Single-nucleotide polymorphisms in ampicillin resistant Escherichia coli K12 strain

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Abstract

Antibiotic resistance is one of the most dangerous phenomena of nowadays. Determination of this condition cause is very important because it leads to changing treatment strategy and more rational antibiotic using. In our investigation, we analysed single-nucleotide polymorphisms in the ampicillin resistant substrain of the Escherichia coli K12 strain. We conclude that mechanism of this strain resistance to ampicillin may be a mutation in a gene of peptidoglycan DD-transpeptidase FtsI. It may lead to changing structure of antibiotics target. In our case problem may be solved by using antibiotics of other groups, as example fluoroquinolones.

Keywords: E.coli, ampicillin resistance, penicillin-binding protein 3, single-nucleotide polymorphisms

1 Introduction

Escherichia coli (E. coli) is a bacterium commonly found in the lower intestine of warm-blooded organisms. Most E. coli strains are harmless, but some strains can cause serious infections. For example, E. coli O157:H7 in humans usually

causes bloody diarrhea and also can cause hemolytic uremic syndrome (HUS), hemolysis, thrombocytopenia, renal failure, and occasionally death [1].

Amplicillin is an antibiotic used to treat certain human infections that are caused by bacteria such as E. Coli. The mechanism of ampicillin action is destroying transpeptidase protein, which builds the cell wall of the bacteria. However, lately the resistance rate of E. Coli to ampicillin has a growth [2].

The antibiotic resistance is one of the most dangerous problem in nowadays healthcare beacuse it means we can't effectively treat bacterial infections. The understanding of mechanisms underlying antibiotic resistance may help to solve this problem.

This work explores hypothetic mechanism of resistance that evolved in E. Coli str. K12 by locating and analyzing mutations in sequence data. The focus will be in single-nucleotide polymorphism (SNP) as the most common type of genetic variation.

2 Methods

The reference genome was get from parental, unevolved E. coli strain K-12 substrain MG1655 as most widely studied strain of E. coli. The sequence data was downloaded from NCBI genomes NCBI genomes. Then E. coli strain that is resistant to the antibiotic ampicillin was selected for SNP analysis. It was sequenced by shotgun sequencing on Illumina. The sequence data was downloaded from figshare. We used fastqc (version 0.11.9) to estimate quality of sequenced data. For improving the overall quality of sequencing reads we used program Trimmomatic (version 0.39) [3]. In our case, it cut bases off the start and the end of a read if quality was below 20. Also it trimmed reads using a sliding window approach, with window size 10 and average quality within the window 20, and then dropped the read if it was below length 20. For aligning sequences to reference we used tool BWA-MEM (version 0.7.17-r1188) [4], that based on Burrows-Wheeler Transformation. Then alignments were compressed, indexed and sorted via Samtools (version 1.16.1) [5].

To differ SNP from sequencing errors we used tool VarScan (version 2.3.9) [6] that found all positions in which more that 10% of reads has non-reference bases. Then for annotation of these positions we used tool SnpEff [7] and for visualization IGV Browser [8].

3 Results

Reads were processed by Trimmomatic for quality arising. In figures 1-4 you may see forward and reverse reads quality before and after trimming respectively. Table 1 contains data about number of the reads at different stages of analysis.

VarScan detected six significant SNPs in the genome material from E.coli str. K-12. Brief information about it is in table 2. One of this SNPs' was the samesense-mutation (GCT substitutes GCC, which code the same amino acid - alanine) and it was excluded from follow-on analysis. One more mutation was

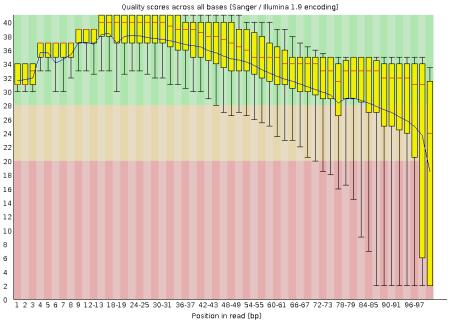

Fig. 1 Forward reads quality before trimming

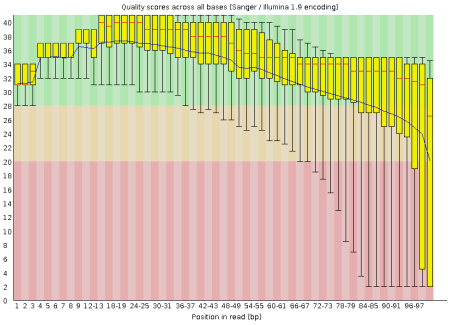

Fig. 2 Reverse reads quality before trimming

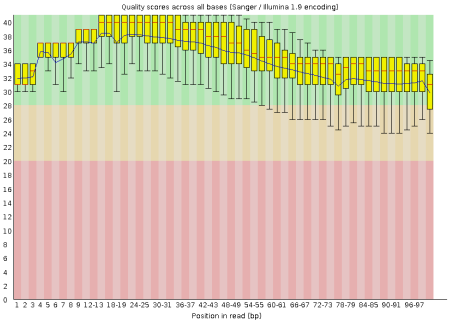

Fig. 3 Forward reads quality after trimming

the error of the VarScan tool and was excluded from the follow-on investigation too.

Thus, we have four single-nucleotide polymorphisms for follow-on investigation: missense-mutations in genes encoding peptidoglycan DD-transpeptidase FtsI, RybA sRNA, manganese efflux pump MntP and sensor histidine kinase EnvZ. Visualizations above-described three SNPs' from IGV are in figures 5-7.

