Detection Of Single Nucleotide Polymorphisms Associated With Ampicillin Resistance In *Escherichia coli*

M. Uzun[a], A. Fedorenko[b]

[a]*Research Center Biotechnology RAS, , Moscow, Russia*
[b]*Skolkovo Institute of Science and Technology, , Moscow, Russia*

Abstract

Studying antibiotic resistance in *Escherichia coli (E. coli)* is crucial due to rising concerns about bacterial resistance to drugs. In this study raw sequencing data from ampicillin-resistant *E. coli* was quality-checked, aligned to the reference genome, and analyzed for variants. Conducted analysis revealed novel SNPs in genes like *ftsI, acrB, rybA, mntP*, and *envZ*, suggesting potential resistance mechanisms.

Keywords: Single nucleotide polymorphisms, antibiotic resistance, *Escherichia coli*, VarScan

1. Introduction

The global surge in antibiotic resistance is an escalating threat to public health, potentially resulting in over 10 million fatalities by 2050, underscoring the urgency of this issue[1]. Ampicillin, a member of the β-lactam antibiotics family, has played a vital role in the treatment of bacterial infections; however, its extensive use has led to the emergence of resistance, challenging its effectiveness[2]. *Escherichia coli (E. coli)*, a commensal bacterium in the human gut, is not exempt from the burgeoning issue of antibiotic resistance. Though essential for digestive health, pathogenic strains of *E. coli* have developed resistance to ampicillin and other β-lactam antibiotics[3].

This study employs next-generation sequencing techniques to explore the genetic basis of ampicillin resistance in *E. coli*, aiming to identify single nucleotide polymorphisms (SNPs) associated with this resistance.

2. Materials and Methods

Basic statistics of raw reads were acquired using `seqkit v.2.5.1`[4]. Raw reads were quality checked with `FastQC v.0.12.1`[5]. Low-quality reads were removed using `Trimmomatic`[6]. Trimmed reads were aligned to the reference genome of *E. coli* strain K-12 substrain MG1655 using `bwa v.0.7.17-r1188` with the "-mem" flag[7]. The generated SAM-format file was compressed to a BAM-formatted file, sorted, and indexed using `samtools v.1.13`[8].

Variant calling was performed using `VarScan v.2.4.0` with a minimum variant frequency of 0.2[9]. SNPs predicted with VarScan were also checked with `snpeff v.5.2`[10]. Data of alignment, annotation, and variant calling were visualized using `IGV v.2.16.11`[11].

3. Results

The downloaded sequencing data comprised 2x101-bp paired-end Illumina reads (84.6 Mb, Table 1). The reads were generally of good quality, successfully passed trimming, and aligned to the reference genome. The obtained sequence reads were aligned against the reference genome sequence of E. coli K12 MG1655 (NC_000913.3) obtained from NCBI. To identify the SNPs potentially related to ampicillin resistance, we performed automated and manual variant calling analysis. As a result, we found four genes with missense mutations, one gene with an upstream gene mutation, and one gene with a silent mutation (Table 2).

Raw reads names	amp_res_1.fastq.gz (forward)	amp_res_2.fastq.gz (reverse)
Number of sequences	455,876	455,876
Summary sequence length	46,043,476	46,043,476
Number of sequences after trimming	446,259	446,259
Summary sequence length after trimming	42,003,868	41,649,154
Percent of mapped	891649 (99.87%)	

Table 1: Statistics for Illumina reads

Gene name	Type of mutation	Mutation	Predicted amino acid change
ftsI	missense	c.1631C>G	p.Ala544Gly
acrB	missense	c.1706A>T	p.Gln569Leu
rybA	upstream gene mutation	c.-4758T>C	-
mntP	missense	c.74G>A	p.Gly25Asp
envZ	missense	c.722T>G	p.Val241Gly
rsgA	silent	c.756C>A	p.Ala252Ala

Table 2: The summary of single nucleotide polymorphisms in *E. coli*.

4. Discussion

Utilizing the next generation sequencing technologies holds the potential to streamline the discovery of genetic variations across bacteria. The application of whole-genome shotgun sequencing allows to detect such spontaneous mutations in *E. coli K12 MG1655* as SNPs. We applied both automated and manual

analyses to identify the genetic alterations by comparing the DNA sequence of interest to reference sequence. In total, we identified five intragenic and one extragenic SNPs in our strain. We observed no information about these mutations (except the mutation in the *envZ* gene Val-241-to-Gly[12]) in the literature means we found novel mutations potentially determining the ampicillin resistance.

