What causes antibiotic resistance?

Voskoboinikov A., Dudkovskaya A.

Abstract

Antibiotics are the main tool for treating bacterial infections nowadays. In our research we studied whole-genome *E.coli* K12 strain Illumina sequencing data to determine its antibiotic resistance properties using bioinformatic methods. We explored the genome of this strain and found 3 missense SNPs, one of which, apparently, is responsible for the ampicillin resistance.

1 Introduction

Nowadays many bacterial infections are successfully treated due to the existence of antibiotics. Unfortunately, during the evolutionary process some bacteria have become resistant to antibiotics. Natural selection supports mutated gene forms in the present of antibiotics as it provides evolutionary fitness for the mutated organism. Bacteria can also acquire a large range of antibiotic resistance genes during the process called horizontal gene transfer. Excessive uncontrolled use of antibiotics caused massive distribution of antibiotic resistance among bacteria. This is now one of the major problems in the treatment of bacterial infections [1]. Being able to understand what causes antibiotic resistance of bacteria will help us treat bacterial infections more effectively.

In our project we studied sequence data of Escherichia coli K12 strain resistant to the antibiotic ampicillin. We examined mutations in this strain and assumed possible mechanisms of antibiotic resistance.

2 Methods

Illumina raw shotgun sequencing data from E.coli K12 strain was obtained from publicly available article [2] and the reference genome was obtained from NCBI FTP. [3]
Reads were filtered using `Trimmomatic` [4]. For Illumina adapters cutting of build-in *TruSeq3-PE.fa*. As a quality threshold we used *Q20* both for start and end trimming. We used sliding window as trimming approach, with window size 10 and average quality within the window 20 and drop-off threshold of 20 for the read length.

Next, we aligned our sequences to the reference genome using `BWA` tools [5]. First, we indexed reference *E.coli* K12 strain genome using `BWA` tools. Then we aligned the reads using the `bwa mem`, and sorted and indexed them using `samtools` [6].

After that we used `samtools` and `VarScan` [7] for variant calling. For SNP detection we set 95% threshold of non-reference bases at a position to be identified as mutation. On the final step we used `SnpEff` [9] for automatic SNP annotation and visualized genomic data in `IGV`[8].

All SNPs discovered during our research were then studied using information from public data sources like PubMed, Google Scholar and UniProt.

3 Results

From below table we can see how changed the amount of the reads during the preparation of our data.

	Raw data	After trimming	After aligning
Reads	911752	892518	892776

Data processing yielded five SNPs and two deletions. Among SNPs one was an upstream gene variant, one – synonymous variant and three were missense variants. Missense mutations occurred in the following genes: *ftsI, acrB* and *envZ*.

First gene, *ftsI*, ecodes Peptidoglycan D,D-transpeptidase enzyme, which is essential for cell wall creation and cell division. It catalyzes cross-linking of the peptidoglycan in the cell wall and is known to be the primary target for penicillin-like antibiotics [10]. This mutation may play a decisive role in ampicillin resistance of a given bacteria strain. Ampicillin binds to and inactivates synthesis of the bacterial cell wall. Interference with the cross-linkage of peptidoglycan chains necessary for bacterial cell wall strength and rigidity. This causes cell lysis [13], [14], [15], [16].

Second gene, *acrB*, encodes subunit in efflux pump complex. Efflux pump is a non-specific bacterial mechanism for substance excretion. It is considered to be a crucial component of antibiotic resistance and may cause multiple non-specific drug resistance to many antibiotics [11].

Third gene, *envZ*, encodes sensor histidine kinase. EnvZ protein is a kinase that phosphorylates OmpR in response to environmental signals [12]. OmpR plays an important role in osmoregulation of the cell. It is a transcription factor that regulates transcription of ompC and ompF genes. So, mutated EnvZ kinase may play an important role regulating cell response to the presence of antibiotics [17].

Overall, all three discovered mutations may play a significant role in bacteria drug resistance as affected genes take part in cell response to antibiotics directly (*ftsI*) or indirectly by regulating cell osmolarity (*acrB* and *envZ*).

4 Discussion

Examination of Illunina data of *E.coli* K12 strain revealed three significant mutations. All of these mutations may cause antibiotic resistance. In all of three mutation the most decisive is mutation in *ftsI*. It is supposed to have a direct effect on penicillin/ampicillin-like antibiotics resistance. Therefore, antibiotics from penicillin group should be excluded as treatment options in this case.

Other mutations (*acrB* and *envZ*) affect cell stress response in terms of osmoregulation and non-specific drug response. These mutations may have an effect on every antibiotic lowering treatment expectations. Probably, higher antibiotic dose for treatment may be required too.

References

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