A SYSTEMATIC STUDY FOR EVOLUTION OF BACTERIAL DRUG RESISTANCE: PHENOTYPE TO GENOTYPE

by

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Abstract

Bacterial drug resistance is a worldwide problem threatening millions of lives. Several studies showed that bacteria develop direct resistance against an antibiotic compound used throughout treatment. However, recent studies demonstrated that resistance to one antibiotic can pleiotropically lead to resistance to other antibiotics, a concept known as cross-resistance, imposing serious limitations for combating against infectious diseases. Therefore, slowing down evolution of cross-resistance is critical and important task for developing effective antibiotic therapies. Despite its importance, mechanisms behind cross-resistance are not well understood due to lack of systematic studies. Here in this systematic study, we aim to provide a better understanding of evolution of antibiotic resistance using state of the art genetic tools. In this study, we evolved 88 initially isogenic *Escherichia coli* populations against 22 different antibiotics for 21 days. For each drug, two populations were evolved under strong selection and two populations were evolved under mild selection. Representative clones from each evolved population were phenotyped against all 22 drugs we used in our experiments and their resistance levels were carefully quantified. Furthermore, these clones were genotyped by Illumina whole genome sequencing and resistance-conferring mutations were identified. Bacterial populations evolved under strong selection acquired stronger resistance against higher number of antibiotics compared

to populations evolved under mild selection. Strongly selected populations also acquired higher number of mutations compared mildly selected populations and there mutations were found to be more pathway specific among strongly selected populations. Finally, populations evolved against aminoglycosides were found to develop hypersensitivity against several other antibiotic classes due to mutations in *trkH* gene, coding for a membrane protein. Our study provides a thorough understanding for phenotype to genotype in the context of antibiotic resistance and demonstrates that selection strength is an important parameter contributing to the complexity of evolution of antibiotic resistance.

ANTİBİYOTİK DİRENCİNİN EVRİMİNE DAİR SİSTEMATİK BİR ÇALIŞMA: FENOTİPTEN GENOTİPE

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Anahtar Kelimeler: Bakteri evrimi, Antibiyotik direnci, Çapraz direnç, Antibiyotik

Özet

Bakteri direnci dünyada çapında sağlığını tehdit eden önemli bir sorundur. Bir çok çalışma bakterilerin tedavi esnasında maruz kaldığı ilaca karşı direnç kazandığını ispatlamıştır. Ancak yeni çalışmalar bakteri hücrelerinin bir antibiyotiğe direnç kazanırken, daha önce maruz kalmadığı başka antibiyotiklere karşı da direnç kazandığını ispatlamıştır. Çapraz direnç denen bu soruna çözüm bulmak günümüzde önemli bir hal almıştır. Bu konuda bir çok çalışma yapılsa dahi sistematik çalışmaların yetersizliğinden ötürü çapraz direncin mekanizması yeterince bilinmemektedir. Bu sistematik çalışma genotipik ve fenotipik bulgularıyla çapraz direnç mekanizmasının daha iyi anlaşılmasını sağlayacaktır. Genetikleri tamamen aynı (izojenik) 88 Escherichia Coli hücresi 22 farklı ilaca 21 gün boyunca maruz bıraktırılarak direnç kazandırıldı. Her ilaç için iki hücreye yüksek miktarda ilaç verilip kuvvetli seçilimle, iki hücreye daha az miktarda ilaç verilip zayıf seçilimle direnç kazandırılarak iki farklı seçilim denenmiştir. Direnç kazanan hücrelere fenotip analizi yapılmış ve diğer ilaçlara karşı direnç seviyelerine bakılmıştır. Ayrıca dirençli hücrelerin tamamının genetik analizi Illumina tüm genom dizilimi ile yapılmıştır. Sonuçlar göstermiştir ki kuvvetli seçilimle direnç kazanan hücreler daha kuvvetli çapraz direnç kazanırken, zayıf seçilimle direnç kazanan hücreler daha zayıf çapraz direnç kazanmıştır. Aynı şekilde kuvvetli dirençle seçilen hücrelerdeki mutasyon sayısı daha fazla olup,

mutasyon yolundaki mutasyon sayısı yine zayıf seçilimle direnç kazananlardan daha fazladır. Bu çalışmanın bir diğer önemli bulgusu aminoglikozit sınıfına direnç kazanan bakterilerin diğer bütün ilaç gruplarına karşı çapraz hassaslık kazanmasıdır. Aynı çapraz direnç gibi, aminoglikozite dirençli bakteriler hiç direnç kazanmamış bakterilere kıyasla daha düşük ilaç konsantrasyonlarında ölebilmektedir. Bunun sebebi olarak da *trkH* genindeki mutasyon tespit edilmiştir. Bu çalışma antibiyotik direncinin genetik sebeplerinin fenotipik özelliklere etkisini göstererek antibiyotik direncinin anlaşılması açısından önemli olup, seçilimin antibiyotik direncini etkileyen önemli bir faktör olduğunu ortaya koymuştur.

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Master is a tough road, we do lots of research, and teaching and we have to decide whether we will continue to PhD or not. Many students change their ideas about PhD after seeing this difficult road. The friendly and happy environment of Toprak Lab makes this decision easier. Our lab was a group of happy people, and this warm environment makes me like science world even more. So I would like to thank Tuğçe Altınuşak, Yusuf Talha Tamer, Sadık Yıldız, Gizem Hazal Şentürk, Tugçe Öz and Enes Karaboğa.

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1 Introduction

Shortly after the introduction of the first antibiotic penicillin, antibiotic resistance became a problem for human health (Levy and Marshall 2004). It is still a major health problem and we still do not have a permanent and effective solution to overcome it (Gootz 2010).

In 1940's Penicillin became available for medical use and in 1967, penicillin resistant bacteria - *Streptococcus pneumoniae* was observed in Australia. (Davies and Davies 2010).

Antibiotic resistance is development of a defense mechanism by the bacterium to evade the activity of a drug which once it was susceptible to(Davies and Davies 2010). Once the microbes become resistant to an antibiotic, it becomes more difficult to inhibit bacteria with the regular drug dose. In some cases bacteria develop resistance to more than one antibiotics, which are called multidrug resistant bacteria (Nikaido 2009).

Antibiotic resistance is a natural process, which is a part of the natural selection of evolution. When bacteria are exposed to an antibiotic, their survival instincts try to find a way to thrive within the environmental stress of the antibiotics(Martinez, et al. 2009; Davies and Davies 2010). They develop some genetically changes that help them to survive, grow in the presence of antibiotics and pass this ability to their progeny (Davies and Davies 2010).

In order to overcome bacterial drug resistance mechanisms, different approaches are developed. Using a synergistic drug combination is one of the most commonly used method which uses more than one drug to work together and allow the antimicrobial effect to take place(Chait, et al. 2007; Cokol, et al. 2011).

Major and most important cause of the acquired antibiotic resistance is repeated exposure to antibiotics. Repeated antibiotic exposure can take place in hospitals, where multi-drug resistant strains are mostly seen, and it can also take place in outpatient circumstances due to over the counter availability of antimicrobial agents(Lee, et al. 2013).

1.1 Antibiotics

Antibiotics are chemicals that are either kills or inhibits bacteria (Kunin 1978). Antibiotics that kill bacteria are called bactericidal, and antibiotics that inhibit bacteria growth are called bacteriostatic(Pankey and Sabath 2004).

According to their mechanism of action there are four major antibiotic classes. These are protein synthesis inhibitors, DNA/RNA repair inhibitors, cell wall biosynthesis inhibitors, and folic acid synthesis inhibitors (Cuddy 1997).

According to specific targets of antibiotics, they have been branched in the classes.

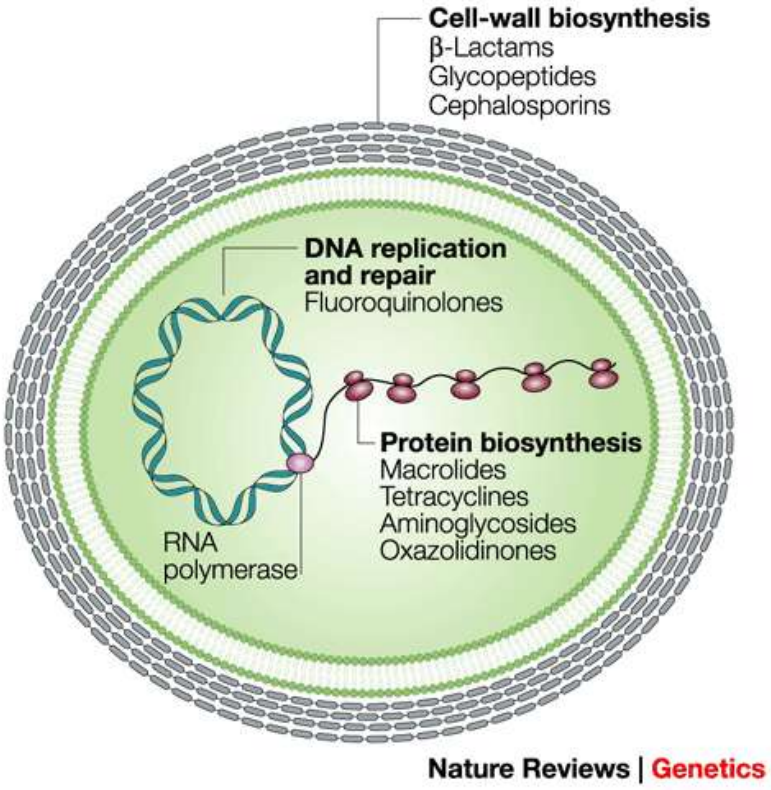


Figure 1-1: Major Antibiotic Classes and their target mechanisms**.** (Miesel, et al. 2003)

1.1.1 Cell Wall Biosynthesis Inhibitors

Cell wall biosynthesis inhibitors (β Lactams) are mostly bactericidal antibiotics and they inhibit synthesis of peptidoglycan layer of bacterial cell wall. Peptidoglycan layer is important for bacterial division; it protects bacteria from lysis, osmotic or mechanical damage, as well as it takes part in bacterial pathogenicity(Ghooi and Thatte 1995). β Lactam Antibiotics binds Penicillin Binding Protein (PBP) in bacteria, and then inhibit cell wall biosynthesis. PBP is an important protein for synthesis of peptidoglycan layer. Inhibition of this protein leads to defective cell wall synthesis, loss of selective permeability and eventual cell death and lysis(Ghooi and Thatte 1995).

β Lactams have two main groups:penicillins and cephalosporins. Bacitracin and Vancomycin also inhibits bacterial cell wall biosynthesis.

Penicillin, ampicillin, penicillin G, penicillin V,amoxicillin, ticarcillin, mezlocillin, piperacillin, and carbenicillin are belongs the class of penicillins(Demain 1991). Cephalosporins are semi synthetic antibiotics, have many members and affect both gram-negative and gram-positive bacteria(Tune and Fravert 1980).

In this study, we used ampicillin, piperacillin and cefoxitin antibiotics to inhibit bacterial growth.

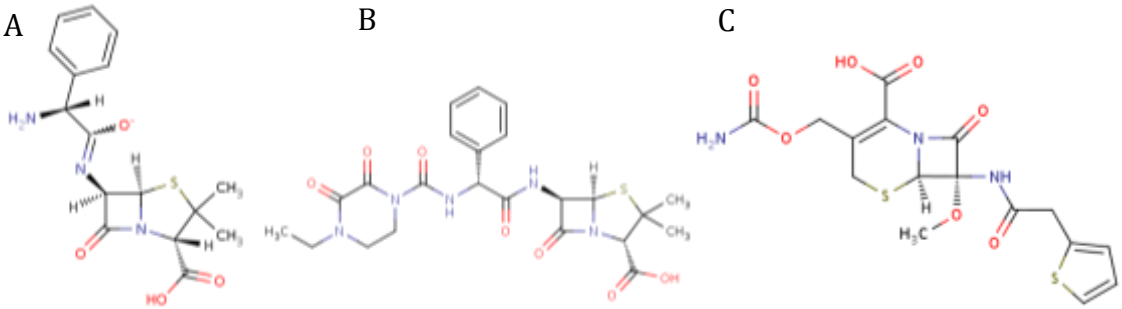


Figure 1-2: Structure of β Lactams. (A) Ampicillin, (B) Piperacillin, (C) Cefoxitin

1.1.2 Protein Synthesis Inhibitors

Protein synthesis inhibitors contain so many different antibiotics and each can exert their effects in different stages of protein synthesis (Coutsogeorgopoulos, et al. 1975). In this study we worked with 3 main groups of this class: 30 S robosomal subunit inhibitors, 50 S ribosomal subunit inhibitors and aminoglycosides.

30S ribosomal subunit inhibitors act via binding to 30 S ribosomal subunits resulting in inhibition of aminoacyl-tRNA - mRNA/ribosome complex binding. We used tetracycline, doxycycline and spectinomycin from this class.

A B C

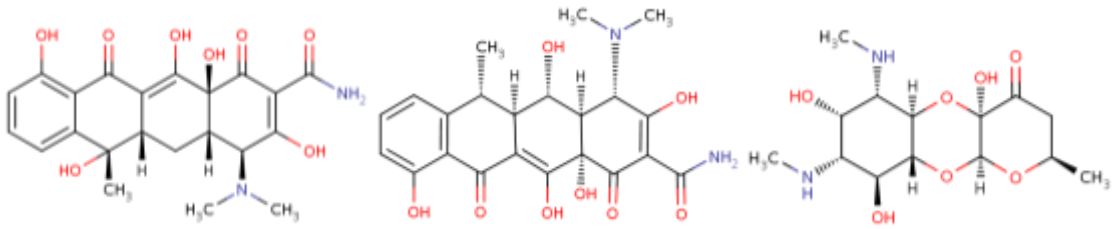


Figure 1-3: Structure of 30 S inhibitors. (A) Tetracycline (B) Doxycycline (C) Spectinomycin.

Aminoglycosides inhibit the protein synthesis via interfering with the elongation of peptide on 30S subunit (Tanaka 1986). We used amikacin, tobramycin, streptomycin and kanamycin from this class.

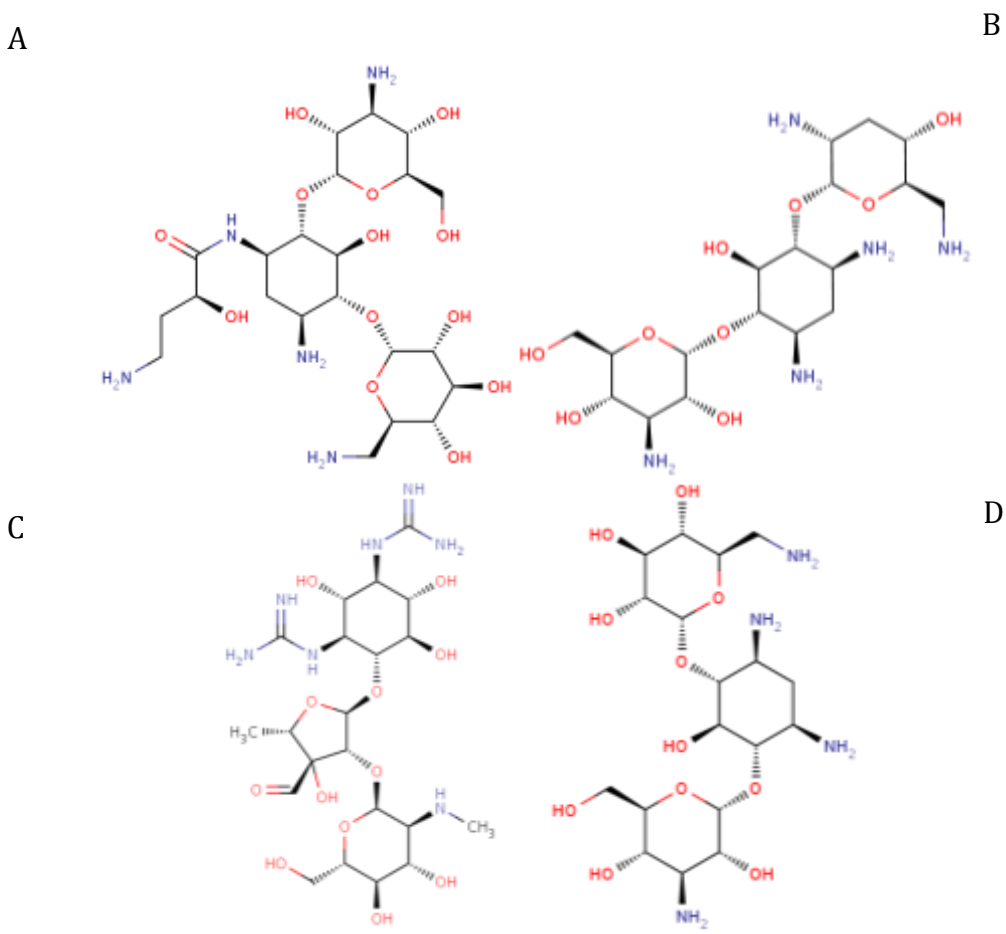


Figure 1-4: Structure of 30S Inhibitors. (A) Amikacin (B) Tobramycin (C) Streptomycin (D) Kanamycin.

50 S inhibitors inhibit bacterial growth by binding 50 S ribosomal subunit and inhibiting peptidyltransferase. We used chloramphenicol, clindamycin, erythromycin, spiramycin and fusidic acid from this class.

Chloramphenicol is one of the important antibiotics because of its wide spectrum(Jardetzky 1963).

Erythromycin is member of sub group macrolides. In order to inhibit protein synthesis, they prevent elongation of peptide chain(Tanaka, et al. 1973).

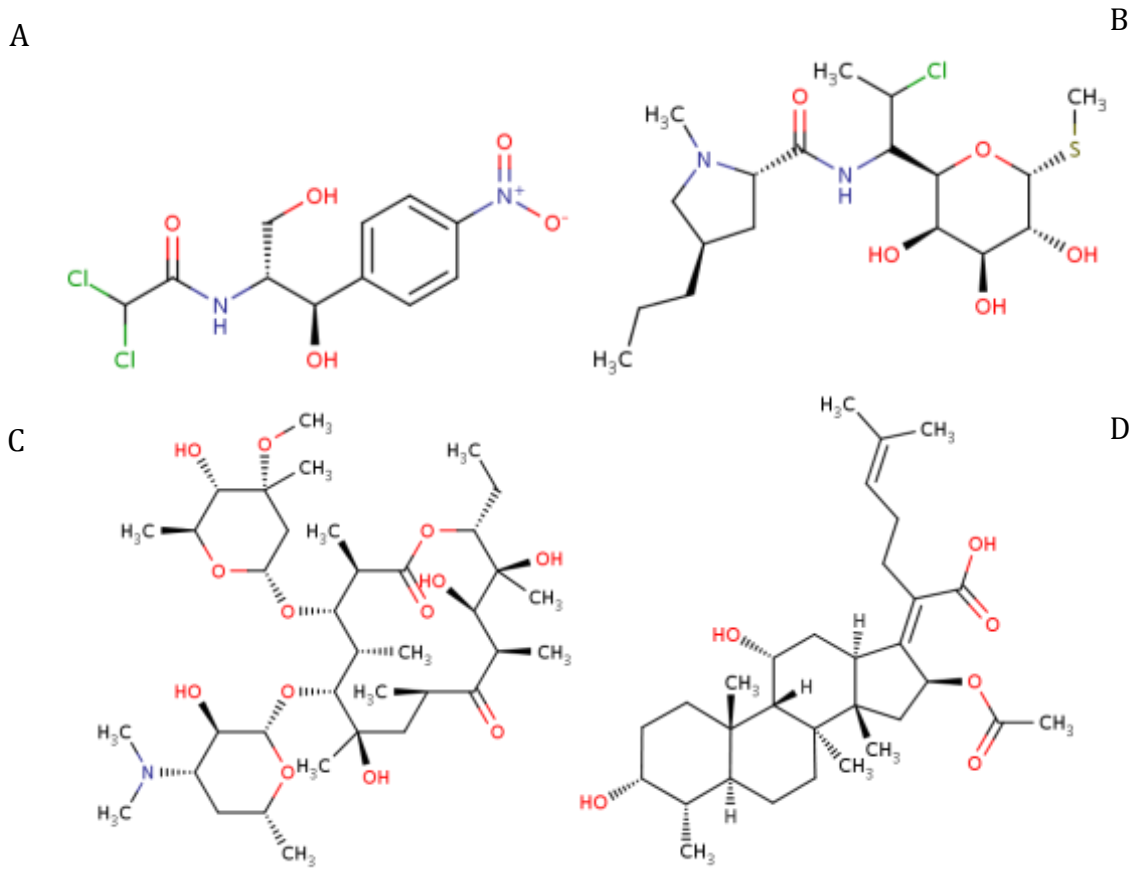


Figure 1-5: Structure of 50S inhibitors. (A) Chloramphenicol (B) Clindamycin (C) Erythromycin (D) Fusidic Acid

1.1.3 DNA/RNA Synthesis Inhibitors

Nucleic acid synthesis inhibitors can either inhibit DNA replication or RNA transcription(Chatterji, et al. 2001). Different antimicrobial from this class have different mechanisms of action. For example some of antimicrobials such as rifampicin binds enzyme that help transcription and stop RNA synthesis(Trnka 1969). Quinolones binds enzyme in DNA synthesis and prevent coiling of DNA strands(Fabrega, et al. 2009).

