Genetic mechanisms of the antibiotics resistance in E.Coli.

Tolmachev M. Student of Bionformatic institute. Saint-Petersburg
Ushakov M. Student of the Bioinformatic institute. Saint-Petersburg

Introduction

Nowadays, microbial resistance is the one of the biggest challenges in medicine. Driving by evolution, mechanisms of antibiotic resistance getting more complicated. For doctors and pharmaceutical companies knowing exact mechanism is crucial. Without that knowledge patients would be treated wrong, hospitals and manufacturers would lose money for inefficient therapy, and microbial resistance problem would be getting bigger and bigger. One way to estimate mechanism is based on getting sequence of DNA\RNA. Knowing the difference in nucleotide position between non resistance E.coli and resistant E.Coli provides information about difference between active proteins, expression e.t.c. And then, we can draw a conclusions about mechanisms and potentially effective drugs.

Pathogenic E.Coli causes diarrhea, urinary tract infections, respirotory illness and many more. Ampicillin, a semi-synthetic β-lactam antibiotics is commonly used in treatment of illnesses caused by E.Coli. Bacteria often resist such antibiotics in the following ways: encodes β-lactamase, changes the target protein in cell wall, reduces the permeability of outer membrane, and increases the expression of drug efflux pump[1]. This changes have been driving by genetic differences such as single nucleotide variants and mutations. By estimating the difference during bioinformatical analysis we can explain mechanism and provide recommendation about treatment.

Materials and methods

We used reference sequence of the parental (unevolved, not resistant to antibiotics) *E. coli* strain[2]. And raw sequence data from our molecular biology department[3,4]. We inspected raw sequence by FastQC[5]. After inspection we decided to filter data by quality. We used Trimmomatic [6] with these options: LEADING:30 TRAILING:30 SLIDINGWINDOW:10:30 MINLEN:20. We aligned sequences to reference

by BWA using mem algorithm and saving results directly in BAM format by samtools[7]. Basic statistics from alignment also provided by samtools. We wrapped up our data in mpileup format by samtools. To call actual variant we used VarScan program by command java -j --min-var-freq 0.50 --variants

Results

Raw data analysis was performed using FastQC. In FastQC report we've got failures in per base sequence quality test of both forward and reverse reads and in per tile sequence quality test on forward reads. So we decided to filter raw data by quality. After filtering there were 4445689 (99.77%) reads.

After filtering, we aligned reads on indexed reference. Almost all reads (99.88%) were mapped. The variant calling results showed that there were 5 mutations in the resistant strain. Exploring these mutations in IGV browser revealed that three of them are missense and locate in protein-coding genes *ftsI, acrB* and *envZ,* fourth occurs in ncRNA-coding gene *rybA* and the last one is synonymous and present in *rsgA* gene *(*Table *1)*.

Table 1 – The mutations analysis result

Gene-id	Position	Base	Codone	Aa	Gene
b0084	93043	C→G	GCC→GGC	*Ala→Gly*	*ftsI*
b0462	482698	T→A	GTC→GAC	*Val→Asp*	*acrB*
b4416	852762	A→G	-	-	*rybA*
b3404	3535147	A→C	CAT→CCT	*His→Pro*	*envZ*

b4161	4390754	G→T	CGG→CGT	*Arg→Arg*	*rsgA*

Information about mutated gene products was taken from annotation, also it can be watched on EcoliWiki [8].

Table 2 – Mutated genes products

Gene	Product
ftsI	Peptidoglycan DD-transpeptidase *ftsI*
acrB	Multidrug efflux pump RND permease *acrB*
rybA	Small RNA *rybA*
envZ	Sensory histidine kinase *envZ*
rsgA	Ribosome small subunit dependent GTPase A

Discussion

To find out how the detected mutations can be associated with resistance we first looked at mutated genes products (Table 2). We decided to look only at protein-coding genes, so we don't consider rybA. Thus we focused on the following three genes: *ftsI*, *acrB* and *envZ*.

Peptidoglycan DD-transpeptidase also known as PBP3 (penicillin-binding protein 3) is the main target of ampicillin. It is involved in bacterial cell wall biosynthesis, namely, the transpeptidation that crosslinks the peptide side chains of peptidoglycan strands [9]. Alteration in this PBP3 can lead to a decrease in the affinity of the ampicillin.

EnvZ and *OmpR* make up the two-component regulatory system that controls permeability of the outer membrane by regulating the expression of the outer membrane porines OmpF and OmpC [10]. We suggest that a mutation in this gene might lead to a decrease in membrane permeability for ampicillin.

In E. coli, the tripartite efflux system AcrA/AcrB/TolC is the pump that extrudes multiple antibiotics, dyes, bile salts and detergents. The inner membrane component A*crB*, a member of the Resistance Nodulation cell Division (RND) family, is the major site for substrate recognition and energy transduction of the entire tripartite system [11]. Mutation in *acrB* may increase ampicillin-binding affinity and improve export of the drug from the cell.

A study in which these 3 mutations were found in ampicillin-resistant E. coli strains reinforces our speculation [1]. Another similar study reports that mutation of the *ftsI* alone did not increase antibiotic resistance, while combination of *envZ and fstI* mutations increased the MIC of antibiotics multiple times [12].

Thus mutations found in genes *ftsI, envZ* and *acrB* are indeed responsible for resistance of the strain to ampicillin. In a study of Mary Adler strain with these and two more mutations acquired high-level carbapenem resistance. Carbopenems are the last-resort antibiotic and apparently they can kill our strain. It can also be assumed that a patient infected with this strain can be treated using non-6-lactam antibiotics with other mechanisms of action, or a combination of several antibiotics.

References

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