resistance: the dual role of tyrosine kinase inhibitors as multitargeting agents. Eur J Med Chem. 2017;142:271-89.
23. Klitgaard RN, Jana B, Guardabassi L, Nielsen KL, Løbner-Olesen A. DNA damage repair and drug efflux as potential targets for reversing low or intermediate ciprofloxacin resistance in E. coli K-12. Front Microbiol. 2018;9:1438.
24. Doncheva NT, Morris JH, Gorodkin J, Jensen LJ. Cytoscape StringApp: network analysis and visualization of proteomics data. J Proteome Res. 2019;18(2):623-32.
25. Webre DJ, Wolanin PM, Stock JB. Bacterial chemotaxis. Curr Biol. 2003;13(2):R47-9.
26. Ahmad A, Majaz S, Nouroz F. Two-component systems regulate ABC transporters in antimicrobial peptide production, immunity and resistance. Microbiology. 2020;166(1):4-20.
27. Karp PD, Billington R, Caspi R, Fulcher CA, Latendresse M, Kothari A, et al. The BioCyc collection of microbial genomes and metabolic pathways. Brief Bioinform. 2019;20(4):1085-93.
28. Oldham ML, Khare D, Quiocho FA, Davidson AL, Chen J. Crystal structure of a catalytic intermediate of the maltose transporter. Nature. 2007;450(7169):515-21.
29. Mächtel R, Narducci A, Griffith DA, Cordes T, Orelle C. An integrated transport mechanism of the maltose ABC importer. Res Microbiol. 2019;170(8):321-37.
30. Nguyen PT, Lai JY, Lee AT, Kaiser JT, Rees DC. Noncanonical role for the binding protein in substrate

uptake by the MetNI methionine ATP Binding Cassette (ABC) transporter. Proc Natl Acad Sci. 2018;115(45):E10596-604.

31. Furuchi T, Kashiwagi K, Kobayashi H, Igarashi K. Characteristics of the gene for a spermidine and putrescine transport system that maps at 15 min on the Escherichia coli chromosome. J Biol Chem. 1991;266(31):20928-33.
32. Narita SI, Tokuda H. Biochemical characterization of an ABC transporter LptBFGC complex required for the outer membrane sorting of lipopolysaccharides. FEBS Lett. 2009;583(13):2160-4.
33. Lundstedt EA, Simpson BW, Ruiz N. LptB-LptF coupling mediates the closure of the substrate-binding cavity in the LptB2FGC transporter through a rigid-body mechanism to extract LPS. Mol Microbiol. 2020;114(2):200-13.
34. Ito Y, Matsuzawa H, Matsuyama SI, Narita SI, Tokuda H. Genetic analysis of the mode of interplay between an ATPase subunit and membrane subunits of the lipoprotein-releasing ATP-binding cassette transporter LolCDE. J Bacteriol. 2006;188(8):2856-64.
35. Yakushi T, Masuda K, Narita SI, Matsuyama SI, Tokuda H. A new ABC transporter mediating the detachment of lipid-modified proteins from membranes. Nat Cell Biol. 2000;2(4):212-8.
36. Crow A, Greene NP, Kaplan E, Koronakis V. Structure and mechanotransmission mechanism of the MacB ABC transporter superfamily. Proc Natl Acad Sci. 2017;114(47):12572-7.
37. Fitzpatrick AW, Llabrés S, Neuberger A, Blaza JN, Bai XC, Okada U, et al. Structure of the MacAB–TolC ABC-type tripartite multidrug efflux pump. Nat Microbiol. 2017;2(7):1-8.
38. Hsiao CD, Sun YJ, Rose J, Wang BC. The crystal structure of glutamine-binding protein fromEscherichia coli. J Mol Biol. 1996;262(2):225-42.
39. Kertesz MA. Bacterial transporters for sulfate and organosulfur compounds. Res Microbiol. 2001;152(3-4):279-90.
40. Mytelka DS, Chamberlin MJ. Escherichia coli fliAZY operon. J Bacteriol. 1996;178(1):24-34.
41. Kobayashi N, Nishino K, Hirata T, Yamaguchi A. Membrane topology of ABC-type macrolide antibiotic exporter MacB in Escherichia coli. FEBS Lett. 2003;546(2-3):241-6.
42. Kobayashi N, Nishino K, Yamaguchi A. Novel macrolide-specific ABC-type efflux transporter in Escherichia coli. J Bacteriol. 2001;183(19):5639-44.