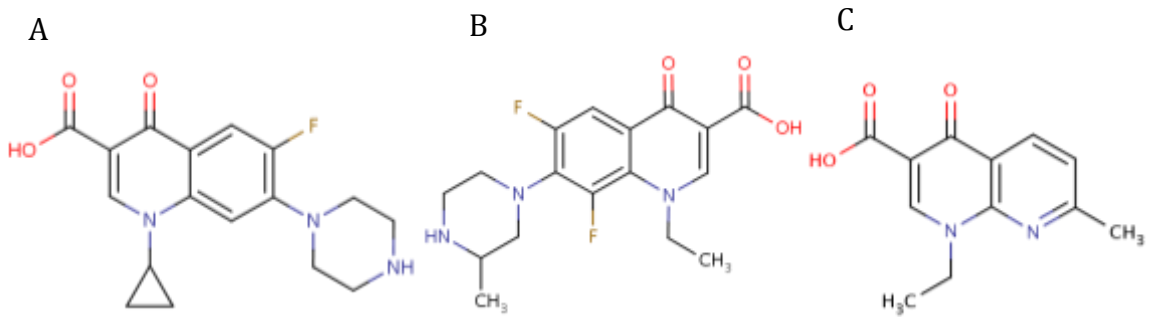


Figure 1-6: Structures of DNA/RNA Synthesis Inhibitors. (A) Ciprofloxacin (B) Lomefloxacin (C) Nalidixic Acid.

In this study we used ciprofloxacin, nalidixic acid and lomefloxacin from this class.

1.1.4 Folic Acid Synthesis Inhibitors

Antifolates are inhibits folic acid synthesis that is necessary for bacteria synthesis of amino acid. Hence inhibition of folate results inhibition of protein synthesis, DNA/RNA synthesis and cell division(Burchall 1973; Bodey, et al. 1982).

Many of the drugs in that class are dihydrofolatereductase inhibitors (DHFR). DHFR inhibitors are also used in cancer treatments. In this project, we used trimethoprim, sulfamethoxazole and sulfamonomethoxine (Bodey, Grose, & Keating, 1982).

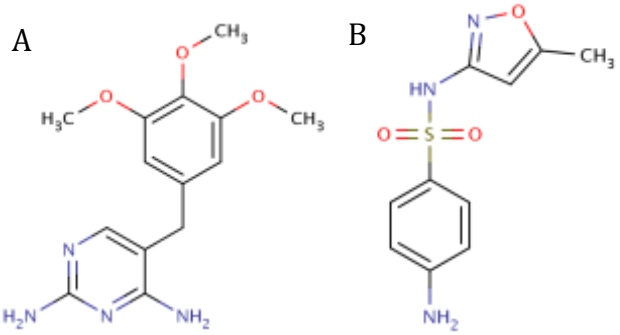


Figure 1-7: Folic Acid Synthesis Inhibitors. (A) Trimethoprim (B) Sulfamethoxazole

1.2 Antibiotic Resistance

Antibiotic resistance is defined as ability to cope with the inhibitory effects of an antibiotic by the bacterium(Davies and Davies 2010). Some bacteria are naturally resistant to certain types of antibiotics; but mostly with repeated exposure, they become resistant to antibiotics by mutations, acquiring resistance genes from its surroundings.

Antibiotic resistance is one of the major health related problems in modern world. More bacteria are gaining resistance due to overuse of antibiotics(Lee, Cho, Jeong, & Lee, 2013). It is especially a serious problem in prolonged hospitalizations, since the bacteria are constantly exposed to antibiotics and the resistant strains cause serious infections.

As demonstrated in**Hata! Başvuru kaynağı bulunamadı.**Bacteria bacterial evolution may depend on environmental stress. When the population exposed to a stress factor such as antibiotics, resistant ones survive and proliferate(Martinez, et al. 2007).

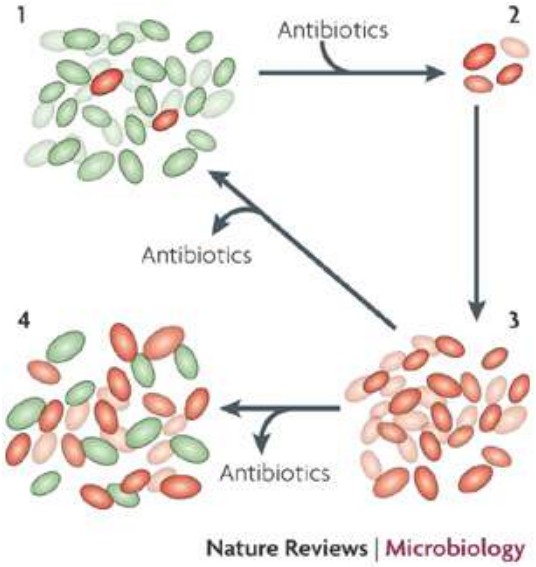


Figure 1-8: Bacterial evolution of drug resistance. In population antibiotic sensitive bacteria (green) dominates population in the absence of antibiotics (1). In presence of antibiotics, antibiotic sensitive wilt type bacteria growth will be inhibited and resistant bacteria (red) survive and proliferate (2) (3). When antibiotics are removed, bacteria may loose its resistance mechanism completely (1) or some bacteria may still have the mechanism and some of them loose (4) in order to growth better. (Martinez, et al. 2007)

1.2.1 Mechanisms of Antibiotic Resistance

There are two general types of antibiotic resistance: intrinsic and acquired (Tenover, 2006).

Intrinsic resistance refers to bacteria's natural ability to neutralize toxic effects of the antibiotic(Cox and Wright 2013). Naturally resistance in bacteria established by being inaccessible to the drug, being able to efflux the internalized drug via pumping mechanisms, lacking the target cellular elements for the drug to exert its effects, naturally occurring enzymes that inactivate the drug(Tenover 2006; Cox and Wright 2013). For example, bacteria that lack mycolic acids are intrinsically resistant to isoniazid, or anaerobic bacteria are resistant to aminoglycosides, which require oxidative metabolism to enter the cell.

Acquired resistance refers to gaining ability to an antimicrobial drug, which the bacteria were susceptible to (Tenover, 2006). Acquiring the ability of non-preexisting resistance can be via mutation of bacterial chromosome, obtaining foreign genetic material that contains resistance genes or combination of both. Sensitive bacteria are dead when exposed to antimicrobial agent. But some of the bacteria successfully develops a resistance mechanism and lives on to pass those resistance genes to its progeny, which is called vertical gene transfer(Martinez, et al. 2009; Davies and Davies 2010). Bacteria also are able to perform horizontal gene transfer, which means acquiring genetic material outside of the bacterium itself. It can be classified in three ways by source of genetic material: bacterial transformation (uptake of genetic material from the environment, which mostly belongs to dead bacteria), transduction (uptake of genetic material from a bacteriophage) and conjugation (transfer of genetic material via sexual pilus between two bacteria)(Martinez, et al. 2009; Davies and Davies 2010).

According to mechanism of action, there are four pathways of antibiotic resistance: prevention of the antimicrobial agent to reach its target, expulsion of the antimicrobial via efflux pumps, inactivation of the drug via modification or degradation, modification of antimicrobial target within the bacteria (Figure **1-9**).

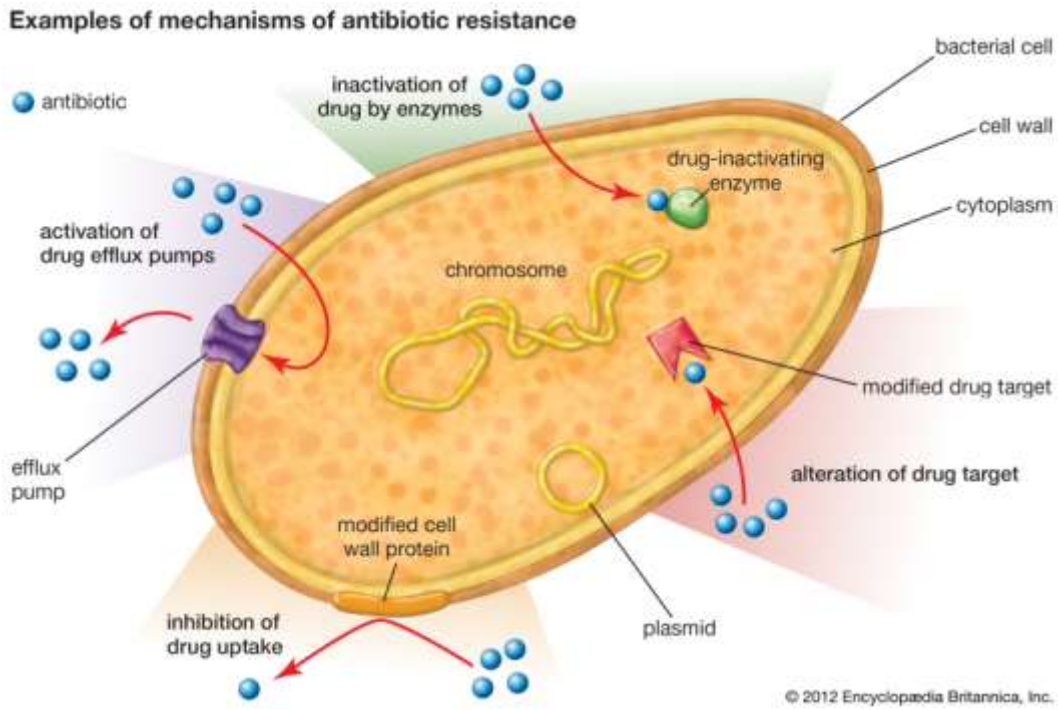


Figure 1-9: Mechanisms of drug resistance in bacteria. (Encyclopædia Britannica Online. Web. 29 May. 2014.)

1.2.2 Cross-resistance and Multi Drug Resistant Bacteria

Since antibiotic resistance become serious public health problem in the world, scientist used alternative antibiotics for treatment. However with this approach, scientist realize new and probably worse problem about drug resistance, which is cross-resistance(Sanders 2001). In 2010 Kohanski made that observation on developing cross-resistance against antimicrobial drugs to which bacteria have never been exposed before(Kohanski, et al. 2010). By helping sequencing now we can make assumption on which changes caused cross-resistance. Kohanski suggests that mutation in multidrug efflux pumps reason of the cross-resistance(Kohanski, et al. 2010). Even though this observation is true, this is not the only reason behind cross-resistance. Cross-resistance can be result of very different gene mutation. In this study we revealed different genes responsible for cross-resistance, even cross sensitivity.

As a result of cross-resistance multiple drug resistant (MDR) bacteria has been aroused. Commonly known MDR bacteria are methicillin resistant Staphylococcus aureus (MRSA), Vancomycin-Resistant Enterococci (VRE) and multi drug resistant tuberculosis. Those super bug causes death in many cases(Rice 2007).

In this study, by exposing antibiotic to bacteria, we produced MDR *Escherichia Coli* and revealed genetic changes that cause this.

1.2.3 Minimize Antibiotic Resistance

There are many different strategies suggested to minimize antibiotic resistance. Since the first antibiotic has been discovered, antibiotics used as a treatment worldwide. However not every patients and every physicians are educated enough to know how to use antibiotics(Baquero and Negri 1997; Lee, et al. 2013). Wrong usage of antibiotics is considered one of the important reasons of antibiotic resistance. Appropriate prescribing antibiotic is very important to slow sown antibiotic resistance(Lee, et al. 2013). Educating the patient is also important since physician cannot control the patient all the time(Lee, et al. 2013).

Studies showed that inappropriate prescription cause rapid increase of antibiotic resistance(Nathwani and Davey 1999).

Development of novel antibiotics is also a way to kill resistant bacteria. Development of new antimicrobial agents is very straightforward way to reduce resistance however bacteria can be resistant eventually even before the new agent released to market(Silver and Bostian 1993). Because of this problem, companies are not willing to invest for this method(Coates and Hu 2007).

Using synergistic drug pair is another suggestion to cope with resistant bacteria. Synergy of antibiotics definition is combination of two antibiotics is significantly more effective then one alone(Yeh, et al. 2006; Bollenbach, et al. 2009; Yeh, et al. 2009; Cokol, et al. 2011). Using synergistic drug pair can be effective on drug resistant bacteria. However some other studies suggest that using synergistic drug pair may increase the rate of bacterial evolution (Chait, et al. 2007; Hegreness and Kishony 2007; Michel, et al. 2008).

Our study aims systematic exploration of antibiotic resistance in order to understand genetic reason behind this problem and find a possible path for resistant mechanism.

2 Methods

2.1 M9 Minimal Media

Minimal Media contains only minimal amount of nutrient that bacteria needs. For 1-liter M9 Minimal media; 11.28 mg M9 salt has been dissolved in 860ml distilled water and autoclaved at 121 C for 15 minutes. Then 40ml, 25X sterile glucose solution, 100ml, 10X sterile amicase solution, 2ml CaCl and 100ul MgS4 added in to M9 salt.

In order to make 25X Glucose, 50gr Glucose has been dissolved in 500ml distilled water and autoclaved for 15 minutes.

In order to make 10X Amicase, 10gr amicase has been dissolved in 500ml-distilled water. Amicase may be denaturate in autoclave so filter sterilization has been applied for sterilization.

2.2 Evolution of Bacterial Strains

At first MG1655 *Escherichia coli* has been spread on to agar plate and incubate at 30 C for 16 hours. Single colony obtained from agar plate and has been growth at minimal media at 30 C for 24 hours.

Minimal inhibitory concentration (MIC) of *Escherichia coli* in different antibiotics has been determined by following method. In 96-well plate, antibiotic concentration has been logarithmically decreased in each 10 well. Each well has half concentration of its left neighbor well. After antibiotics in minimal media added in to plate, bacteria has been added in to each well. Plate has been put in to shaker in the incubator for 24 hours. After 24 hours, OD measurement has been done by using Tecan. The lowest concentration that has no growth is MIC

22 different antibiotics have been selected. MIC of each antibiotic has been determined. MG1655 strain has been exposed to antibiotics separately, with 2 biological replicas, with 2 different strategies for each drug.

First day drug concentration have been prepared in 6 culture tube with 3ml minimal media in it. First tube has one eight of MIC of drug. Second tube has one four MIC, third has half MIC, and others has higher concentration accordingly. Then bacteria have been added, as final OD was 0.0001. Then culture tubes placed in to incubator with shaker for ~22 hours. 4 replicates have been done at first day.

At second day, growth can be observed at first three concentration tubes. Growing cultures observed by visual examination or measured by spectrometer if the growth was not clear on eyes. Starting from second day we evolved populations as two different strategies. Two isogenic population evolved under strong selection, other two population evolved under mild selection (Figure **2-1**).

Strong selection means that cells were taken from half MIC concentration. For second day we made new concentration gradient, this time the lowest concentration tube has half MIC of drug. So each day we are expecting better survival since bacteria exposed high amount of drug and survived.

Mild selection means that cells were taken from one eight MIC. Which means that we select bacteria from one four lower concentration of drug comparing to strong selection. Again taken concentration will be the lowest concentration tube for second day.

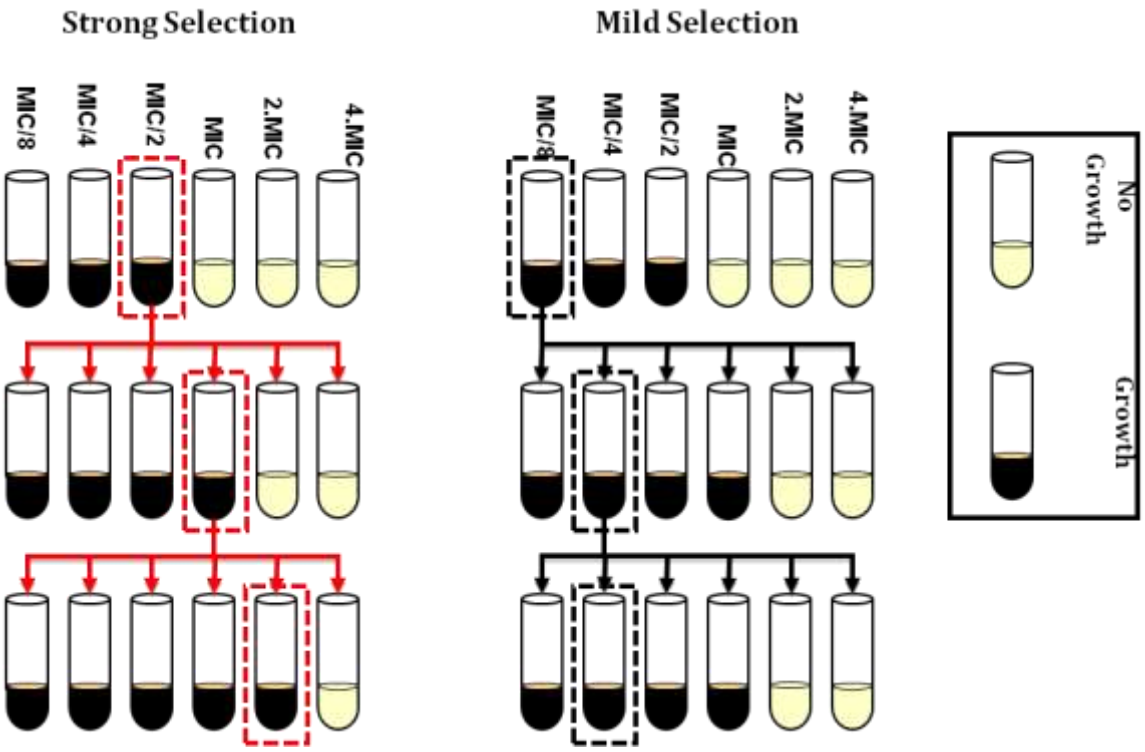


Figure 2-1: Evolution experiment in liquid culture under strong selection. Bacterial populations were grown in several tubes with increased drug concentrations that span the expected minimal inhibitory concentration (MIC) of the population. Populations were grown for ~22 hours and the populations surviving in the highest drug concentration were transferred to new culture vials (yielding 60X dilution, 6-7 generations per day if new mutants do not appear) with increasing drug concentrations.

The minimum drug concentration that inhibited growth (ODfinal< 0.1) was daily recorded as MIC of the population (Table 3.1). At the end of each day 30ul bacteria taken from growth culture and transferred fresh media tubes with different antibiotic concentration.

This experiment was made for 21 days with 22 different antibiotics. Each antibiotic has two different strategies with two replicas, so we had 88 different populations (Figure **2-1**).

At the ends of 21 days, each population MIC shifted higher concentration comparing to wild type.

On a daily basis, 1 ml of cells were frozen and stored at −80°C in 15% glycerol for further characterization.

