BIOINFORMATICS INSTITUTE

Case report: Investigation of Escherichia Coli ampicillin resistance using NGS data

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Project 1

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1 Abstract

This study focuses on the example of pathogenic E. coli that has developed resistance to ampicillin. Through analysis of next-generation sequencing (NGS) data, we revealed that resistance have been caused by mutations in the genes ftsI and acrB. They encode enzymes: transpeptidase responsible for synthesizing peptidoglycans and multidrug pump, respectively. Finally, we propose potential strategy to increase treatment effectiveness.

2 Introduction

Antibiotic resistance is one of the major problems in healthcare today[1]. There are 4 mane types of antibiotic resistance: modification of antibiotic molecule, decreased antibiotic penetration and efflux, changes in target site, global cell adaptations[2].The development of NGS has made a significant contribution to the further investigation of bacterial mutation mechanisms and made targeted therapies possible.

In our study, we use contemporary bioinformatic tools to analyse pathogenic E. coli k12 strain shotgun sequencing data. The aim of our study was to find the cause of resistance of the bacterium. This work is noteworthy not only because it highlights possible genetic causes of antibiotic resistance, but also demonstrates the potential of the bioinformatic tools we have used.

3 Methods

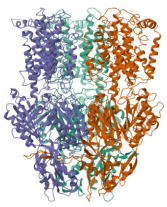
In this case report, we performed identification of possible causes of antibiotic resistance. The overall pipeline included reads filtering, aligning sequences, variant calling and automatic SNP annotation.

Briefly,reference sequence of the parental (not resistant to antibiotics) E. coli strain and fastq files of resistant E.coli strain were given. Sample reads were filtered by Trimmomatic tool [3] with threshold coverage 20, then they were aligned to indexed reference by bwa tool[4]. Aligned reads were sorted and indexed by Samtools[5], variant calling was performed by VarScan[6] with p-value threshold 0.01 and minimal variant frequency 0.4. Automatic SNP annotation was performed by snpEff. For variant effect prediction IGV browser was used[7]. Information on altered proteins was obtained from the UniProt and RCSB PDB.

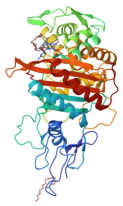
All used commands and environment preparations can be viewed on github[8].

4 Results

Five variants were called, two of them seemed to be causal for antibiotic resistance. The first one encodes the protein ftsI (fig. b), and this mutation changes Ala to Gly in 544 position of the protein. The second one encodes acrB protein (fig. a), and the mutation changes Gln(polar uncharged) to Leu(hydrophobic) in 569 position.



(a) acrB protein



(b) ftsI protein

5 Discussion

We have revealed two possible mechanisms of antibiotical resistance of E.coli k12 strain to ampicillin.

In the first case changing Ala to flexible Gly aminoacid in FtsI may lead to feature changes of ftsI protein. FtsI (also called Penicillin Binding Protein 3, PBP3) of Escherichia coli is a transpeptidase required for synthesis of peptidoglycan in the division septum and is one of several proteins that localize to the septal ring. Ampicillin acts as an irreversible inhibitor of the enzyme transpeptidase, which is needed by bacteria to make the cell wall. Thus, changing the target site may be the leading cause of resistance[9].

In the second case change of polar Gln to hydrophobic Leu in acrB may be responsible for changes in acrB. The E. coli AcrB protein is a transporter that is energized by proton-motive force and that shows the widest substrate specificity among all known multidrug pumps, ranging from most of the currently used antibiotics, disinfectants, dyes, and detergents to simple solvents.Thus, improving the AcrB efflux pump may be the second mechanism of antibiotic resistance[10].

These hypotheses need to be tested in future studies with help of molecular dynamics, X-ray crystallography and other approaches. Potential treatment options include non beta-lactam antibiotics, because target site has been changed. Moreover, it will be challenging to specify the drug, because it is needed to be studied acrB efflux of other antibiotics in k12 strain.

References

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