4.1. rsgA gene

The peptidoglycan DD-transpeptidase *ftsI* gene, also known as *PBP3*, encodes a critical protein involved in bacterial cell wall synthesis and integrity. *FtsI* is responsible for catalyzing the final steps in peptidoglycan cross-linking, which is crucial for maintaining the structural integrity of the bacterial cell wall[13]. This gene plays a pivotal role in the process of cell division, where it participates in septal peptidoglycan synthesis. *FtsI* is a primary target of β-lactam antibiotics, such as penicillin and cephalosporins[14]. These antibiotics inhibit *FtsI*'s transpeptidase activity by binding irreversibly to the active site. Consequently, this interference disrupts the formation of new peptidoglycan cross-links and weakens the cell wall. The inhibition of *FtsI*'s function by beta-lactam antibiotics ultimately leads to bacterial cell lysis and death[15]. Mutations in the *FtsI* gene can lead to antibiotic resistance, which poses a major challenge in combating bacterial infections.

4.2. acrB gene

The *acrB* gene encodes a crucial component of the AcrAB-TolC efflux pump, a major player in bacterial antibiotic resistance. This protein functions as a multidrug efflux transporter, actively pumping a wide range of toxic compounds, including antibiotics, out of bacterial cells[16]. *AcrB*'s role in antibiotic resistance is particularly notable, as it actively extrudes penicillin-like antibiotics, preventing their lethal effects by expelling them from the cell. The upregulation or mutation of *acrB* can significantly contribute to antibiotic resistance[17], making it a key target in understanding and combating bacterial drug resistance. *AcrB*'s efflux pump function is central to bacterial survival and the challenge of overcoming antibiotic therapies[17].

4.3. rybA gene

The *rybA* gene encodes a small regulatory RNA molecule in bacteria, and it plays a critical role in the post-transcriptional regulation of gene expression. Specifically, *rybA* is involved in regulating the translation and stability of various mRNA molecules such as downregulation of *aroL* and *aroF*, thereby influencing the overall protein production within the cell[18]. This regulation impacts a variety of cellular functions, including stress response, metabolism, and adaptation to changing environmental conditions. Moreover, *rybA*'s influence on mRNA stability and translation can indirectly affect bacterial antibiotic resistance, as it may modulate the expression of genes involved in resistance mechanisms.

4.4. mntP gene

The *mntP* gene in bacteria encodes a manganese transporter protein that plays a crucial role in maintaining intracellular manganese homeostasis. Manganese is an essential micronutrient involved in various cellular processes, including protection against oxidative stress. This manganese efflux pump, controlled by the yybP-ykoY riboswitch, contributes to protecting the cell from oxidative damage and preventing toxic intracellular accumulation[19,20].

4.5. envZ gene

The *envZ* gene encodes a crucial protein involved in bacterial two-component signal transduction systems, primarily responsible for monitoring and responding to changes in the osmolarity of the surrounding environment[12]. As an osmosensor, *EnvZ* regulates the activity of the *OmpR* transcription factor, which, in turn, controls the expression of outer membrane proteins21. This includes porins, such as *OmpC* and *OmpF*, which are pivotal for the influx and efflux of various solutes across the bacterial outer membrane. In terms of antibiotic resistance, the envZ gene's role is interconnected with its impact on outer membrane protein composition. Changes in the expression of porins can influence the permeability of the bacterial cell envelope, potentially affecting susceptibility to antibiotics like penicillins, which rely on efficient transport into the bacterial cell[22].

4.6. Genes Involved in Antibiotic Resistance

These results suggest the mutations of *ftsI*, *acrB*, and *envZ* genes involved in the antibiotic resistance mechanisms. Therefore, it may be recommended to use methods of influencing these genes but other domains to treat patients or their targets as for *envZ* gene. For instance, *envZ* affects the OmpC and OmpF porins that can be a highly attractive antibacterial target due to mutation in *envZ*. Because of the *EnvZ/OmpR* two-component system, OmpR also influences the *envZ*-mediated resistance. However, *OmpC* and *OmpF* genes can be affected by antimicrobial peptides (AMPs) to handle antibiotic resistance[23]. Another approach is to use the other group of antibiotics to affect the bacterial cell. One of the promising alternatives are AMPs supposing damage to the membrane of bacteria, especially Gram-positive bacteria. The AMPs are targeted for precursors of cell wall components, particularly the highly conserved lipid II. Consequently, these peptides do not inhibit peptidoglycan synthesis by binding to proteins, as conventional antibiotics do. Instead, they form a complex with the precursor molecule, which can additionally promote pore formation and membrane disruption. Thus, the multifaceted mode of action renders AMPs superior compared to antibiotics that exclusively target a single specific entity[24]. It makes AMPs a potential way of fighting antibiotic resistance.

5. References

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