As a negative control wild type *Escherichia coli* exposed to minimal media without antibiotic for 21 days.

2.3 Selection of Representative Colony

Mixed cultures of 21st day of each drug were spread on agar plate in order to isolate single colonies. 10 single colony isolated for each replica, 40 colony isolated for a single drug. MIC values determined for each single colonies. . Resistance levels of these colonies did not show much variations in their MIC values comparing with population MIC, therefore, one colony from all evolved populations were assigned as representative colonies to carry out all future genotyping and phenotyping experiments.

Each representative colony named according to drug name, selection strength (strong or mild) and replica order. For example; AMP-S-1 means Ampicillin strong number 1.

2.4 Phenotypic Characterization

88 representative colonies has been growth separately in minimal media and placed in to 96 well plates with glycerol. This master plate used for our cross-resistance experiments.

For cross-resistance experiments 96 well plates prepared with different drug concentration for each drug. At least ten different drug concentration 96 well plates were prepared. Drug concentration of these plates ranged from drug free to the highest concentration that we can dissolve in growth medium Drug concentrations across plates were diluted by a factor of 101/2 ([drug]n−1 = 101/2× [drug]n). However if the colony's resistance level is not very high comparing to wilt type, in that case a dilution factor of 21/2 was used in order to observe more delicate range.

After concentration gradient plate with 150ul volume of minimal media was prepared, colonies from master plate transferred in to those 96 well plates with antibiotic by using 96-pinner (V&P Scientific) and were grown for 22 hours with rapid shaking at 30°C.

Final optical densities of the cells were measured using a plate reader (Tecan M200). Phenotyping experiments were performed in duplicates for every drug and the mean values of these measurements were used for MIC calculations. Background corrected ODfinal reads from phenotyping experiments were used to calculate the MIC values of the evolved strains. We calculated mean ODfinal values for every strain in every drug concentration we used. The MIC values were calculated by interpolating the drug concentrations corresponding to mean ODfinal reads corresponding to 0.03.

2.5 Constructing Cross-resistance Networks

MIC observation experiment applied for each resistance strain against 22 different antibiotics by using master plate, as described above. MIC values saved and normalized for analysis and building cross-resistance network. Those values then converted to -1, 0, 1, respectively antibiotic sensitivity, no change in resistance, and antibiotic resistance. For both strongly selected and mildly selected strains, strains are grouped according to drug classes and their cross-resistance frequencies (f_{CR}) and antibiotic susceptibility frequencies (f_{AS}) against each drug class are calculated. Moreover, the mean cross-resistance ($0 \leq CR \leq 1$; 1 being the strongest possible resistance) and antibiotic susceptibility ($-1 \leq AS \leq 0$; -1 being 20 fold less resistance compared to the wild type ancestor) values are calculated for each cluster. A seven by seven matrix has been created(Figure **3-5**) with frequency and cross-resistance (or antibiotic sensitivity) values for strongly selected (panels on the left) and mildly selected (panels on the right) strains. The 22 by 88 trinary matrix is then randomly shuffled for 10^5 times and the actual $f_{CR}$and f_{AS}values for each group is recorded (histograms in panels). Finally, we calculated the probability (p) of randomly getting a frequency higher than the actual $f_{CR}$and f_{AS}values. We consider the phenotypic changes

within clusters which have p values less than 0.05 as significant and score these interactions as increased cross-resistance or increased antibiotic susceptibility.

2.6 Genotypic Characterization

In order to understand genetic changes and mutations in the evolved strains bacterial cells were genotyped by Illumina whole genome sequencing using a HiSeq platform. Cells prepared for sequencing in agar stabs and were submitted to Genewiz Incorporation for sequencing service. Service from Genewiz included genomic DNA extraction, library preparation, multiplexing, sequencing, and data delivery. Sequencing was performed on the Illumina HiSeq2000 platform, in a 2x100bp paired end configuration, with at least 100X coverage for each sample. We aligned resulting reads onto the MG1655 reference chromosome (NC_000913.2) using the Bowtie 2 toolkit (Langmead and Salzberg 2012).

Aligned sequences were analyzed for genetic changes by using SAMtools and BRESEQ software (Barrick et al., 2009; Li et al., 2009). Both tools gave similar results for finding SNPs, however BRESEQ is better for finding insertions and deletions. If there is detected mutation by only one tool, visual inspection has been used to confirm the mutation.

Six strains have been sequenced twice in order to confirm accuracy of sequencing.

MG1655 wild type bacteria also sequenced to examine if there is contamination during experiment. There was no contamination between species however we wanted to make sure if there is any contamination between our selected colonies, so that we compared all genetic changes in all strains. All strains have different mutations accept TMP-M-1 and TMP-S-2. However the mutations, that both have, are pathway specific folA mutation, which are expected to observed in TMP resistant bacteria.

Cefoxitin resistant strains; CEF-S1 and CEF-S-2 interestingly have more then 200 mutations. It requires deep and separate analyze to understand all those mutations. Therefore we exclude their mutation, during analyzing our data.

2.7 Functional Classification

In order to understand and analyzed mutations, we used EcoCyc gene database for the bacterium *Escherichia coli* K-12 MG1655. EcoCyc we giving properities of that gene, and according to information on EcoCyc we have decided wheter the mutation on that gene is pathways specific or not. Pathway specific means that; such mutations are directly effect of mechanism of the drug.

3 Results

3.1 Evolution Experiment

First part of the project was evolving wilt type *Escherichia coli* against 22 different antibiotics. For each antibiotic we had 2 different evolution strategies: strong selection, mild selection. For each selection we made 2 biological replicas. At the end of 21 days, we had 88 different strains that are resistant to 22 different antibiotics (Figure **3-1**).

Concentration of drug increased day by day if necessary according to our method. However Fusidic Acid has been reached its maximum solubility (3200ug/ml), at day fifth, so Fusidic Acid concentration remained say for the rest of experiment.

Drug	Solvent	MIC (μg/ml) for Wild Type E. coli	Maximum dose used for selection	Clinical Dose (μg/ml/day)	Highest MIC Reported in Literature (μg/ml)	Mechanism of Action	Phenotypic Effect
Chloramphenicol (CHL)	Ethanol	6.1	256	100	512	Protein Synthesis, 50S	Bacteriostatic/Bactericidal
Clindamycin (CLI)	DMSO	97.5	1280	120	NA	Protein Synthesis, 50S	Bacteriostatic/Bactericidal
Erythromycin (ERY)	DMSO	65.80	1280	100	NA	Protein Synthesis, 50S	Bacteriostatic/Bactericidal
Spiramycin (SPR)	Ethanol	260	2560	75	NA	Protein Synthesis, 50S	Bacteriostatic
Fusidic Acid (FUS)	Water	647	3200	37.5	NA	Protein Synthesis, 50S	Bacteriostatic
Amikacin (AMK)	Water	14.36	640	37.5	32	Protein Synthesis, 30S (Aminoglycoside)	Bactericidal
Tobramycin (TOB)	Water	1.14	512	0.1875	32	Protein Synthesis, 30S (Aminoglycoside)	Bactericidal
Streptomycin (STR)	Water	15.2	163840	25	512	Protein Synthesis, 30S (Aminoglycoside)	Bactericidal
Kanamycin (KAN)	Water	11.70	1280	37.5	512	Protein Synthesis, 30S (Aminoglycoside)	Bacteriocidal
Tetracycline (TET)	Ethanol	1.23	4.8	5	512	Protein Synthesis, 30S	Bacteriostatic
Doxycycline (DOX)	Water	1.70	16	5	128	Protein Synthesis, 30S	Bacteriostatic
Spectinomycin (SPT)	Water	61	40960	50	512	Protein Synthesis, 30S	Bactericidal
Piperacillin (PIP)	Water	1.88	128	375	≥ 512	β-lactam, Cell Wall	Bactericidal
Ampicillin (AMP)	Water	4.4	40	50	512	β-lactam, Cell Wall	Bactericidal
Cefoxitin (CEF)	Water	1.9	2048	300	128	β-lactam, Cell Wall	Bactericidal
Nalidixic Acid (NAL)	Chloroform	7.9	300	100	512	DNA Gyrase	Bactericidal
Lomefloxacin (LOM)	Water	0.3	6.4	10	NA	DNA Gyrase	Bactericidal
Ciprofloxacin (CIP)	HCL	0.015	2.56	37.5	128	DNA Gyrase	Bactericidal
Sulfamonomethoxine (SMO)	Acetone	1.40	410	NA	NA	Folic Acid Synthesis	Bacteriostatic
Trimethoprim (TMP)	DMSO	4.83	614	5	≥ 512	Folic Acid Synthesis	Bacteriostatic/Bactericidal
Sulfamethaxozole (SUL)	Acetone	2.45	640	20	≥ 512	Folic Acid Synthesis	Bacteriostatic
Nitrofurantoin (NIT)	DMSO	4.75	320	10	128	Multiple Mechanisms	Bacteriostatic/Bactericidal

Table 3-1: List of all drugs that have been used in project. Drug names and abbreviations, solvent, MIC values for wild type Escherichia Coli MG1655, maximum dose that used in experiment, daily clinical dose average (taken from **http://www.globalrph.com**), higher MIC reported in EUCAST, mechanism of action and phenotypic effect: bacteriostatic or bactericidal. (Oz & Guvenek & Yildiz 2014)

Strong selections and mild selections act differently in some cases, such as; Tobramycin, Kanamycin, Spectinomycin, Cefoxitin, Ciprofloxacin and Nitrofurantoin. For those drugs resistance level of strong selection and mild selection are far from each other. However in other drugs, resistance level of strong selection and mild selection are same or very close with each other.

Resistance pathway of each strain may show differences, both phenotypically and genotypically. When we look at Spectinomycin strong selection strains and mild selection strains get very different level of resistance. However two replicates of strong selection strain act similar.

When we look at Streptomycin both 4 strains resistance levels are same in the end, however their behaviors are different than each other.

We can say that strongly selected strains have relatively higher resistance level. In some cases strong and mild selection strains have same resistance level. But there is no case such, mild resistant strains have higher resistance level.

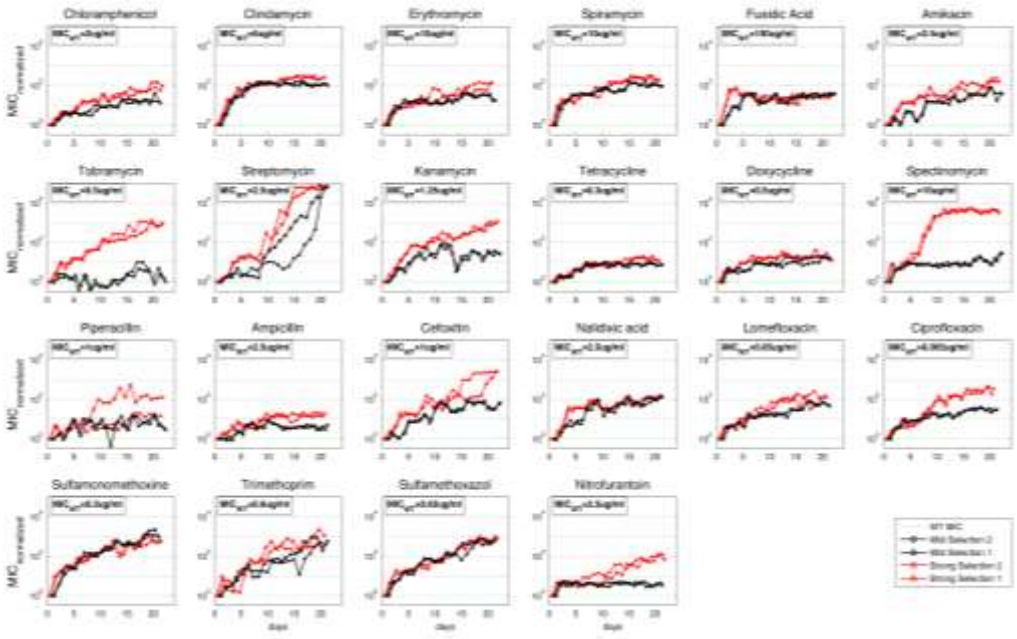


Figure 3-1: MIC level of resistance strains. Daily-recorded MIC values of resistant strains strongly selected (red circle and red triangle) and mildly selected strains(black circle and clack triangle) for each drug. X axis stands for days and Y axis stands for minimum inhibitory concentration of drug. (Oz & Guvenek & Yildiz 2014)

3.2 Cross-resistance Experiment

After observing changes in MIC level against corresponding antibiotics, we design a cross-resistance experiment in order to build a cross-resistance network.

We expect resistant strains were pleiotropically developed cross-resistance against other antibiotics. Our expectation was antibiotics that are in the same class should have developed cross-resistance against each other. In order to build this map, we did concentration gradient for all 22 drugs in order to calculate MIC level of the resistant strains (Methods).

In this cross-resistance map, we used Mat Lab for visualization. We compared MIC of the strain with MIC of wild type.Figure **3-2**-A shows MIC of 3 different resistant strains, and

wilt type in Chloramphenicol. As it shown Chloramphenicol resistant strain has higher MIC (~60 times) then wilt type MIC, as expected. Doxycycline resistant strain also shows higher MIC. Doxycycline resistant strain has never been exposed to Chloramphenicol during evolution experiment. However a cross resistant occurred in that strain.

On the other hand, Tobramycin resistant strain sensitivity against Chloramphenicol has been decreased, as can be seen in Figure **3-2-**A. This was an interesting result. Understanding why a strain become even more sensitive then wild type against other drug was one of the important questions of this project.

Finally we build up a cross-resistance map, for all strains (Figure **3-2-**B, C). Figure 3-2 B shows cross-resistance behavior of strongly selected strains. Figure 3-2-C shows cross-resistance behavior of mildly selected strains. Similar behaviors can be observed at both maps. Red color represents if the strain has at least 3 times higher MIC then wilt type. Blue color shows if the strain has at least 3 times lower MIC then wilt types. White colors means that strain has same MIC as wilt type. By looking these maps, we can say that resistance behavior is relatively higher in strongly selected strains.

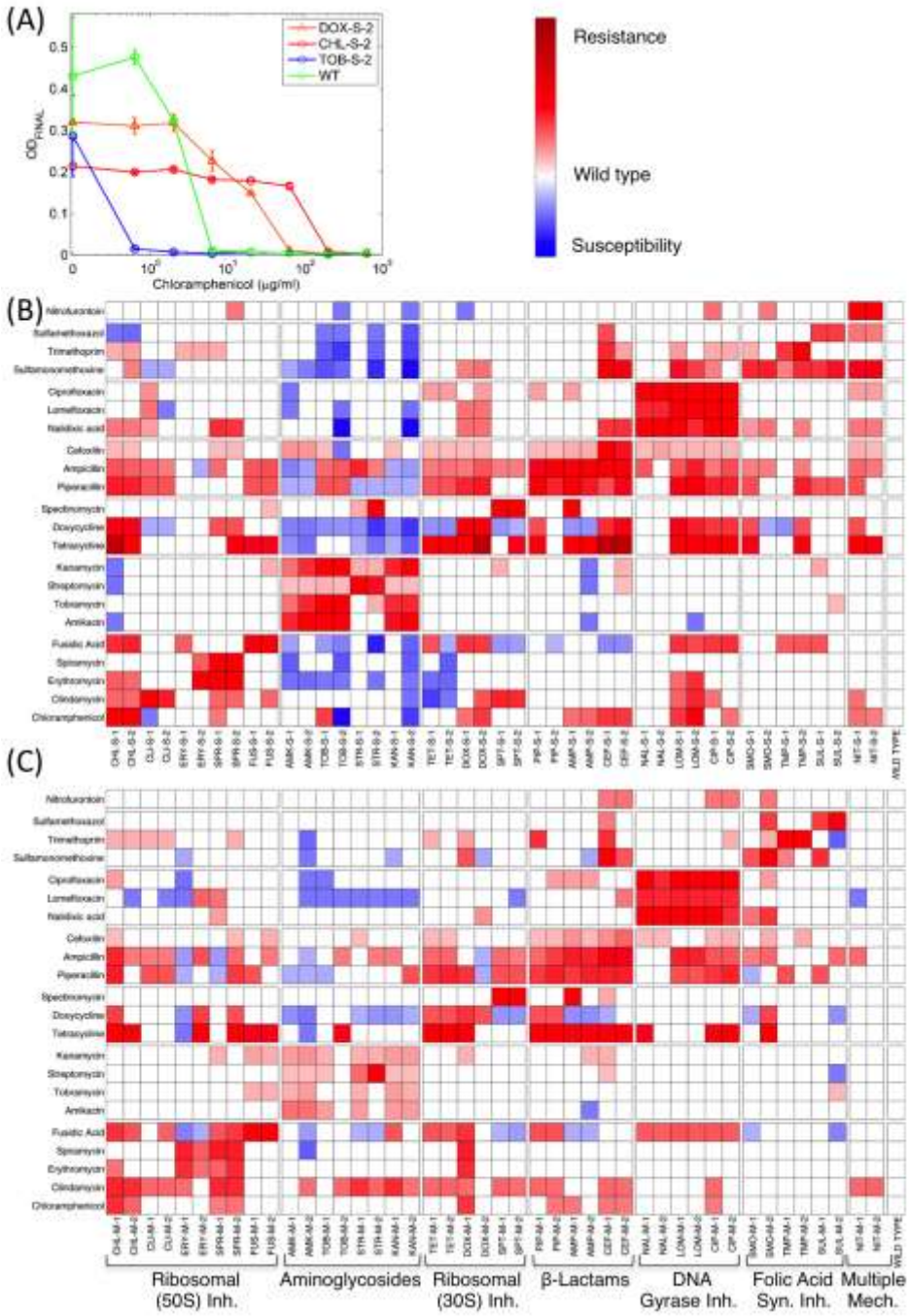


Figure 3-2: Cross-resistance measurement of all strains. (A) Representative strains for extreme examples. Chloramphenicol resistance of wild type ancestor strain (green circles), a strain evolved against doxycycline (DOX-S-2, orange triangles), a strain evolved against chloramphenicol (CHL-S-2, red circles), and a strain against kanamycin (TOB-S-2, blue circles) were measured. (B) Cross-resistance map of strains evolved under strong selection. (C) Cross-resistance map of strains evolved under mild selection. (Oz & Guvenek & Yildiz 2014)

In order to understand behavior of a antibiotic class against other classes we built a cross-resistance network for both strong and mild selection. (Figure **3-3**)

Figure **3-3**-A shows resistance/sensitivity behavior with in the antibiotic classes and if there is general trend between classes. Again red represent increased cross-resistance and blue represent increased cross sensitivity, and intensity of the color in a line represents the frequency of increased cross-resistance or antibiotic susceptibility against a drug or drug class.

Increased cross-resistance is very common within the antibiotic class. Almost all of the antibiotic resistant strains gain resistant to other antibiotic in its own class, although, there were two exceptions of this trend. Such interaction cannot be observed for Folic acid synthesis inhibitors and Ribosomal 30S Inhibitors.

Very important observation of this project is increased sensitivity of Aminoglycoside resistant strains against other antibiotic classes. On both Figure **3-2**and Figure **3-3**we observed that resistant strains of Aminoglycoside (Tob, Str, Amk, Kan) have increased resistance against each other, but increased sensitivity against other drug classes. This observation on their phenotype led us to discover a specific gene mutation, when we analyze the sequencing results. Another thing is, addition to this unique behavior of aminoglycoside, none of the other drug classes developed resistance against aminoglycoside.

Folic acid synthesis inhibitors were another interesting observation of this study. As mentioned above, they didn't developed resistance within the group. Also they didn't developed resistance against other drugs from other classes. So we can say that their resistance mechanisms can be an independent mechanism.

