Cambridge Default Journal (2022), **00**, 1–3

CAMBRIDGE
UNIVERSITY PRESS

HW

What causes ampicillin resistance in E.Coli K12 strain.

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Abstract

After sequencing the genome of the new ampicillin-resistant strain of E. Coli and analysing the data three potentially important mutations were found, which can induce such a resistance. AcrB(acrE), envZ(ompB, perA, tpo) and ftsI (pbpB) genes were influenced. AcrB is a multidrug efflux pump subunit of RND superfamily, envZ is involved in acid stress response. Peptidoglycan D,D-transpeptidase FtsI catalyzes cross-linking of the peptidoglycan cell wall, it is usually targeted by beta-lactam antibiotics.

Keywords: E.Coli, ampicillin, RND, OmpR, antibiotic resistance, FtsI

1. Introduction

Studying antibiotic resistant bacteria is extremely important today, as, firstly, resistance might become one of the greatest problems of the humanity in the nearest future. In order to fight it scientists must know the exact mutation and mechanism behind new resistance. Secondly, such knowledge is necessary for doctors to choose a correct treatment according to the exact resistance (remember that different antibiotics have different side effects too). That might help some patients immediately.
Ampicillin from the penicillin group of beta-lactam antibiotics is able to penetrate gram-positive and some gram-negative bacteria, including E. Coli. This bacteria is a normal part of human gut microbiome, however it may be virulent and potentially dangerous. That is why it is inevitable sometimes to use antibiotics on it. Usually ampicillin acts like a bacteriolytic antibiotic: it blocks the enzyme transpeptidase, acting in the third and final stage of bacterial cell wall synthesis. At the end it causes cell lysis(Delcour AH, 2009).
However, bacterias can become antibiotic resistant through the evolution process. Mutations occur randomly, but some of them might be very effective. One example is changing the structure of lactam-binding site of transpeptidase (Peptidoglycan D,D-transpeptidase FtsI).

2. Methods

The reference sequence was an E.coli strain K-12 substrain MG1655 genome, published as `GCF_000005845.2_ASM584v2/` from 2022-10-28, and the analysed raw data was Illumina sequencing reads from shotgun sequencing of an E. coli strain that is resistant to ampicillin: https://doi.org/10.6084/m9.figshare.10006541.v3 (forward and reverse sequences). 455 876 reads in each file. The data was quickly looked through manually(using bash) and with fastqc. The last one showed a lack of quality in some reads, but the general quality was sufficient to go on with the analysis. Sequences were aligned with BWA-MEM, the alignment SAM file was converted to BAM, sorted and indexed. Variant calling was made through VarScan, and with the snpEff the automatic SNP annotation was made. It showed 6 SNP's, 3 of them seem to play some role in ampicillin resistance.

3. Results

Multiple mutations have occured in the genome of the bacteria, but, according to snpEff only six of them deserve our attention. All of them are SNP's and these are:

Table 1. SNP mutations

POS	ID	REF	ALT	QUAL	FILTER	NAME1	NAME2
93043	.	C	G	.	PASS	NP_414626.1	peptidoglycan DD-transpeptidase FtsI *
482698	.	T	A	.	PASS	NP_414995.1	multidrug efflux pump RND permease AcrB *
852762	.	A	G	.	PASS	ECK0806	small RNA RybA
1905761	.	G	A	.	PASS	NP_416335.4	manganese efflux pump MntP
3535147	.	A	C	.	PASS	NP_417863.1	sensor histidine kinase EnvZ *
4390754	.	G	T	.	PASS	NP_418585.4	ribosome small subunit-dependent GTPase A

Table note

* Is associated with antibiotic interaction

It can be seen with IGV visualisation that occured in small RNA RybA mutation cannot even be translated (it follows stop-codons in all three possible reading frames), as well as manganese efflux pump MntP. They appear to be not important.

FtsI mutated in the periplasmic domain, ArcB - in 5th helical transmembrane region. MntP mutated in the periplasmic domain, the 24th aminoacid was changed.

4. Discussion

The most obvious mutation that has something to do with this antibiotic resistance case is the change in peptidoglycan DD-transpeptidase FtsI, it is blocked with beta-lactam ring, which is one of the main mechanisms of cell wall synthesis inhibitors (Houba-Hérin N, 1985). It is not clear if the changed region interacts with ampicillin, though. The region, interacting with penicillin, beta-lactam antibiotic as well, is situated closer to the C-end of the protein too, but this periplasmatic region is not described well enough.

Another SNP mutation appeared in the AcrB coding gene. This protein is one of the most important subunits of a drug efflux protein complex AcrA-AcrB-AcrZ-TolC with broad substrate specificity that uses the proton motive force to export substrates. Even a small change in this subunit can influence the whole complex functioning.(Li, 2015) This complex belongs to resistance-nodulation-division (RND) superfamily, which also takes part in stress response and pathogenicity.(Fernando DM, 2013) AcrB was changed in the 6th transmembrane region, which seemed to increase it's efficiency in pumping ampicilling out of the cell, through both of membranes.

Osmoregulatory system EnvZ/OmpR is involved in transporting beta-lactam antibiotics inside of the cell. The amount of the substance depends on it's structure(Ghai I, 2018). The EnvZ protein mutated in the 3rd cytoplasmic reagion, similar changes can cause resistance to some antibiotics, for instance, waldiomycin(Eguchi Y, 2017). Thus, it is brobable, that EnvZ mutation decreased the amount of ampicillin, transported in a cell.

5. Citations

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