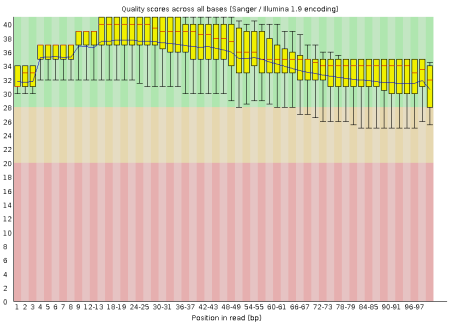

Fig. 4 Reverse reads quality after trimming

Table 1 The number of reads at the different stages of investigation

	Forward	Reverse	All
Reads at start of the work	455876	455876	911752
Reads after trimming	446259	446259	892518
Alignmented reads	-	-	891649

Fig. 5 IGV visualization of SNP in peptidoglycan DD-transpeptidase FtsI gene

4 Discussion

The first one of the analysed mutations was missense-mutation in CDS of the ftsI gene, encoding peptidoglycan DD-transpeptidase FtsI also known as penicillin-binding protein 3 (PBP3). It is known, that this protein is a lethal target for penicillin-like antibiotics and an essential protein for changing cellular wall, while bacterial cell divides [9]. The changing of the aminoacid sequence

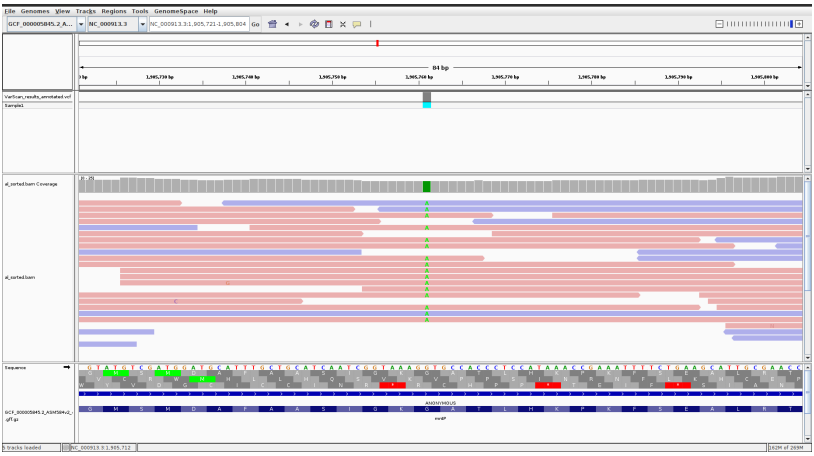

Fig. 6 IGV visualization of SNP in manganese efflux pump MntP

Fig. 7 IGV visualization of SNP in sensor histidine kinase EnvZ

of this protein may cause its spatial characteristics and does its resistant to ampicillin's action.

The second mutation is SNP in gene, encoding RybA small RNA. This sRNA regulates genes of the biosynthesis of aromatic amino acids under peroxide stress [10]. We haven't any evidence to declare connection between mutations in RybA and ampicillin resistance.

The next SNP is a missense-mutation in the CDS of a gene, encoding probable manganese efflux pump MntP. The probable function of this protein is magnesium efflux. MntP helps to regulate the intracellular level of manganese in the bacterial cell [11]. It can be helpful for resistance to oxidative stress because the exact regulation of magnesium level in the cell can effectively regulate the activity of manganese superoxide dismutase (MnSOD). This fact may be the cause of lesser susceptibility to oxidative stress. Some investigators connect this fact with the presence of non-antibiotic resistance [12]. The

Table 2 Brief data about SNPs in ampicillin resistant E.coli

Gene id	Protein id (if exists)	Function of transcript	Nucleotide substitution	Type of mutation	Amino acid substitution (for proteins)
gene-b0084	NP_414626.1	Links tails of peptidoglycans	C to G	Missense	alanine to glycine
gene-b0462	NP_414995.1	Transports antibiotics from the cell (cause of multidrug resistance)	A to A	Artifact	glutamine to glutamine
gene-b4416	-	Regulates biosynthesis of aromatic amino acids under peroxide stress	T to G	-	-
gene-b1821	NP_416335.4	Transports manganese from the cell	G to A	Missense	glycine to aspartic acid
gene-b3404	NP_417863.1	Regulates an expression of porins in response to osmotic stress	T to C	Missense	valine to alanine
gene-b4161	NP_418585.4	GTP hydrolytic protein	C to T	Samesense	alanine to alanine

absence of exact mechanisms make hypothesising about connections between antibiotic resistance and MntP mutations doubtful and speculative.

The last SNP we analyzed is a missense-mutation in CDS of a sensor histidine kinase EnvZ gene. This protein regulates an expression of porins in response to osmotic stress [13]. It is indicated, that reducing of this gene expression is associated with resistance to other antibiotics group - aminoglycosides [14].

We have at least one mutation, which may cause E.coli antibiotic resistance to ampicillin. It is the alteration of the ampicillin's target site - peptidoglycan DD-transpeptidase FtsI, also known as PBP3.

It needs to concentrate on the checking our hypothesis in further investigation. It includes biochemical and x-ray-structure analyse of mutated peptidoglycan DD-transpeptidase FtsI and, if possible, direct comparison of two E.coli colonies, which differs exactly by one this SNP (we hypothetically omitted the contribution of random mutations).

The suggested clinical solution in adult patients with infection caused by E. coli K-12 with the above-described phenotype may be the usage of fluoroquinolones (ciprofloxacin, levofloxacin, and others), which influences the bacterial DNA-gyrase. Fluoroquinolones have another molecular target and more efficient against gram-minus bacteria (E. coli is gram-minus). Using of aminoglycosides is not indicated, because EnvZ mutation can potentially initiate resistance for this group. Nowadays we need to use antibiotics more rational and answerable, only by indications. It can give us chance to avoid major accident in the world healthcare.

Declarations

- All authors declare that they have no conflicts of interest.

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