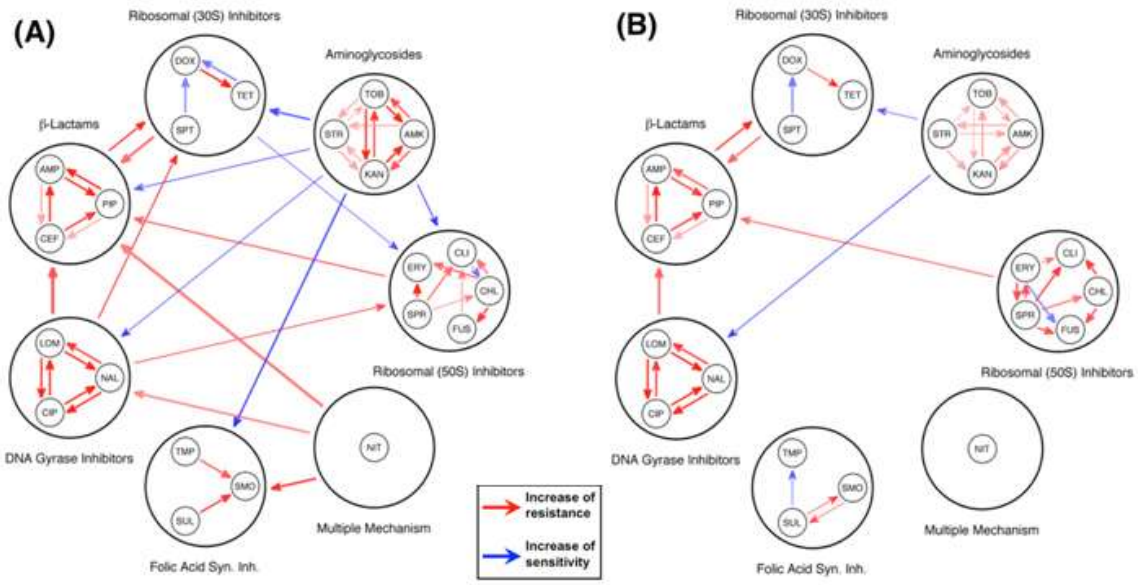


Figure 3-3: Cros resistance network. (A) Network for strains evolved under strong selection. (B) Network for strains evolved under mild selection. Red lines represent cross-resistance and blue lines represent sensitivity. Resistance or sensitivity activity of a strain against other drugs in its class is shown in each circle. Resistance or sensitivity of all strains in one class against other drug classes are shown between circles. (Oz & Guvenek & Yildiz 2014)

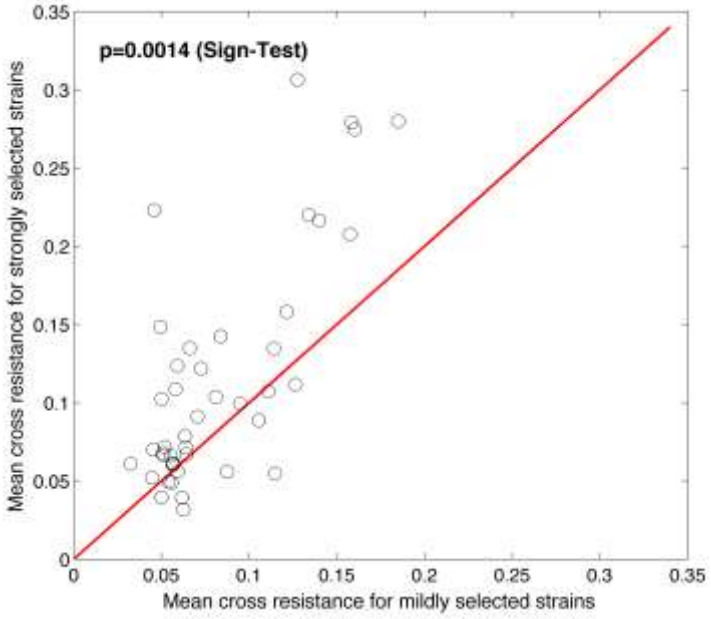


Figure 3-4: For every evolved strain, we calculated direct-resistance values and mean cross-resistance values. Using these values we calculated Pearson's linear correlation coefficients and p values separately for (left panel) strongly selected strains (R=0.28, p=0.064), (middle panel) mildly selected strains (R=0.047, p=0.76), and (right panel) strongly selected and mildly selected strains together (R=0.23, p=0.033). Direct-resistance values are plotted against mean cross-resistance values (black and red circles are used for mildly and strongly selected strains respectively) for all 88 evolved strains and blue lines show the best linear fit. (Oz & Guvenek & Yildiz 2014)

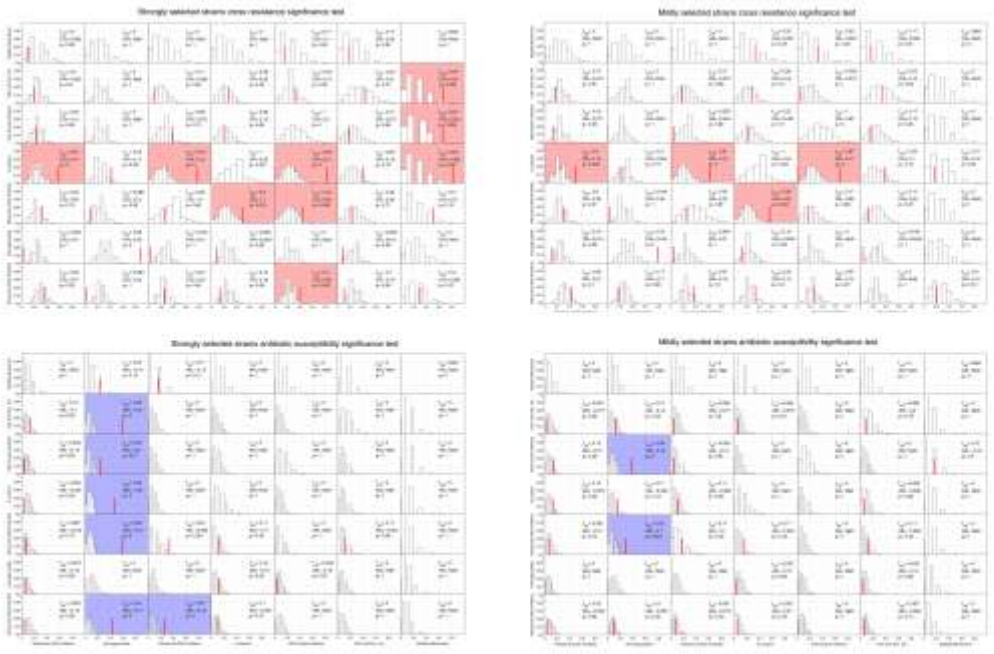


Figure 3-5: Frequency and cross-resistance levels of strains evolved against drug classes. Normalized values of cross-resistance converted to -1, 0, 1, respectively antibiotic sensitivity, no change in resistance, and antibiotic resistance. For both strongly selected and mildly selected strains, strains are grouped according to drug classes and their cross-resistance frequencies (fCR) and antibiotic susceptibility frequencies (fAS) against each drug class are calculated. Moreover, the mean cross-resistance ($0 \leq CR \leq 1$; 1 being the strongest possible resistance) and antibiotic susceptibility ($-1 \leq AS \leq 0$; -1 being 20 fold less resistance compared to the wild type ancestor) values are calculated for each cluster. A seven by seven matrix has been createdwith frequency and cross-resistance (or antibiotic sensitivity) values for strongly selected (panels on the left) and mildly selected (panels on the right) strains. The 22 by 88 trinary matrix is then randomly shuffled for 105 times and the actual fCR and fAS values for each group is recorded (histograms in panels). Finally, we calculated the probability (p) of randomly getting a frequency higher than the actual fCR and fAS values. We consider the phenotypic changes within clusters which have p values less than 0.05 as significant and score these interactions as increased cross-resistance or increased antibiotic susceptibility. (Oz & Guvenek & Yildiz 2014)

3.3 Genotypic Characterization

In order to understand genetic changes on evolved strains, 88 evolved strains has been sequenced. All the genetic changes can are available on Appendix A. In addition to 88 strains, we sequenced two wild type, 4 replicas of randomly selected colonies, and 2 wild type strains who has been growth in minimal media for 21 days, without any antibiotic.

Two strains that exposed nothing but minimal media have same genetic changes. There were deletions of 82 base pair in the *pyrE-rph* operon in both strains. In order to understand if that mutation has any effect on bacteria we compared growth rates of all 88 evolved strains, media adapted 2 strains, and wilt type MG1655. Doubling time for MG1655 was 70±4 minutes (mean ± standard deviation), as well as the doubling time for minimal media adapted strain was 48±3 minutes, which means that *pyrE-rph*deletion causes an elevation in growth rate. This mutation was previously reported as a minimal media adaptation related mutation(Conrad, et al. 2009). This result led us to understand changes in growth rate in some resistant strains.

On Figure **3-6** green line represent growth rate of MG1655, and blue line represent growth rate of media adapted strains. Without knowing the effect of *pyrE-rph*deletion it would be difficult to understand the strains have better growth rate then wild type. Mutations in the *rph-pyrE*operon were observed in 29 of the resistant strains and majority of these strains (24 out of 29) were growing significantly faster (Figure **3-6**, $p<0.05$, Wilcoxon rank-sum test) than the wild type ancestor strain. Again, majority of fast growing strains were mildly selected strains (20 out of 24).

There were 17 strains that have significantly lower growth rate (twelve strongly selected and five mildly selected. When we compared growth rate of strongly selected and mildly selected strains, average growth rate for the strains evolved under strong selection was 71±16 minutes whereas the average growth rate for the strains evolved under mild selection was 59±12 minutes.

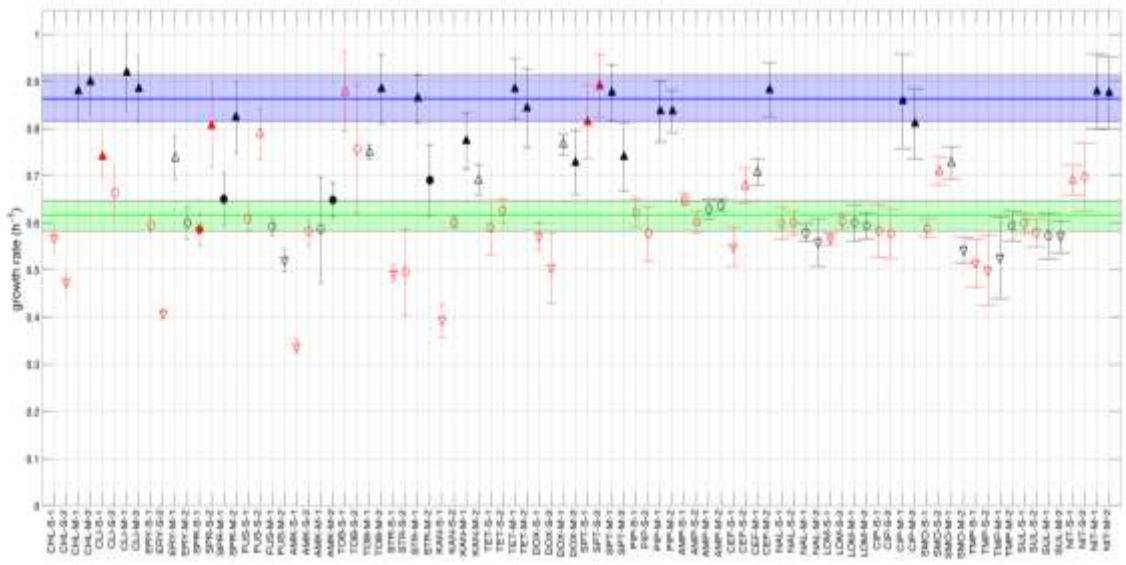


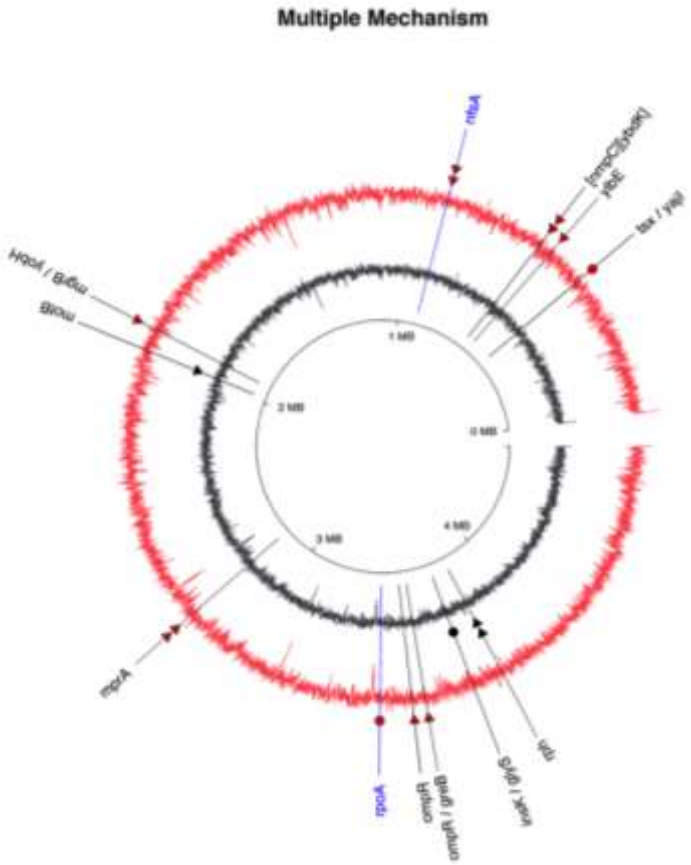
Figure 3-6: Growth rate of each evolved strains in 30°C in M9 minimal medium. Red color represents strong selection strains and black color represents mildly selected strains. Green rectangle represent mean growth rate of wild type ancestor MG1655 and blue rectangle represents strains evolved in minimal media for 21 days. Error bars show the standard deviations of 6 growth rate measurements per strain. Upward triangle used for strains that growth rate is higher than ancestor strains and downward triangle used for strains that growth rate is lower than ancestor strains. Filled markers represent strains that carry deletions of 82 base pair in the pyrE-rph operon. (Oz & Guvenek & Yildiz 2014)

All the mutations in all strains are provided in Appendix A. We observed total 215 mutations, 113 of them were SNPs and 102 of them were indels. In order to better understand those mutations, mutations were grouped according to their antibiotic class inFigure 3-7. In Figure 3-7 the genetic changes found in strains has been shown by radially distributing mutations on circular plots according to mutations' locations on E. coli reference genome. Indels has shown as filled red and black triangles and SNPs has shown as filled red and black circles. Strongly selected strains had 124 mutations in total (111 in coding regions, 13 in intergenic regions) and mildly selected strains had 91 mutations (83 in coding regions, 8 in intergenic regions).

Two of the strains (CEF-S-1 and CEF-S-2) have 558 mutations in total, so they were excluded from all analyses. Out of 558 mutations 139 of them were synonymous mutations.

According to drug's mechanism of action, we classified mutations in to two; pathway specific and off pathway mutation. In Figure 3-7pathway specific mutations are shown in blue color. Outer red circle represents mutations of strains evolved under strong selection and inner black circle represents mutation evolved under mild selection. If a mutation has been seen more than once, it can also be detected on Figure 3-7.

