Mutations that probably cause ampicillin resistance in E.Coli.

Darya Grechishkina

Abstract:
Sequencing data from a strain of *E. Coli* resistant to ampicillin were analyzed using sequence alignment and variant calling methods. Three missense mutations were found in genes associated with antibiotic resistance(ftsI, acrB, envZ). Several pieces of advice about threatening methods were given.

Introduction:
There are four general mechanisms of antibiotic resistance and first is *inactivation of the antibiotic molecule*, which includes (i)modification of the molecule by producing special enzymes, which are capable of alter molecule and (ii)destruction of the molecule, for example by producing β-lactamases to destroy β-lactams .

Second mechanism is *reducing the amount of drug in the cell*, which includes (i)decreased permeability, which especially affects hydrophilic molecules and (ii)efflux pumps, which are complex systems used to pump the drug out of the cell.

Third mechanism is *alteration of the target site* by (i) target protection, so antibiotics can't reach target and bind, or (ii)target site modification(1). And, finally, the fourth way is *altering the metabolic pathway* to compensate drug influence.

Methods:
E.Coli strain K-12 substrain MG1655(2) was used as a reference sequence of unevolved *E.Coli* strain. Raw Illumina sequencing reads(3,4) of ampicillin resistant *E.Coli* strain were analysed using FastQC(5). Were noticed that reads have low per base sequence quality, so reads were filtered using TrimmomaticPE(6) with sliding window approach(window size 10, average quality 20), cutting bases from the start and the end if quality is lower than 20 and minimal length 20.

Filtered reads were indexed and aligned using bwa(7). Then aligned reads were compressed, sorted, indexed and piled up using samtools(8). Resulting mpileup file was scanned using VarScan(9) with minimum variant frequency *0.9*, *--variants* option and *.vcf* output. Results were analyzed using IGV(10).

Results:

Main result of the work is finding three missense mutations, one per three genes(ftsI, acrB, envZ).

FtsI is a penicillin binding protein, binding β-lactam antibiotics to it suppresses FtsI activity and is lethal(11).

AcrB is an inner membrane transporter, part of multidrug efflux pump AcrAB-TolC, which is considered to be a major contributor to antibiotic resistance(12).

EnvZ products are required for the expression of outer membrane proteins OmpF and OmpC(13), which form pores in the outer membrane, so molecules like β-lactams may get into the cell(14).

Discussion:

I assume that antibiotic resistance was received with one (or all three) of described mutations. If mutation in ftsI is involved, then it's *target site modification* mechanism of antibiotic resistance, and patients should be treated with non-beta-lactam antibiotics (as all of them bind to PBP), eg Vancomycin. If mutation in acrB is involved, inhibitors of efflux pumps should be used.(15) If mutation in envZ is involved, then it's decreased permeability mechanism of antibiotic resistance, and combination of antibiotic and a permeabilizing agent could be used.(16)

Supplementary materials:

https://github.com/DespairedController/bioinf_lab_journal

Citations:

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