A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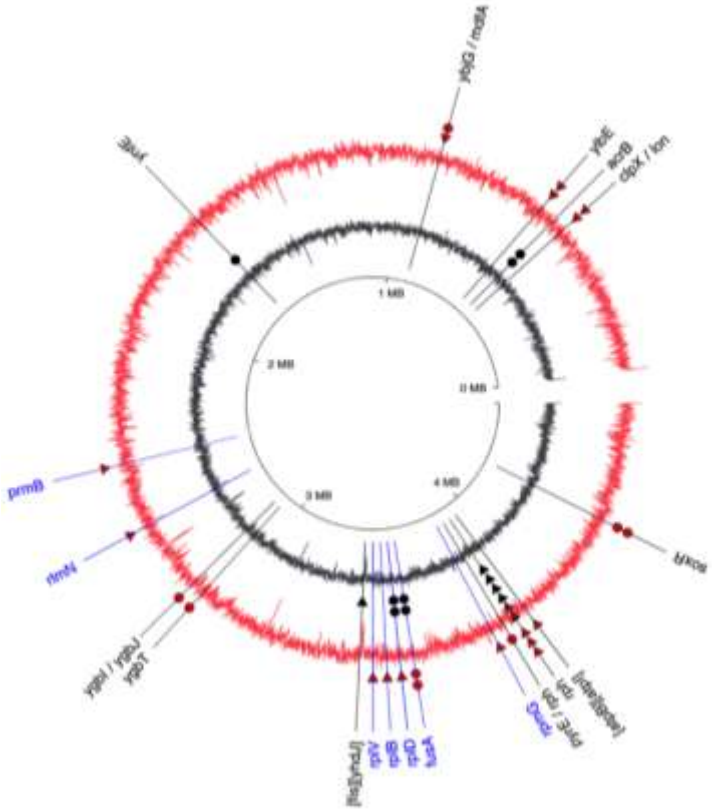
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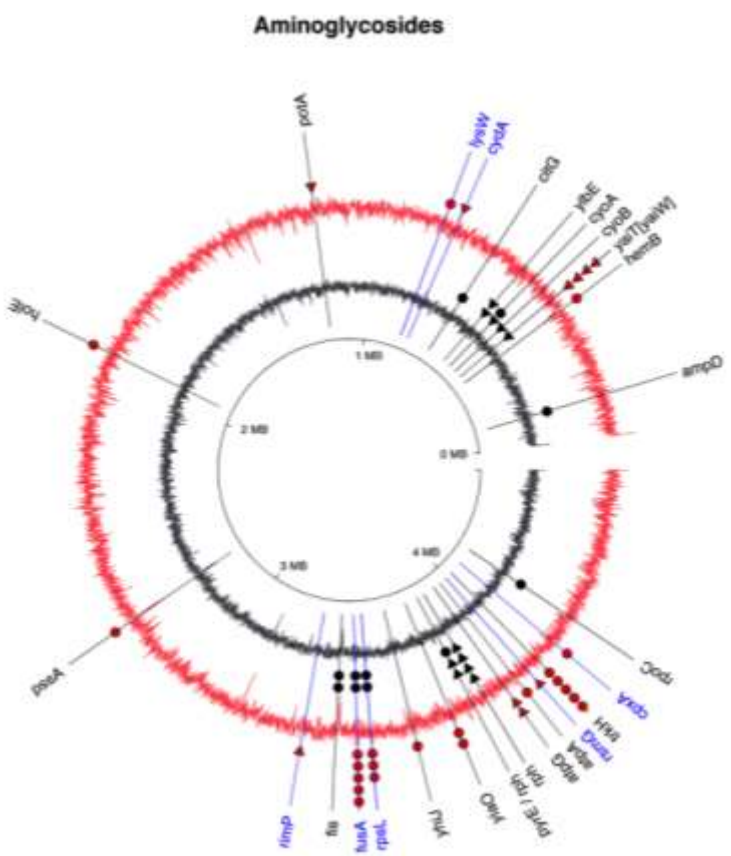
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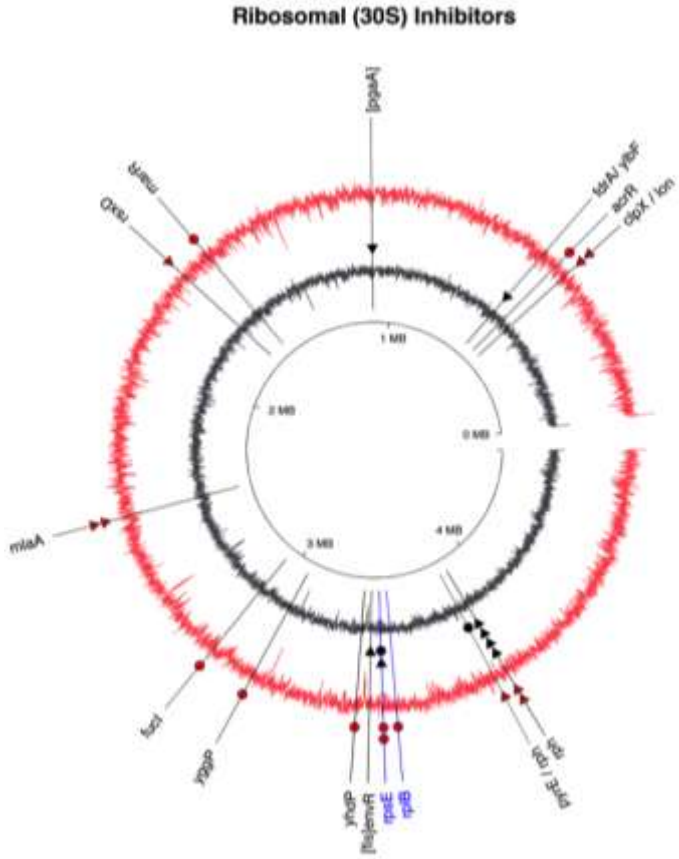
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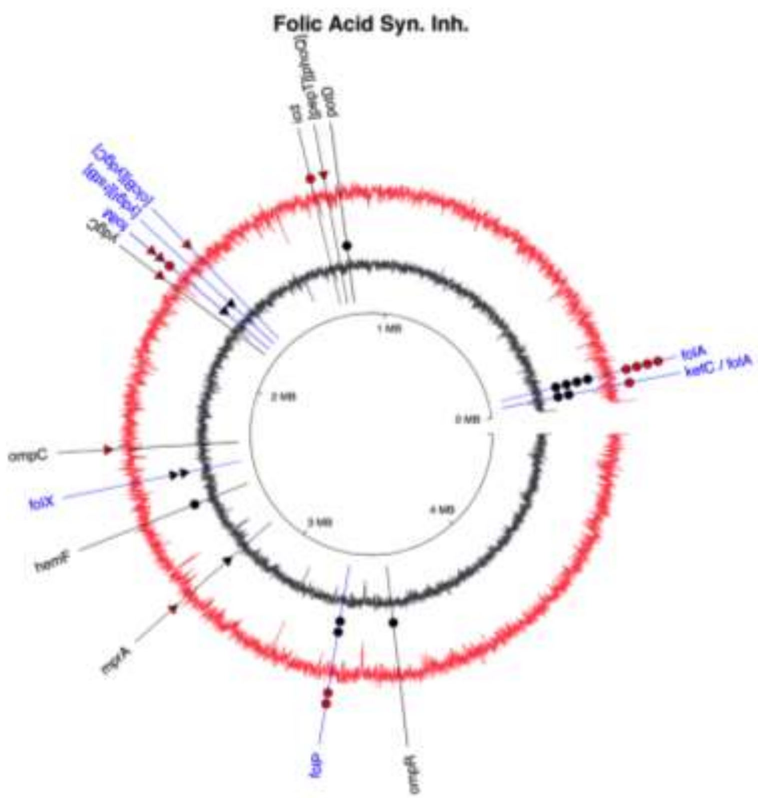
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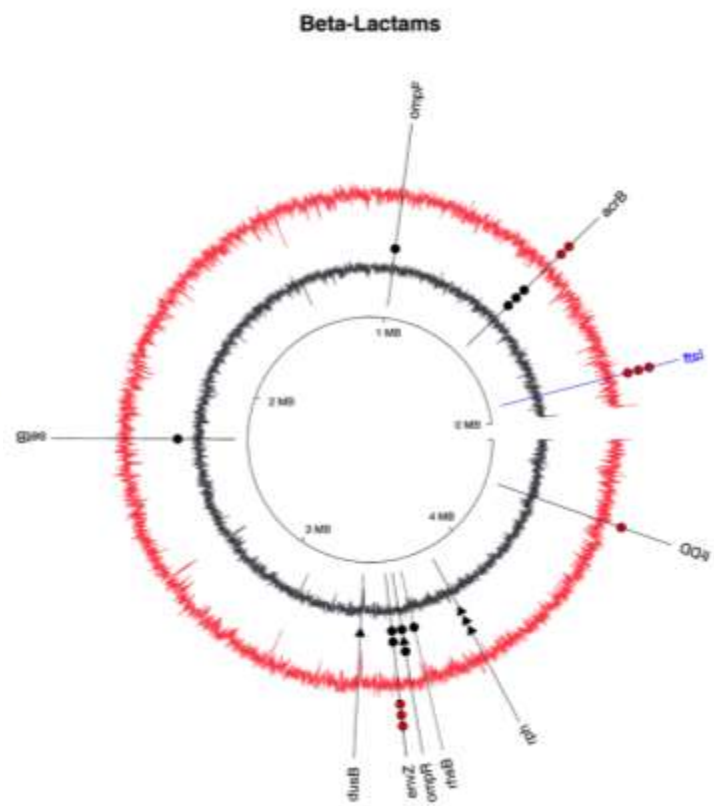
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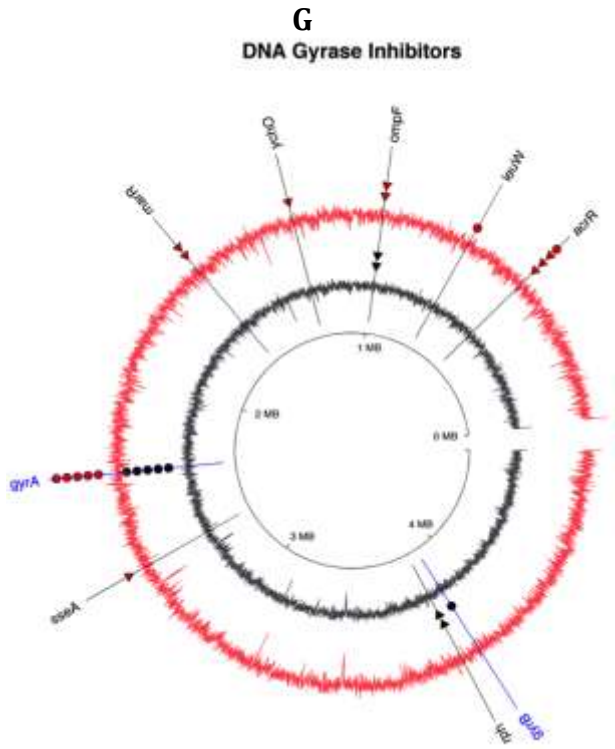

Figure 3-7: Mutations found in strains evolved against a drug class under strong selection (outer red circle) and mild selection (inner black circle) are shown with filled red and black markers respectively. SNPs are shown with filled circles and insertions/deletions are shown with filled triangles. Mutated genes' names are printed using standard annotations; however mutations are printed as "unknown" if there are no annotated genes found in literature. Pathway-specific mutations are printed in blue. **(A)** Mutations found in strains evolved against antibiotics with multiple mechanisms (nitrofurontain). **(B)** Mutations found in strains evolved against 50S ribosomal inhibitors. **(C)** Mutations found in strains evolved againstaminoglycosides. The*TrkH* gene, which is mutated in five aminoglycoside resistant strains, is shown with a magenta arrow. (D) Mutations found in strains evolved against 30S ribosomal inhibitors. (E) Mutations found in strains evolved against beta-lactams. (F) Mutations found in strains evolved against DNA gyrase inhibitors. (G) Mutations found in strains evolved against folic acid synthesis inhibitors. (Oz & Guvenek & Yildiz 2014)

In order to understand our results better, we made Table 3.2, which allows us to see mutations that belong to a specific drug group. However Table 3.2 only contains mutations that occur more than one times. In Table 2 pathway specific and off pathway mutation for each drug can be seen. For all of the drugs we used in evolution experiments (except chloramphenicol, doxycycline and tetracycline), we were able to identify several pathway-specific gene mutations in evolved strains. Mutated gene names marked with asterisks are genes that previously reported in literature to be involved antibiotic resistance studies.

For example SNP in *folA* has been reported to be involved with trimethoprim resistance in *Escherichia Coli* (Keith Miller 2004).

We conclude that since mutations in Table 2 has been observed more than one time, more than one strain, these entire mutations can ben related with drug resistance.

Off pathway mutations were interesting observation of this study. They are obviously related with drug resistance behavior of our resistance strains. There are 71 off pathway mutation in strongly selected strains and 38 off pathway mutation in mildly selected strains (Figure 3.8). Again mutations that previously reported in literature to be involved antibiotic resistance studies have been shown with asterisk on the gene name.

Number of mutation belonging to major pathways of strong selection and mild selection for each class has been demonstrated in Figure 3-8-A. Figure 3-8-B shows number of pathways specific mutations accordingly.

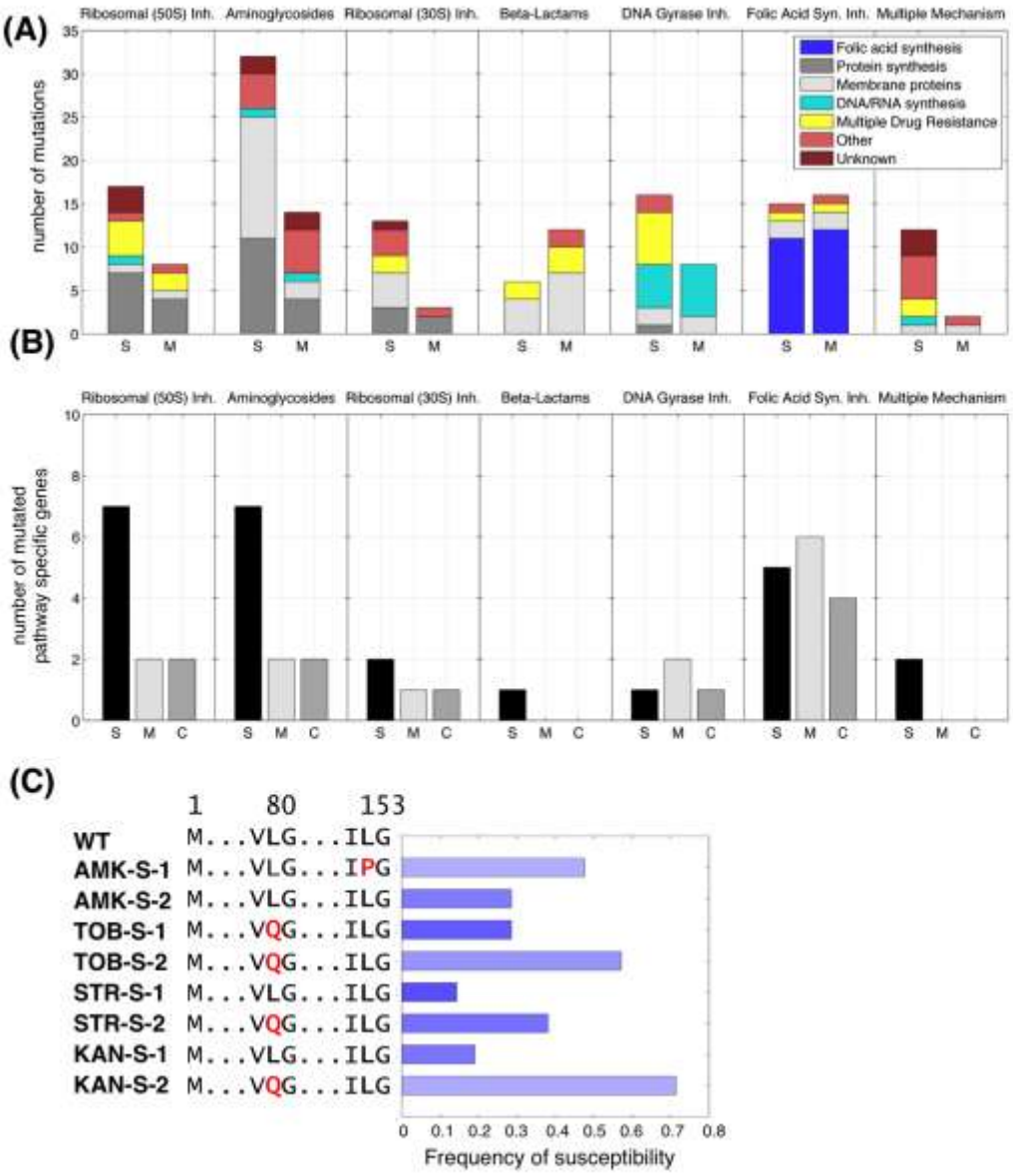

Figure 3-8: Effect of selection strength on genetic diversity. (A) Number of mutation belongs major cellular pathways in strongly selected (S) and mildly (M) selected strains. Strains clustered according to major antibiotic classes. (B) Pathway specific mutations per classes for strongly selected (S), and mildly selected (M) strains. (C) *trkH* mutations and drug sensitivity on aminoglycosides. Blue color weight of bars indicated strength of sensitivity. (Oz & Guvenek & Yildiz 2014)

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One of the most important finding of this study was aminoglycoside resistant strains behavior against other drugs. Aminoglycoside resistant strains were resistant to other drugs in their class but susceptible to other drugs from other class. We found out that six of the eight aminoglycoside resistant strains have mutation in *trkH* gene (Figure 3.8-B).

Drug Class	Drug	Pathway-specific mutations	Off-pathway mutations
50S, Protein synthesis inhibitor	Chloramphenicol		[soxR]*; [mdfA]*
50S, Protein synthesis inhibitor	Clindamycin	[prmB]; [rplB]*; [rpmG]	
50S, Protein synthesis inhibitor	Fusidic Acid	[fusA]*	[ylbE]
50S, Protein synthesis inhibitor	Spiramycin	[rplD]*; [rlmN]*	
50S, Protein synthesis inhibitor	Erythromycin	[rplV]*	[acrB]*; [fis], [ylbE]
Aminoglycosides	Streptomycin	[lysW]; [rimP]; [rpsL]*	[trkH]
Aminoglycosides	Amikacin	[cydA]; [fusA]*	[trkH], [ylbE]
Aminoglycosides	Kanamycin	[cpxA]*; [fusA]*	[trkH]; [fis]
Aminoglycosides	Tobramycin	[fusA]*	[trkH]; [fis]; [atpG]; [yiaO], [ylbE]
30S, Protein synthesis inhibitor	Doxycycline		[acrR]*; [fis]; [marR]*
30S, Protein synthesis inhibitor	Spectinomycin	[rpsE]*; [rplB]*	
30S, Protein synthesis inhibitor	Tetracycline		[mlaA];
Cell wall synthesis inhibitors	Ampicillin	[ftsI]*	[acrB]*; [envZ]*
Cell wall synthesis inhibitors	Cefoxitin	[ftsI]*	[acrB]*; [envZ]*; [ompR]*; [ompF]*
Cell wall synthesis inhibitors	Piperacillin	[ftsI]*	[envZ]*;[ompR]*
DNA gyrase inhbibitors	Ciprofloxacin	[gyrA]*; [gyrB]*	[acrR]*; [ompF]*
DNA gyrase inhibitors	Nalidixic Acid	[gyrA]*	
DNA gyrase inhbibitors	Lomefloxacin	[gyrA]*	[acrR]*; [marR]*
Folic acid synthesis inhibitor	Trimethoprim	[folA]*	
Folic acid synthesis inhibitor	Sulfamethaxozole	[folP]*; [folM]*; [folX]*	

Table 3-2: Drug classes, drugs used for selection, mutated pathway-specific genes, mutated off-pathway genes. Genes that are reported in literature to be related to antibiotic resistance are marked with asterisks. (Oz & Guvenek & Yildiz 2014)

3.4 Mutants Behavior on Different Temperature

Slow growth in mutant strains has been observed in previous studies before (Blackburn and Davies 1994). In this project some resistant strains such as: AMK-S1, KAN-S-2, ERY-S-1, CHL-S1,S-2 have significantly slower growth rate comparing to their ancestor wild type. Slower growth rate in resistant strains has been observed before, even when the stress factor has been remove, resistant bacteria turn back to be sensitive because of cost-benefit

optimization (Dekel and Alon 2005). The interesting observation of this study was some strains were growing better than its ancestor (Figure 3.6). In order to understand those fast growing strains we first tested all resistant strains in different temperature. Resistant strains and their wild type ancestor has been growth at 9 different temperature between 28 °C and 42 °C (Figure 3.9). For almost all strains 37 °C were optimal temperature except NIT-S-1 (Figure 3.10). NIT S-1 was an interesting strain that cannot grow temperatures above 37 °C. KAN-M-1 growth rate was also dramatically decreased temperatures above 39 °C. All these different behaviors at different temperatures should be investigated more in future studies.

About faster growing strains, we observed rph-pyrE mutations majority of those strains. We sequenced two strains that were propagated for 28 days in the absence of any antibiotics in minimal media, and those two also had deletion on rph-pyrE operon. And those media adapted strains also grow faster than its ancestor. We come up with a conclusion that this mutation is related with faster growing behavior. In literature pyrE previously reported with its relation with minimal media adaptation (Jensen 1993; Conrad, et al. 2009). Considering our result with Jensen's study, deletion in rph-pyrE operon should be related with our observation.

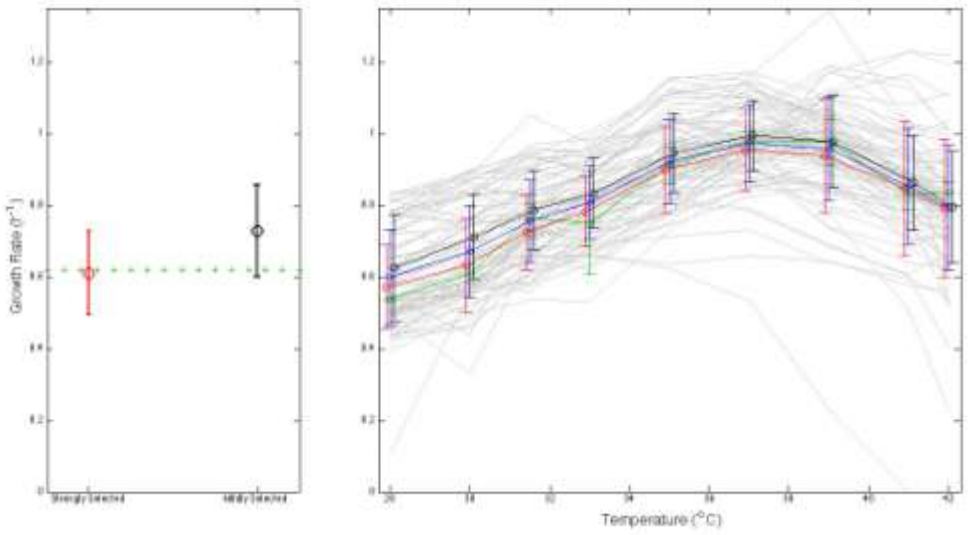

Figure 3-9: Effect of selection strength on growth rate at different temperatures. (A) Mean of growth rates. Red marker indicates strongly selected strains; black marker indicates mildly selected strains and green lines indicates growth rate of wild type. (B) Mean of growth rates in different temperatures. Red marker indicates strongly selected strains; black marker indicates mildly selected strains; green marker indicates growth rate of wild type and blue marker indicates mean of all strains.

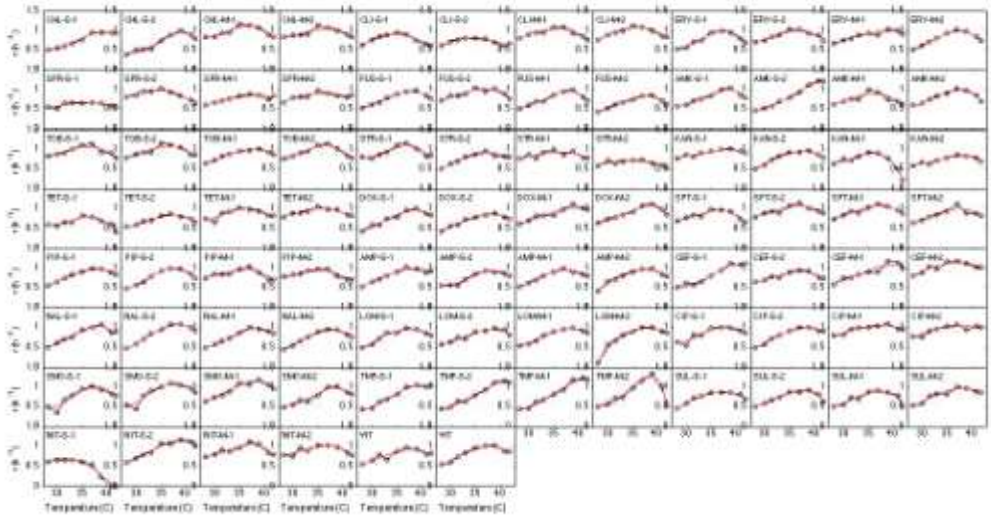

Figure 3-10: Growth rate of all strains at different temperatures between 28 °C and 42 ^{o}C. Each circle represents growth rate of different temperatures.

4 Discussion

In this study we accomplish systematic study of antibiotic resistance of *Escherichia Coli*. We pointed out the affect of selection strength as an important factor on bacterial evolution resistance mechanism. We combined phenotypic observation with genotypic observation and revealed important facts about evolutionary mechanisms. Bacteria developed resistance under stronger selection developed cross-resistance against several other drugs. Bacteria developed resistance under mild selection also developed cross-resistance, however that was significantly lower comparing to strong selection strains.

Strength selection has important effect on genetic diversity. Strong selection bacteria have more mutation in both number and diversity. Strongly selected strains have more pathway specific mutations comparing to mild selection. However pathway specific mutation and probably multidrug resistance gene mutation cost is higher, so fitness is lower. If mutated genes are important genes that effect cellular machinery, changes have huge fitness cost. An example of higher genetic diversity in strongly selected strain is aminoglycosides. Strongly selected aminoglycosides have 32 mutations in total and 13 of them were pathway specific mutation. Whereas mildly selected aminoglycosides have 22 mutations in total and only 4 of them were pathway specific. On the other hand folic acid synthesis inhibitors does not show such diversity. Strongly selected strains and mildly selected strains almost have same number of mutation. However when we look at the evolutionary experiment (Figure 3.1) we saw that evolutionary pathway of strongly selected strains and mildly selected strains are not very different on this group. Another interesting observation about folic acid synthesis inhibitors that TMP-S-2 and TMP-M-1 have exactly same mutations, and all strains have mutation in *folA* gene. This result in not surprising since pathway specific DHFR mutation has been observed in TMP resistance strains in previous studies (Toprak, et al. 2012).

Collateral sensitivity of aminoglycoside was another important discovery of this study. Recently another research group also discovered same collateral sensitivity of

aminoglycosides(Imamovic and Sommer 2013). In Imamovic's study they applied strong selection in order to evolved bacteria. Similarity between Imamovic's work and this project is not surprising since we observed stronger collateral sensitivity in strongly selected strains. They demonstrate phenotype of collateral sensitivity but their study was lack of genetic data. Meanwhile another group Lazar *et al* evolved Escherichia Coli against several antibiotics including aminoglycosides and sequenced resistant strain and discovered *trkH* mutation behind this sensitivity, similar to our findings(Lazar, et al. 2013). In addition to their findings we contribute these finding by studying selection strength. This collateral sensitivity can be a new strategy to minimize antibiotic resistance. In future research combined therapy of aminoglycoside with antibiotics that are not member of aminoglycosides should be tested.

5 Conclusions

In this study we pointed out a hidden factor in antibiotic resistance, which is selection strength. We concluded that selection strength is an important parameter that affects complexity of resistance evolution. We observed that population evolved in high concentration of drug acquired significantly higher cross-resistance. This result can lead new perspective on evolution of resistance, since physicians prefer to use highest concentration of drug in order to minimize cross-resistance. High concentration is useful the drug kills all the population, however in case of survival, bacteria will develop stronger cross-resistance.

To minimize cross-resistance, cross sensitivity aminoglycoside can be used in clinic, although it requires further investigation. During the treatment combination therapy can be used for patient, not because synergistic effect of drugs, but because cross sensitivity properties of aminoglycosides. During antibiotic treatment in specific days aminoglycoside can be used to slow down the resistance. This kind of study has not been done yet, however it may give promising result for resistance evolution.

Our study highlighted important and newly discovered facts about resistance evolution and further studies about selection strength and cross sensitivity will give better understanding about this area.

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7 Appendices

7.1 Appendix A

Genetic changes found in all sequenced strains except CEF-S-1 and CEF-S-2.Sequence ID, strain ID, drug class used for selection, genome position of the mutation, nucleotide change, annotation of the mutation, mutated gene(s), description of the mutated gene(s), gene function, selection strength, exclusivity (exclusive: mutation found in only mildly selected or strongly selected strains, common: mutation found in both mildly selected and strongly selected strains), pathway-specifity

Sequence ID	Strain ID	Antibiotic Class	Position	Mutation	Annotation	Gene	Description	Gene Function	Selection	Exclusivity	Pathway Specific
ET01	**SPR-S1**	50S inhibitor	2641864	Δ1 bp	coding (443/1155 nt)	***rlmN*** ←	dual specificity 23S rRNA m(2)A2503, tRNA m(2)A37 methyltransferase, SAM-dependent	Protein synthesis	strong	exclusive	Yes
ET01	**SPR-S1**	50S inhibitor	3450111	Δ6 bp	coding (199-199/606 nt)	***rplD*** ←	50S ribosomal subunit protein L4	Protein synthesis	strong	common	Yes
ET01	**SPR-S1**	50S inhibitor	3813953	Δ82 bp		***rph***	defective ribonuclease PH - adaptation to minimal media	Not classified	strong	common	No
ET02	**SPR-S2**	50S inhibitor	3813953	Δ82 bp		***rph***	defective ribonuclease PH - adaptation to minimal media	Not classified	strong	common	No
ET03	**SPR-M1**	50S inhibitor	3450113	C→A	G66C (GGC→TGC)	***rplD*** ←	50S ribosomal subunit protein L4	Protein synthesis	mild	common	Yes
ET03	**SPR-M1**	50S inhibitor	3813953	Δ82 bp		***rph***	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No
ET04	**SPR-M2**	50S inhibitor	3450112	C→T	G66D (GGC→GAC)	***rplD*** ←	50S ribosomal subunit protein L4	Protein synthesis	mild	common	Yes
ET04	**SPR-M2**	50S inhibitor	3813953	Δ82 bp		***rph***	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No
ET06	**CLI-S1**	50S inhibitor	2445968	Δ9 bp	coding (487-495/993 nt)	***prmB*** ←	3(5)-glutamate methyltransferase	protein synthesis	strong	exclusive	Yes
ET06	**CLI-S1**	50S inhibitor	3809327	Δ1 bp	coding (114/168 nt)	***rpmG*** ←	50S ribosomal subunit protein L33	Protein synthesis	strong	exclusive	Yes
ET06	**CLI-S1**	50S inhibitor	3813847	G→T	intergenic (-364/-39)	***pyrE*** → / ← ***rph***	orotate phosphoribosyltransferase/defective ribonuclease PH- adaptation to minimal media	adaptation to minimal media	strong	exclusive	No
ET08	**CLI-S2**	50S inhibitor	3449681	Δ3 bp	coding (704-706/822 nt)	***rplB*** ←	50S ribosomal subunit protein L2	Protein synthesis	strong	exclusive	Yes
ET08	**CLI-S2**	50S inhibitor	3813953	Δ82 bp		***rph***	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	strong	common	No
ET09	**CLI-M1**	50S inhibitor	3813953	Δ82 bp		***rph***	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No
ET10	**CLI-M2**	50S inhibitor	3813953	Δ82 bp		***rph***	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No

ET12	**ERY-S2**	50S inhibitor	2959372	C→A	intergenic (-116/-93)	***ygbI* ←/→ *ygbJ***	putative transcriptional regulator, DeoR family/putative dehydrogenase, with NAD(P)-binding Rossmann-fold domain	Other	strong	exclusive	No
ET12	**ERY-S2**	50S inhibitor	3447953	Δ3 bp	coding (301-303/333 nt)	***rplV*** ←	50S ribosomal subunit protein L22	Protein synthesis	strong	exclusive	Yes
ET13	**ERY-M1**	50S inhibitor	461479	G→A	R717C (CGT→TGT)	***acrB*** ←	multidrug efflux system protein	Multi Drug Resistance	mild	exclusive	No
ET13	**ERY-M1**	50S inhibitor	3460405	Δ617 bp		***[fis]-[yhdJ]***	*[fis], [yhdJ]*	Other	mild	exclusive	No
ET14	**ERY-M2**	50S inhibitor	547832	+G	coding (478/1259 nt)	***ybbE*** →	Unknown	Unknown	strong	exclusive	No
ET14	**ERY-M2**	50S inhibitor	461479	G→A	R717C (CGT→TGT)	***acrB*** ←	multidrug efflux system protein	Multi Drug Resistance	mild	exclusive	No
ET16	**CHL-S1**	50S inhibitor	458014	12186 (+) +6 bp : Δ1	intergenic (+98/-93)	***clpX*** →/→ ***lon***	ATPase and specificity subunit of ClpX-ClpP ATP-dependent serine protease/DNA-binding ATP-dependent protease La	Unknown	strong	exclusive	No
ET16	**CHL-S1**	50S inhibitor	882776	12230 (+) +2 bp	intergenic (-165/-119)	***ybjG*** ←/→ ***mdfA***	undecaprenyl pyrophosphate phosphatase/multidrug efflux system protein	Multi Drug Resistance	strong	exclusive	No
ET16	**CHL-S1**	50S inhibitor	3919571	Δ513 bp		***[atpB]-[atpI]***	*[atpB], [atpI]*	Membrane proteins	strong	exclusive	No
ET16	**CHL-S1**	50S inhibitor	4275808	C→T	A146V (GCA→GTA)	***soxR*** →	DNA-binding transcriptional dual regulator, Fe-S center for redox-sensing	Multi Drug Resistance	strong	exclusive	No
ET17	**CHL-S2**	50S inhibitor	458016	12186 (-) +4 bp	intergenic (+92/-93)	***clpX*** →/→ ***lon***	ATPase and specificity subunit of ClpX-ClpP ATP-dependent serine protease/DNA-binding ATP-dependent protease La	unknown	strong	exclusive	No
ET17	**CHL-S2**	50S inhibitor	882884	C→A	intergenic (-273/-12)	***ybjG*** ←/→ ***mdfA***	undecaprenyl pyrophosphate phosphatase/multidrug efflux system protein	Multi Drug Resistance	strong	exclusive	No
ET17	**CHL-S2**	50S inhibitor	2877629	G→C	P56A (CCT→GCT)	***ygbT*** ←	multifunctional endonuclease Cas1, CRISPR adaptation protein; DNA repair enzyme	DNA/RNA synthesis	strong	exclusive	No
ET17	**CHL-S2**	50S inhibitor	4275919	G→A	G143D (GGT→GAT)	***soxR*** →	DNA-binding transcriptional dual regulator, Fe-S center for redox-sensing	Multi Drug Resistance	strong	exclusive	No
ET18	**CHL-M1**	50S inhibitor	3813953	Δ62 bp		***rph***	defective ribonuclease PH- adaptation to minimal media	adaptation to minimal media	mild	common	No

ET19	**CHL-M2**	50S inhibitor	3813953	Δ82 bp		***cyh***	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No
ET21	**KAN-S2**	Aminoglycosides	392191	Δ5,466 bp	IS3-mediated	***yaiT-[yaiW]***	*yaiT, yaiV, aegA, ybeA, [yaiW]*	Unknown	strong	exclusive	No
ET21	**KAN-S2**	Aminoglycosides	3469722	A→C	P605L (TTT→TTG)	***fusA*** ←	protein chain elongation factor EF-G, GTP-binding	Protein synthesis	strong	common	Yes
ET21	**KAN-S2**	Aminoglycosides	4031406	T→A	L80Q (CTG→CAG)	***trkH*** →	potassium transporter	Membrane proteins	strong	exclusive	No
ET21	**KAN-S2**	Aminoglycosides	4102440	C→G	A107P (GCA→CCA)	***cpxA*** ←	sensory histidine kinase in two-component regulatory system with CpxR	Membrane proteins	strong	exclusive	Yes
ET22	**KAN-M1**	Aminoglycosides	118793	T→A	C21S (TGC→AGC)	***ampD*** →	1,6-anhydro-N-acetylmuramyl-L-alanine amidase, Zn-dependent, murein amidase	Cell wall synthesis	mild	exclusive	No
ET22	**KAN-M1**	Aminoglycosides	450605	IS5 (-) +4 bp	coding (227-230/948 nt)	***cyoA*** ←	cytochrome o ubiquinol oxidase subunit II	Other	mild	exclusive	No
ET22	**KAN-M1**	Aminoglycosides	3469903	A→G	I345T (ATC→ACC)	***fusA*** ←	protein chain elongation factor EF-G, GTP-binding	Protein synthesis	mild	common	Yes
ET22	**KAN-M1**	Aminoglycosides	3813953	Δ82 bp		***cyh***	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No
ET23	**KAN-M2**	Aminoglycosides	3409806	G→T	R5L (CGC→CTC)	***fis*** →	global DNA-binding transcriptional dual regulator	Other	mild	exclusive	No
ET24	**SUL-S1**	Folic Acid inhibitor	1195468	T→C	L375M (TTA→CTG)	***icd*** →	e14 prophage; isocitrate dehydrogenase, specific for NADP+	Cell wall synthesis	strong	exclusive	No
ET24	**SUL-S1**	Folic Acid inhibitor	1679046	G→T	R16L (CGT→CTT)	***folM*** →	dihydromonapterin reductase, NADPH-dependent, dihydrofolate reductase isozyme	Folic acid synthesis	strong	common	Yes
ET24	**SUL-S1**	Folic Acid inhibitor	3322744	G→A	P64S (CCA→TCA)	***folP*** ←	7,8-dihydropteroate synthase	Folic acid synthesis	strong	exclusive	Yes
ET25	**SUL-S2**	Folic Acid inhibitor	1679653	Δ4 bp	coding (654-657/723 nt)	***folM*** →	dihydromonapterin reductase, NADPH-dependent, dihydrofolate reductase isozyme	Folic acid synthesis	strong	common	Yes
ET25	**SUL-S2**	Folic Acid inhibitor	3322744	G→A	P64S (CCA→TCA)	***folP*** ←	7,8-dihydropteroate synthase	Folic acid synthesis	strong	common	Yes
ET26	**SUL-M1**	Folic Acid inhibitor	2419514	Δ1 bp	coding (169/363 nt)	***folX*** →	D-erythro-7,8-dihydroneopterin triphosphate 2'-epimerase and dihydroneopterin aldolase	Folic acid synthesis	mild	exclusive	Yes

ST26	**SUL-M1**	Folic Acid inhibitor	3322744	G→A	P948 (CCA→TCA)	***folP*←**	7,8−dihydropteroate synthase	Folic acid synthesis	mild	common	Yes
ST27	**SUL-M2**	Folic Acid inhibitor	2419378	IS3 (+) +5 bp	coding (32−363/363 nt)	***folX*→**	D−erythro−7,8−dihydroneopterin triphosphate 2'−epimerase and dihydroneopterin aldolase	Folic acid synthesis	mild	exclusive	Yes
ST27	**SUL-M2**	Folic Acid inhibitor	2551265	C→T	Q5* (CAG→TAG)	***hemF*→**	coproporphyrinogen III oxidase	Other	mild	exclusive	No
ST27	**SUL-M2**	Folic Acid inhibitor	3322050	A→T	F29L (TTT→TTA)	***folP*←**	7,8−dihydropteroate synthase	Folic acid synthesis	mild	common	Yes
ST28	**CIP-S1**	DNA Gyrase inhibitor	405391	Δ1 bp	coding (405/1041 nt)	***acrR*→**	DNA−binding transcriptional repressor	Multi Drug Resistance	strong	exclusive	No
ST28	**CIP-S1**	DNA Gyrase inhibitor	985571	Δ1 bp	coding (635/1089 nt)	***ompF*←**	outer membrane porin 1a (Ia),F)	Membrane proteins	strong	common	No
ST28	**CIP-S1**	DNA Gyrase inhibitor	2337195	G→A	S83L (TCG→TTG)	***gyrA*←**	DNA gyrase (type II topoisomerase), subunit A	DNA/RNA synthesis	strong	common	Yes
ST31	**CIP-S2**	DNA Gyrase inhibitor	986179	IS (+) 1bp	FrameShift	***[ompF]***	outer membrane porin 1a (Ia),F)	Membrane proteins	strong	common	No
ST31	**CIP-S2**	DNA Gyrase inhibitor	405103	Δ12 bp	coding (119−130/948 nt)	***acrR*→**	DNA−binding transcriptional repressor	Multi Drug Resistance	strong	common	No
ST31	**CIP-S2**	DNA Gyrase inhibitor	2337195	G→A	S83L (TCG→TTG)	***gyrA*←**	DNA gyrase (type II topoisomerase), subunit A	DNA/RNA synthesis	strong	common	Yes
ST31	**CIP-S2**	DNA Gyrase inhibitor	2650804	Δ1 bp	coding (289/944 nt)	***nusA*→**	3−mercaptopyruvate sulfurtransferase	Other	strong	exclusive	No
ST32	**CIP-M1**	DNA Gyrase inhibitor	985734	+G	coding (472/1089 nt)	***ompF*←**	outer membrane porin 1a (Ia),F)	Membrane proteins	mild	common	No
ST32	**CIP-M1**	DNA Gyrase inhibitor	2337195	G→A	S83L (TCG→TTG)	***gyrA*←**	DNA gyrase (type II topoisomerase), subunit A	DNA/RNA synthesis	mild	common	Yes
ST32	**CIP-M1**	DNA Gyrase inhibitor	3813953	Δ82 bp		***rph***	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No
ST33	**CIP-M2**	DNA Gyrase inhibitor	985632	Δ4 bp	coding (571−574/1089 nt)	***ompF*←**	outer membrane porin 1a (Ia),F)	Membrane proteins	mild	common	No
ST33	**CIP-M2**	DNA Gyrase inhibitor	3976752	C→A	D46Y (TCT→TAT)	***gyrB*←**	DNA gyrase, subunit B	DNA/RNA synthesis	mild	exclusive	Yes
ST33	**CIP-M2**	DNA Gyrase inhibitor	3813953	Δ82 bp		***rph***	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No

ST34	**TET-S1**	XII inhibitor	1707843	Δ4 bp	coding (679-683/1059 nt)	***mcsD* →**	electron transport complex protein required for the reduction of EutB, predicted membrane protein	Membrane protein	strong	exclusive	No
ST34	**TET-S1**	XII inhibitor	2462896	I21 (+) +6 bp	coding insertion	***mlaA* ←**	ABC transporter maintaining OM lipid asymmetry, OM lipoprotein component	Membrane protein	strong	exclusive	No
ST34	**TET-S1**	XII inhibitor	2955304	T→C	W567R (TGG→CGG)	***fkpA* →**	L-fucose isomerase	Other	strong	exclusive	No
ST35	**TET-S2**	XII inhibitor	2462896	I21 (+) +6 bp	coding insertion	***mlaA* ←**	ABC transporter maintaining OM lipid asymmetry, OM lipoprotein component	Membrane protein	strong	exclusive	No
ST36	**TET-M1**	XII inhibitor	3013953	Δ62 bp		***rph***	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No
ST37	**TET-M2**	XII inhibitor	3013953	Δ62 bp		***rph***	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No
ST38	**DOX-M1**	XII inhibitor	3409407	Δ2,396 bp		***[fis]-cnrR***	*[fis], yhdT, yhdT, cnrR*	Other	mild	exclusive	No
ST39	**DOX-M2**	XII inhibitor	1091488	Δ1,348 bp		***fpgmA]***	-polysaccharide PGA secretin; OM porin; poly-beta 1,6-N-acetyl-D-glucosamine	Transport	mild	exclusive	No
ST39	**DOX-M2**	XII inhibitor	3013953	Δ62 bp		***rph***	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No
ST40	**DOX-S1**	XII inhibitor	458014	I1186 (+) +6 bp ::Δ 1	stop gain (+90/-93)	***clpX* → / → *ion***	ATPase and specificity subunit of ClpX-ClpP ATP-dependent serine protease/DNA-binding ATP-dependent protease La	Other	strong	exclusive	No
ST40	**DOX-S1**	XII inhibitor	465076	C→A	E31Y (GCC→TAC)	***acrR* →**	DNA-binding transcriptional repressor	Multi Drug Resistance	strong	exclusive	No
ST40	**DOX-S1**	XII inhibitor	3393629	G→A	Q218* (CAG→TAG)	***yhdT* ←**	conserved membrane protein, predicted transporter	Membrane protein	strong	exclusive	No
ST41	**DOX-S2**	XII inhibitor	458014	I1186 (+) +6 bp ::Δ 1	stop gain (+90/-93)	***clpX* → / → *ion***	ATPase and specificity subunit of ClpX-ClpP ATP-dependent serine protease/DNA-binding ATP-dependent protease La	Other	strong	exclusive	no
ST41	**DOX-S2**	XII inhibitor	1617223	G→C	R27P (CGC→CCC)	***marR* →**	DNA-binding transcriptional repressor of multiple antibiotic resistance	Multi Drug Resistance	strong	exclusive	No

ET42	**TOB-S1**	Aminoglycosides	302191	Δ3,006 bp	IE1-mediated	***yaiT-fhuA)***	*yaiT, yaiP, ampH, ybeA, yaiW, yaiT, yaiZ, sikA, yaiP, fhuA)*	Other	strong	exclusive	No
ET42	**TOB-S1**	Aminoglycosides	2722072	C→T	R442C (CGC→TGC)	***pssA →***	phosphatidylserine synthase (CTP--dacylglycerol--serine O-phosphatidyltransferase)	Membrane proteins	strong	exclusive	No
ET42	**TOB-S1**	Aminoglycosides	3471108	C→A	G117C (GGT→TGT)	***fusA ←***	protein chain elongation factor EF-G, GTP-binding	Protein synthesis	strong	common	Yes
ET42	**TOB-S1**	Aminoglycosides	3744558	C→A	E148E (GGC→AGC)	***ybiO →***	L-dehydroascorbate transporter, periplasmic binding protein for TRAP (TRIpartite ATP-independent Periplasmic) family transporter TakBP)	Membrane proteins	strong	exclusive	No
ET42	**TOB-S1**	Aminoglycosides	3915554	Δ1 bp	coding (733/864 nt)	***atpG ←***	F1 sector of membrane-bound ATP synthase, gamma subunit	Membrane proteins	strong	exclusive	No
ET42	**TOB-S1**	Aminoglycosides	4031406	T→A	L80Q (CTG→CAG)	***nhaM →***	potassium transporter	Membrane proteins	strong	exclusive	No
ET43	**TOB-S2**	Aminoglycosides	302191	Δ3,466 bp	IE1-mediated	***yaiT-fusB)***	*yaiT, yaiP, ampH, ybeA, fusB)*	Other	strong	exclusive	No
ET43	**TOB-S2**	Aminoglycosides	1184514	Δ1 bp	coding (302/1137 nt)	***pstA ←***	polyamine transporter subunit	Membrane proteins	strong	exclusive	No
ET43	**TOB-S2**	Aminoglycosides	3471108	C→A	G117C (GGT→TGT)	***fusA ←***	protein chain elongation factor EF-G, GTP-binding	Protein synthesis	strong	common	Yes
ET43	**TOB-S2**	Aminoglycosides	3629733	T→C	D204G (GAC→GGC)	***yhiJ ←***	hypothetical protein	Unknown	strong	exclusive	No
ET43	**TOB-S2**	Aminoglycosides	3744558	C→A	E148E (GGC→AGC)	***ybiO →***	L-dehydroascorbate transporter, periplasmic binding protein for TRAP (TRIpartite ATP-independent Periplasmic) family transporter TakBP)	Membrane proteins	strong	exclusive	No
ET43	**TOB-S2**	Aminoglycosides	3915559	+AC	coding (730/864 nt)	***atpG ←***	F1 sector of membrane-bound ATP synthase, gamma subunit	Membrane proteins	strong	exclusive	No
ET43	**TOB-S2**	Aminoglycosides	4031406	T→A	L80Q (CTG→CAG)	***nhaM →***	potassium transporter	Membrane proteins	strong	exclusive	No
ET44	**TOB-M1**	Aminoglycosides	449164	+CACAGT	coding (702/1092 nt)	***cysB ←***	cytochrome c ubiquinol oxidase subunit I	Membrane proteins	mild	exclusive	No
ET44	**TOB-M1**	Aminoglycosides	3409506	G→T	R3L (CGC→CTC)	***fis →***	global DNA-binding transcriptional dual regulator	Other	mild	exclusive	No
ET45	**TOB-M2**	Aminoglycosides	547032	+G	coding (476/1259 nt)	***ybbE →***	Unknown	Unknown	mild	exclusive	No
ET45	**TOB-M2**	Aminoglycosides	3013080	+C	intergenic (+89/-6)	***pyrE 6-/4-rph***	orotate phosphoribosyltransferase/defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No
ET45	**TOB-M2**	Aminoglycosides	3013083	Δ52 bp		*rph*	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No

ET49	**SMO-S1**	Folic Acid inhibitor	1679517	Δ6 bp	coding (518-523723 nt)	***folM*** **→**	dihydromonapterin reductase, NADPH-dependent, dihydrofolate reductase enzyme	Folic acid synthesis	strong	common	Yes
ET49	**SMO-S1**	Folic Acid inhibitor	2310941	Δ1 bp	coding (133/1104 nt)	***ompC*** **←**	outer membrane porin protein C	Membrane proteins	strong	exclusive	No
ET51	**SMO-S2**	Folic Acid inhibitor	1677915	+GTTG	coding(75/396)	***ydgC*** **←**	inner membrane protein, GlpM family	Membrane proteins	strong	exclusive	No
ET51	**SMO-S2**	Folic Acid inhibitor	1663609	Δ15,179 bp		***[ckdB]-[ydgC]***	*17 genes [ckdB], ynfE, mlc, ynfL, ynfM, ncr, ydgU, ydgZ), mdtE, mdtJ, tgcA, ynfB, ynfA, ydgN, ydgL, folM, [ydgC]*	Folic acid synthesis	strong	exclusive	Yes
ET51	**SMO-S2**	Folic Acid inhibitor	2809566	Δ2 bp	coding (475-476/531 nt)	***mprA*** **→**	DNA-binding transcriptional repressor of microcin B17 synthesis and multidrug efflux	Multi Drug Resistance	strong	common	No
ET52	**SMO-M1**	Folic Acid inhibitor	1677907	Δ3,012 bp		***[ydgI]-[mtfA]***	*[ydgI], folM, ydgC, mtA, [mtfA]*	Folic acid synthesis	mild	exclusive	Yes
ET52	**SMO-M1**	Folic Acid inhibitor	2809566	Δ2 bp	coding (475-476/531 nt)	***mprA*** **→**	DNA-binding transcriptional repressor of microcin B17 synthesis and multidrug efflux	Multi Drug Resistance	mild	common	No
ET53	**SMO-M2**	Folic Acid inhibitor	1191136	G→T	P306Q (CCA→CAA)	***pmiB*** **←**	polyamine transporter subunit	Membrane proteins	mild	exclusive	No
ET53	**SMO-M2**	Folic Acid inhibitor	1679513	Δ4 bp	coding (654-657/723 nt)	***folM*** **→**	dihydromonapterin reductase, NADPH-dependent, dihydrofolate reductase enzyme	Folic acid synthesis	mild	common	Yes
ET53	**SMO-M2**	Folic Acid inhibitor	3534219	C→A	E96D (GAG→GAT)	***cpxR*** **←**	DNA-binding response regulator in two-component regulatory system with EnvZ	Membrane proteins	mild	exclusive	No
ET54	**LOM-S1**	DNA Gyrase inhibitor	405556	C→A	A135D (GCC→GAC)	***acrR*** **→**	DNA-binding transcriptional repressor	Multi Drug Resistance	strong	exclusive	No
ET54	**LOM-S1**	DNA Gyrase inhibitor	1617410	Δ13 bp	coding (267-279/435 nt)	***marR*** **→**	DNA-binding transcriptional repressor of multiple antibiotic resistance	Multi Drug Resistance	strong	common	No
ET54	**LOM-S1**	DNA Gyrase inhibitor	2337104	C→A	D87Y (GAC→TAC)	***gyrA*** **←**	DNA gyrase (type-II topoisomerase), subunit A	DNA/RNA synthesis	strong	common	Yes
ET56	**LOM-S2**	DNA Gyrase inhibitor	405201	125 (-) +4 bp	coding (217-220/948 nt)	***acrR*** **→**	DNA-binding transcriptional repressor	Multi Drug Resistance	strong	common	No
ET56	**LOM-S2**	DNA Gyrase inhibitor	604269	G→T	noncoding (2/65 nt)	***leuV*** **←**	tRNA-Leu	Protein synthesis	strong	exclusive	No
ET56	**LOM-S2**	DNA Gyrase inhibitor	1617375	14 bp x 2	duplication	***marR*** **→**	DNA-binding transcriptional repressor of multiple antibiotic resistance	Multi Drug Resistance	strong	exclusive	No

ET57	**LOM-M1**	DNA Gyrase inhibitor	2337195	G→A	S83L (TCG→TTG)	***gyrA*** ←	DNA gyrase (type II topoisomerase), subunit A	DNA/RNA synthesis	mild	common	Yes
ET58	**LOM-M2**	DNA Gyrase inhibitor	2337195	G→A	S83L (TCG→TTG)	***gyrA*** ←	DNA gyrase (type II topoisomerase), subunit A	DNA/RNA synthesis	mild	common	Yes
ET59	**NIT-S1**	Multi	547882	+G	coding (476/1259 nt)	***ylbE*** →	Unknown	Unknown	strong	exclusive	No
ET59	**NIT-S1**	Multi	433499	G→A	intergenic (-262/+37)	***tsx*** ← / ← ***yajI***	nucleoside channel, receptor of phage T6 and colicin K/putative lipoprotein	Unknown	strong	exclusive	No
ET59	**NIT-S1**	Multi	575009	Δ30,955 bp	IS5-mediated	***[ompC]–[ybdK]***	*34 genes [ompC], essD, rrrD, rzpD, rzoD, borD, ybcV, ybcW, ylcI, nohD, nmpD, pfsD, ybcY, ybcY, tfaX, appY, ompT, pauD, envY, ybcH, nfrA, nfrB,* [illegible]	Other	strong	exclusive	No
ET59	**NIT-S1**	Multi	890552	Δ1 bp	coding (146/723 nt)	***nfsA*** →	nitroreductase A, NADPH-dependent, FMN-dependent	Other	strong	exclusive	Yes
ET59	**NIT-S1**	Multi	1906836	IS5 (–) +4 bp	intergenic (-44/-110)	***mgrB*** ← / → ***yobH***	regulatory peptide for PhoPQ, feedback inhibition/hypothetical protein	Other	strong	exclusive	No
ET59	**NIT-S1**	Multi	2809160	+TT	coding (369/531 nt)	***mprA*** →	DNA-binding transcriptional repressor of microcin B17 synthesis and multidrug efflux	Multi Drug Resistance	strong	exclusive	No
ET59	**NIT-S1**	Multi	3438945	C→T	G36D (GGC→GAC)	***rpoA*** ←	RNA polymerase, alpha subunit	DNA/RNA synthesis	strong	exclusive	Yes
ET59	**NIT-S1**	Multi	3534678	IS5 (+) +4 bp	intergenic (-72/-153)	***ompR*** ← / → ***greB***	DNA-binding response regulator in two-component regulatory system with EnvZ/transcript cleavage factor	Unknown	strong	exclusive	No
ET60	**NIT-S2**	Multi	575009	Δ31,250 bp	IS5-mediated	***[ompC]–[ybdK]***	*34 genes [ompC], essD, rrrD, rzpD, rzoD, borD, ybcV, ybcW, ylcI, nohD, nmpD, pfsD, ybcY, ybcY, tfaX, appY, ompT, pauD, envY, ybcH, nfrA, nfrB,* [illegible]	Other	strong	exclusive	No
ET60	**NIT-S2**	Multi	890790	Δ1 bp	coding (384/723 nt)	***nfsA*** →	nitroreductase A, NADPH-dependent, FMN-dependent	Other	strong	exclusive	Yes
ET60	**NIT-S2**	Multi	2809147	Δ1 bp	coding (356/531 nt)	***mprA*** →	DNA-binding transcriptional repressor of microcin B17 synthesis and multidrug efflux	Multi Drug Resistance	strong	exclusive	No
ET60	**NIT-S2**	Multi	3534075	Δ7 bp	coding (526-532/720 nt)	***ompR*** ←	DNA-binding response regulator in two-component regulatory system with EnvZ	Membrane proteins	strong	exclusive	No
ET61	**NIT-M1**	Multi	3813953	Δ82 bp		***rph***	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No
ET62	**NIT-M2**	Multi	1973719	Δ1 bp	coding (561/927 nt)	***motB*** ←	protein that enables flagellar motor rotation	Membrane proteins	mild	exclusive	No

ET62	**NIT-M2**	Multi	3720257	T→A	intergenic (+185/+94)	***insE*** **→ / ← *ilvY***	IS150 transposase B/glycine tRNA synthetase, beta subunit	Other	mild	exclusive	No
ET62	**NIT-M2**	Multi	3813053	Δ82 bp		***rph***	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No
ET63	**PIP-S1**	Cell Wall Synth. Inhibit	93439	G→A	D343N (GAC→AAC)	***ftsI*** **→**	transpeptidase involved in septal peptidoglycan synthesis (penicillin-binding protein 3)	Cell wall synthesis	strong	exclusive	Yes
ET63	**PIP-S1**	Cell Wall Synth. Inhibit	3533169	A→C	V341G (GTA→GGA)	***envZ*** **←**	sensory histidine kinase in two-component regulatory system with OmpR	Membrane proteins	strong	common	No
ET64	**PIP-S2**	Cell Wall Synth. Inhibit	4377058	A→T	V111D (GTC→GAC)	***fhlD*** **←**	fumarate reductase (anaerobic), membrane anchor subunit	Membrane proteins	strong	exclusive	No
ET65	**PIP-M1**	Cell Wall Synth. Inhibit	3813053	Δ82 bp		***rph***	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No
ET66	**PIP-M2**	Cell Wall Synth. Inhibit	3534564	G→A	R15C (CGC→TGC)	***ompR*** **←**	DNA-binding response regulator in two-component regulatory system with EnvZ	Membrane proteins	mild	exclusive	No
ET66	**PIP-M2**	Cell Wall Synth. Inhibit	3813053	Δ82 bp		***rph***	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No
ET69	**CEF-M1**	Cell Wall Synth. Inhibit	401611	C→T	E673K (GAA→AAA)	***acrB*** **←**	multidrug efflux system protein	Multi Drug Resistance	mild	common	No
ET69	**CEF-M1**	Cell Wall Synth. Inhibit	905123	G→A	Q361* (CAG→TAG)	***ompF*** **←**	outer membrane porin 1a (Ia,b,F)	Membrane proteins	mild	exclusive	No
ET69	**CEF-M1**	Cell Wall Synth. Inhibit	3489255	153 (-)+9 bp	coding (954-961/966 nt)	***dusB*** **→**	tRNA-dihydrouridine synthase B	Other	mild	exclusive	No
ET69	**CEF-M1**	Cell Wall Synth. Inhibit	3534564	Δ1 bp	coding (21/720 nt)	***ompR*** **←**	DNA-binding response regulator in two-component regulatory system with EnvZ	Membrane proteins	mild	exclusive	No
ET70	**CEF-M2**	Cell Wall Synth. Inhibit	401611	C→T	E673K (GAA→AAA)	***acrB*** **←**	multidrug efflux system protein	Multi Drug Resistance	mild	common	No
ET70	**CEF-M2**	Cell Wall Synth. Inhibit	2262723	G→A	G286D (GGT→GAT)	***setB*** **→**	lactose/glucose efflux system	Membrane proteins	mild	exclusive	No
ET70	**CEF-M2**	Cell Wall Synth. Inhibit	3534600	C→A	E3* (GAG→TAG)	***ompR*** **←**	DNA-binding response regulator in two-component regulatory system with EnvZ	Membrane proteins	mild	exclusive	No
ET70	**CEF-M2**	Cell Wall Synth. Inhibit	3620862	A→G	R121G (CGA→GGG)	***rhsB*** **→**	rhsB element core protein RshB	Other	mild	exclusive	No

ET70	**CEF-M2**	Cell Wall Synth. Inhibitor	301,265.0	Δ62 bp		***rph***	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No
ET71	**NAL-S1**	DNA Gyrase Inhibitor	1273621	Δ1 bp	coding (615/1395 nt)	***pdhO*** →	putative enzyme	Other	strong	exclusive	No
ET71	**NAL-S1**	DNA Gyrase Inhibitor	2337195	G→A	S83L (TCG→TTG)	***gyrA*** ←	DNA gyrase (type II topoisomerase), subunit A	DNA/RNA synthesis	strong	common	Yes
ET72	**NAL-S2**	DNA Gyrase Inhibitor	2337195	G→A	S83L (TCG→TTG)	***gyrA*** ←	DNA gyrase (type II topoisomerase), subunit A	DNA/RNA synthesis	strong	common	Yes
ET73	**NAL-M1**	DNA Gyrase Inhibitor	2337195	G→A	S83L (TCG→TTG)	***gyrA*** ←	DNA gyrase (type II topoisomerase), subunit A	DNA/RNA synthesis	mild	common	Yes
ET74	**NAL-M2**	DNA Gyrase Inhibitor	2337194	C→T	D87N (GAC→AAC)	***gyrA*** ←	DNA gyrase (type II topoisomerase), subunit A	DNA/RNA synthesis	mild	common	Yes
ET75	**TMP-S1**	Folic Acid Inhibitor	49084	C→T	P31L (CCG→CTG)	***folA*** →	dihydrofolate reductase	Folic acid synthesis	strong	common	Yes
ET75	**TMP-S1**	Folic Acid Inhibitor	49089	C→T	A29V (GCC→GTC)	***folA*** →	dihydrofolate reductase	Folic acid synthesis	strong	common	Yes
ET75	**TMP-S1**	Folic Acid Inhibitor	1185837	Δ2,556 bp		***[secY]-[folA]***	*[secY], secD, [folA]*	Other	strong	exclusive	No
ET76	**TMP-S2**	Folic Acid Inhibitor	49765	C→T	intergenic (+134/-58)	***kefC*** → / → ***fkl A***	potassium/proton antiporter/dihydrofolate reductase	Folic acid synthesis	strong	common	Yes
ET76	**TMP-S2**	Folic Acid Inhibitor	49903	T→A	D27E (GAT→GAA)	***folA*** →	dihydrofolate reductase	Folic acid synthesis	strong	common	Yes
ET76	**TMP-S2**	Folic Acid Inhibitor	49910	T→G	W30G (TGG→GGG)	***folA*** →	dihydrofolate reductase	Folic acid synthesis	strong	common	Yes
ET77	**TMP-M1**	Folic Acid Inhibitor	49765	C→T	intergenic (+134/-58)	***kefC*** → / → ***fkl A***	potassium/proton antiporter/dihydrofolate reductase	Folic acid synthesis	mild	common	Yes
ET77	**TMP-M1**	Folic Acid Inhibitor	49903	T→A	D27E (GAT→GAA)	***folA*** →	dihydrofolate reductase	Folic acid synthesis	mild	common	Yes
ET77	**TMP-M1**	Folic Acid Inhibitor	49910	T→G	W30G (TGG→GGG)	***folA*** →	dihydrofolate reductase	Folic acid synthesis	mild	common	Yes

ET78	**TMP-M2**	Folic Acid Inhibitor	49765	C→T	intergenic (+134/-58)	***hepC*** **→ / →** ***folA***	potassium:proton antiporter/dihydrofolate reductase	Folic acid synthesis	mild	common	Yes
ET78	**TMP-M2**	Folic Acid Inhibitor	49910	T→C	W30R (TGG→CGG)	***folA*** **→**	dihydrofolate reductase	Folic acid synthesis	mild	common	Yes
ET78	**TMP-M2**	Folic Acid Inhibitor	50273	T→G	Y151D (TAT→GAT)	***folA*** **→**	dihydrofolate reductase	Folic acid synthesis	mild	common	Yes
ET79	**FUS-S1**	50S inhibitor	547882	+G	coding (476/1259 nt)	***yhdL*** **→**	Unknown	Unknown	strong	exclusive	No
ET79	**FUS-S1**	50S inhibitor	3470224	A→T	L439Q (CTG→CAG)	***fusA*** **←**	protein chain elongation factor EF-G, GTP-binding	Protein synthesis	strong	common	Yes
ET80	**FUS-S2**	50S inhibitor	3470224	A→T	L439Q (CTG→CAG)	***fusA*** **←**	protein chain elongation factor EF-G, GTP-binding	Protein synthesis	strong	common	Yes
ET81	**FUS-M1**	50S inhibitor	3470224	A→T	L439Q (CTG→CAG)	***fusA*** **←**	protein chain elongation factor EF-G, GTP-binding	Protein synthesis	mild	common	Yes
ET82	**FUS-M2**	50S inhibitor	1656421	A→G	D110G (GAT→GGT)	***ynfE*** **→**	putative selenate reductase, periplasmic	Membrane proteins	mild	exclusive	No
ET82	**FUS-M2**	50S inhibitor	3471160	A→C	V126G (GTA→GGA)	***fusA*** **←**	protein chain elongation factor EF-G, GTP-binding	Protein synthesis	mild	common	Yes
ET83	**AMK-S1**	Aminoglycosides	392191	Δ5,466 bp	IS-mediated	***yaiT-[yaiW']***	*yaiT, yaiV, aegA, ybaA, [yaiW']*	Other	strong	common	No
ET83	**AMK-S1**	Aminoglycosides	770729	Δ1 bp	coding (49/1569 nt)	***cydA*** **→**	cytochrome d terminal oxidase, subunit I	Membrane proteins	strong	exclusive	Yes
ET83	**AMK-S1**	Aminoglycosides	1023333	C→T	R68C (CGT→TGT)	***holE*** **→**	DNA polymerase III, theta subunit	DNA/RNA synthesis	strong	exclusive	No
ET83	**AMK-S1**	Aminoglycosides	3469504	G→A	A679V (GCA→GTA)	***fusA*** **←**	protein chain elongation factor EF-G, GTP-binding	Protein synthesis	strong	common	Yes
ET83	**AMK-S1**	Aminoglycosides	4031625	T→C	L153P (CTC→CCC)	***mkH*** **→**	potassium transporter	Membrane proteins	strong	exclusive	No
ET84	**AMK-S2**	Aminoglycosides	3470425	C→A	R371L (CGT→CTT)	***fusA*** **←**	protein chain elongation factor EF-G, GTP-binding	Protein synthesis	strong	common	Yes

ET84	**AMK-S2**	Aminoglycosides	3916025	C→A	Q353H (CAG→CAT)	***atpA*** ←	F1 sector of membrane-bound ATP synthase, alpha subunit	Membrane proteins	strong	exclusive	No
ET85	**AMK-M1**	Aminoglycosides	392191	Δ5,406 bp	IS3-mediated	***yaiT*-*[yaiW]***	*yaiT, yaiV, ampH, sbmA, [yaiW]*	Other	mild	common	No
ET85	**AMK-M1**	Aminoglycosides	4186006	A→C	K1172Q (AAA→CAA)	***rpoC*** →	RNA polymerase, beta prime subunit	DNA/RNA synthesis	mild	exclusive	No
ET86	**AMK-M2**	Aminoglycosides	547032	+G	coding (476/1259 nt)	***ybhK*** →	Unknown	Unknown	mild	exclusive	No
ET86	**AMK-M2**	Aminoglycosides	3469576	A→T	I654N (ATC→AAC)	***fusA*** ←	protein chain elongation factor EF-G, GTP-binding	Protein synthesis	mild	common	Yes
ET86	**AMK-M2**	Aminoglycosides	3813847	G→T	intergenic (-56/+39)	***pyrE*** ← / ← ***rph***	orotate phosphoribosyltransferase/defective ribonuclease PH- adaptation to minimal media	adaptation to minimal media	mild	exclusive	No
ET87	**AMP-S1**	ell Wall Synth. Inhibit.	92512	T→A	L367Q (CTG→CAG)	***ftsI*** →	transpeptidase involved in septal peptidoglycan synthesis (penicillin-binding protein 3)	Cell wall synthesis	strong	exclusive	Yes
ET87	**AMP-S1**	ell Wall Synth. Inhibit.	482636	G→T	P331Q (CCG→CAG)	***acrB*** ←	multidrug efflux systemprotein	Multi Drug Resistance	strong	common	No
ET87	**AMP-S1**	ell Wall Synth. Inhibit.	3533169	A→C	V241G (GTA→GGA)	***envZ*** ←	sensory histidine kinase in two-component regulatory system with OmpR	Membrane proteins	strong	common	No
ET88	**AMP-S2**	ell Wall Synth. Inhibit.	93043	C→G	A544G (GCC→GGC)	***ftsI*** →	transpeptidase involved in septal peptidoglycan synthesis (penicillin-binding protein 3)	Cell wall synthesis	strong	exclusive	Yes
ET88	**AMP-S2**	ell Wall Synth. Inhibit.	481922	T→A	Q569L (CAG→CTG)	***acrB*** ←	multidrug efflux systemprotein	Multi Drug Resistance	strong	common	No
ET88	**AMP-S2**	ell Wall Synth. Inhibit.	3533169	A→C	V241G (GTA→GGA)	***envZ*** ←	sensory histidine kinase in two-component regulatory system with OmpR	Membrane proteins	strong	common	No
ET89	**AMP-M1**	ell Wall Synth. Inhibit.	482636	G→T	P331Q (CCG→CAG)	***acrB*** ←	multidrug efflux systemprotein	Multi Drug Resistance	mild	common	No
ET89	**AMP-M1**	ell Wall Synth. Inhibit.	3533169	A→C	V241G (GTA→GGA)	***envZ*** ←	sensory histidine kinase in two-component regulatory system with OmpR	Membrane proteins	mild	common	No
ET90	**AMP-M2**	ell Wall Synth. Inhibit.	3533169	A→C	V241G (GTA→GGA)	***envZ*** ←	sensory histidine kinase in two-component regulatory system with OmpR	Membrane proteins	mild	common	No

ET91	**SPT-S1**	30S inhibitor	3075037	T→G	M245L (ATG→CTG)	***rpsB*** **←**	putative dehydrogenase	Unknown	strong	exclusive	No
ET91	**SPT-S1**	30S inhibitor	3443179	C→A	V25F (GTT→TTT)	***rpsE*** **←**	30S ribosomal subunit protein S5	Protein synthesis	strong	common	Yes
ET91	**SPT-S1**	30S inhibitor	3448974	C→T	G130D (GGT→GAT)	***rplB*** **←**	50S ribosomal subunit protein L2	Protein synthesis	strong	exclusive	Yes
ET91	**SPT-S1**	30S inhibitor	3613880	+C	intergenic (+99/-6)	***pyrE*** **←/←*rph***	orotate phosphoribosyltransferase/defective ribonuclease PH- adaptation to minimal media	adaptation to minimal media	strong	common	No
ET91	**SPT-S1**	30S inhibitor	3613953	Δ82 bp		***rph***	defective ribonuclease PH- adaptation to minimal media	adaptation to minimal media	strong	common	No
ET92	**SPT-S2**	30S inhibitor	3443175	T→A	K26I (AAA→ATA)	***rpsE*** **←**	30S ribosomal subunit protein S5	Protein synthesis	strong	common	Yes
ET92	**SPT-S2**	30S inhibitor	3613953	Δ82 bp		***rph***	defective ribonuclease PH- adaptation to minimal media	adaptation to minimal media	strong	common	No
ET93	**SPT-M1**	30S inhibitor	547032	+G	intergenic (+262/-18)	***fdnH-/←yhiF***	unknown	unknown	mild	exclusive	No
ET93	**SPT-M1**	30S inhibitor	3443179	C→A	V25F (GTT→TTT)	***rpsE*** **←**	30S ribosomal subunit protein S5	Protein synthesis	mild	common	Yes
ET93	**SPT-M1**	30S inhibitor	3613947	G→T	intergenic (-56/+30)	***pyrE*** **←/←*rph***	orotate phosphoribosyltransferase/defective ribonuclease PH- adaptation to minimal media	adaptation to minimal media	mild	exclusive	No
ET94	**SPT-M2**	30S inhibitor	3443182	Δ3 bp	coding (68-70/504 nt)	***rpsE*** **←**	30S ribosomal subunit protein S5	Protein synthesis	mild	common	Yes
ET94	**SPT-M2**	30S inhibitor	3613953	Δ82 bp		***rph***	defective ribonuclease PH- adaptation to minimal media	adaptation to minimal media	mild	common	No
ET95	**STR-S1**	Aminoglycosides	310757	G→T	D83Y (GAT→TAT)	***hemB*** **←**	5-aminolevulinate dehydratase (porphobilinogen synthase)	Other	strong	exclusive	No
ET95	**STR-S1**	Aminoglycosides	3315877	Δ3 bp	coding (140-152/493 nt)	***rimP*** **←**	ribosome maturation factor for 30S subunit	Protein synthesis	strong	exclusive	Yes
ET95	**STR-S1**	Aminoglycosides	3472447	T→G	K43T (AAA→ACA)	***rpsL*** **←**	30S ribosomal subunit protein S12	Protein synthesis	strong	common	Yes
ET96	**STR-S2**	Aminoglycosides	710133	G→A	nsecoding (69/756 nt)	***lysW*** **→**	tRNA-Lys	Protein synthesis	strong	exclusive	Yes
ET96	**STR-S2**	Aminoglycosides	3472319	G→T	K88I (GGT→ATT)	***rpsL*** **←**	30S ribosomal subunit protein S12	Protein synthesis	strong	common	Yes

ET96	**STR-S2**	Aminoglycosides	3472510	G→A	P22L (CCT→CTT)	***rpsL*** ←	30S ribosomal subunit protein S12	Protein synthesis	strong	common	Yes
ET96	**STR-S2**	Aminoglycosides	3921670	I21 (+) +6 bp	coding (27-34624 nt)	***rsmG*** ←	16S rRNA m(7)G527 methyltransferase, SAM-dependent, glucose-inhibited cell-division protein	Protein synthesis	strong	exclusive	Yes
ET96	**STR-S2**	Aminoglycosides	4031406	T→A	L80Q (CTG→CAG)	***mkH*** →	potassium transporter	Membrane proteins	strong	exclusive	No
ET97	**STR-M1**	Aminoglycosides	450635	C→T	W67* (TGG→TAG)	***cyoA*** ←	cytochrome o ubiquinol oxidase subunit II	Membrane proteins	mild	exclusive	No
ET97	**STR-M1**	Aminoglycosides	3472312	T→C	K88R (AAA→AGA)	***rpsL*** ←	30S ribosomal subunit protein S12	Protein synthesis	mild	common	Yes
ET97	**STR-M1**	Aminoglycosides	3813880	+C	intergenic(+89/-6)	***pyrE*** ←/→ ***rph***	orotate phosphoribosyltransferase/defective ribonuclease PH- adaptation to minimal media	adaptation to minimal media	mild	common	no
ET97	**STR-M1**	Aminoglycosides	3813953	Δ62 bp		***rph***	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No
ET98	**STR-M2**	Aminoglycosides	645970	T→G	T255P (ACC→CCC)	***coaD*** ←	2-(3''-triphosphoribosyl)-3'-dephosphocoenzyme-A synthase	Other	mild	common	No
ET98	**STR-M2**	Aminoglycosides	3472446	T→G	K43R (AAA→AAC)	***rpsL*** ←	30S ribosomal subunit protein S12	Protein synthesis	mild	common	Yes
ET98	**STR-M2**	Aminoglycosides	3813953	Δ62 bp		***rph***	defective ribonuclease PH - adaptation to minimal media	adaptation to minimal media	mild	common	No

7.2 Appendix B

Daily MIC values of 88 strains.

ug/ml	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
CHL-S-1	2	4	8	8	8	16	32	32	32	32	64	32	64	64	64	64	64	128	128	128	128
CHL-S-2	2	4	8	8	8	16	32	32	32	32	64	32	64	64	128	128	128	128	128	256	256
CHL-M-1	2	4	8	8	8	8	8	8	16	16	16	16	16	32	32	32	32	32	32	32	32
CHL-M-2	2	4	8	8	8	8	8	8	16	16	16	16	16	32	32	32	32	32	32	64	32
TET-S-1	0.3	0.6	0.6	0.6	1.2	1.2	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	4.8	4.8	4.8	4.8	4.8	4.8	4.8
TET-S-2	0.3	0.6	0.6	0.6	1.2	1.2	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	4.8	4.8	4.8	4.8	4.8	4.8
TET-M-1	0.3	0.6	0.6	0.6	1.2	1.2	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
TET-M-2	0.3	0.6	0.6	0.6	1.2	1.2	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
NIT-S-1	2.5	10	10	10	10	10	20	20	40	40	40	40	80	80	80	80	160	160	160	320	320
NIT-S-2	2.5	10	10	10	10	10	10	10	10	10	20	20	40	40	80	80	80	160	160	160	320
NIT-M-1	2.5	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
NIT-M-2	2.5	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
SUL-S-1	0.62	2.5	10	10	10	10	20	20	20	40	40	40	80	80	160	160	320	320	640	320	320
SUL-S-2	0.62	2.5	10	10	10	10	20	20	20	40	40	40	80	80	160	160	320	320	640	320	320
SUL-M-1	0.62	2.5	5	10	10	10	20	40	40	40	40	80	80	80	40	80	160	320	320	320	320
SUL-M-2	0.62	2.5	5	10	10	10	20	40	40	40	40	80	80	160	320	320	320	320	640	320	320
KAN-S-1	1.25	2.5	5	20	40	80	80	80	80	160	320	320	320	160	320	320	320	640	640	640	1280
KAN-S-2	1.25	2.5	5	20	40	80	80	80	160	160	160	160	320	160	320	320	320	640	640	1280	1280
KAN-M-1	1.25	2.5	5	5	10	20	40	20	40	20	80	80	80	5	20	20	40	40	20	40	40
KAN-M-2	1.25	2.5	5	5	10	20	20	80	40	40	80	80	40	5	20	20	40	40	20	40	40
DOX-S-1	0.5	2	2	2	4	4	8	8	8	8	8	8	16	16	8	8	8	8	8	8	8
DOX-S-2	0.5	2	2	2	4	4	8	8	8	8	8	8	16	16	8	8	8	8	16	8	16
DOX-M-1	0.5	2	2	2	2	2	4	4	4	4	4	4	8	8	8	8	8	8	8	8	8
DOX-M-2	0.5	2	2	2	2	2	4	4	4	4	4	4	4	4	4	4	4	4	4	8	8
CIP-S-1	0.005	0.01	0.02	0.04	0.02	0.02	0.04	0.08	0.16	0.32	0.64	0.32	0.64	0.64	0.64	1.28	1.28	1.28	1.28	2.56	1.28
CIP-S-2	0.005	0.01	0.02	0.04	0.02	0.02	0.04	0.08	0.16	0.32	0.64	0.32	0.64	0.64	0.64	1.28	1.28	1.28	1.28	2.56	1.28
CIP-M-1	0.005	0.02	0.02	0.04	0.04	0.04	0.04	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.16	0.16	0.16	0.16	0.16	0.16	0.16
CIP-M-2	0.005	0.02	0.02	0.04	0.04	0.04	0.04	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.16	0.16	0.16	0.16	0.16	0.16	0.16
LOM-S-1	0.05	0.2	0.2	0.2	0.4	0.8	0.8	1.6	1.6	1.6	3.2	3.2	6.4	6.4	6.4	12.8	6.4	6.4	12.8	6.4	6.4
LOM-S-2	0.05	0.2	0.2	0.2	0.4	0.4	0.8	0.8	0.8	1.6	1.6	3.2	3.2	3.2	6.4	1.6	6.4	1.6	3.2	6.4	6.4
LOM-M-1	0.05	0.2	0.2	0.4	0.2	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	1.6	1.6	1.6	3.2	3.2	3.2	3.2	3.2
LOM-M-2	0.05	0.2	0.2	0.4	0.2	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	1.6	1.6	1.6	3.2	3.2	3.2	3.2	3.2
CLI-S-1	5	20	80	160	320	320	640	640	640	640	640	640	1280	1280	1280	1280	1280	1280	1280	1280	1280
CLI-S-2	5	20	80	160	320	320	640	640	640	640	640	640	1280	1280	1280	1280	1280	1280	1280	1280	1280
CLI-M-1	5	20	80	160	160	320	640	640	640	640	640	640	640	640	640	640	640	640	640	640	640
CLI-M-2	5	20	80	160	160	320	640	640	640	640	640	640	640	640	640	640	640	640	640	640	640
SMO-S-1	0.2	1.6	3.2	6.4	6.4	12.8	12.8	12.8	25.6	25.6	51.2	51.2	102.4	25.6	25.6	102.4	51.2	102.4	204.8	204.8	102.4
SMO-S-2	0.2	1.6	3.2	6.4	6.4	12.8	12.8	12.8	25.6	25.6	51.2	51.2	102.4	25.6	51.2	102.4	51.2	51.2	102.4	102.4	102.4
SMO-M-1	0.2	0.8	3.2	6.4	6.4	12.8	25.6	25.6	25.6	25.6	51.2	51.2	102.4	102.4	102.4	102.4	102.4	204.8	204.8	204.8	204.8
SMO-M-2	0.2	0.8	3.2	6.4	6.4	12.8	25.6	25.6	25.6	25.6	51.2	51.2	102.4	102.4	102.4	102.4	102.4	204.8	409.6	409.6	102.4
SPR-S-1	10	80	160	160	160	320	320	320	320	320	640	1280	1280	1280	2560	2560	2560	2560	2560	2560	2560
SPR-S-2	10	80	160	160	160	320	320	320	640	640	640	1280	1280	1280	2560	2560	2560	2560	2560	2560	2560
SPR-M-1	10	80	160	320	320	320	320	320	640	640	640	640	640	640	1280	1280	1280	1280	1280	1280	1280
SPR-M-2	10	80	160	320	320	320	320	320	640	640	640	640	640	640	1280	1280	1280	1280	1280	1280	1280
ERY-S-1	10	40	80	80	80	160	160	320	160	160	160	320	320	320	640	640	1280	1280	1280	1280	1280
ERY-S-2	10	40	80	80	80	80	160	160	320	640	640	320	320	320	320	640	320	640	640	640	1280
ERY-M-1	10	40	80	160	160	160	160	160	160	160	160	160	160	160	320	320	320	320	320	320	160
ERY-M-2	10	40	80	160	160	160	160	160	160	160	160	160	160	160	320	320	320	320	320	320	160
NAL-S-1	2.5	2.5	10	80	80	80	80	200	200	150	75	75	150	150	300	150	150	150	300	300	300
NAL-S-2	2.5	5	10	80	80	80	80	200	200	75	75	75	150	150	300	150	150	300	300	300	300
NAL-M-1	2.5	5	10	20	20	20	80	100	200	75	75	75	150	150	300	150	150	150	150	300	300
NAL-M-2	2.5	5	10	10	20	20	80	200	200	37	75	75	150	150	300	150	150	300	300	300	300
STR-S-1	2.5	5	5	20	40	40	80	40	40	40	160	1280	10240	10240	40960	163840	163840	163840	163840	163840	163840
STR-S-2	2.5	5	5	20	40	40	40	40	80	640	320	640	2560	5120	40960	81920	81920	163840	163840	163840	163840
STR-M-1	2.5	5	5	10	5	5	10	10	20	20	20	20	10	20	40	80	160	320	1280	40960	163840
STR-M-2	2.5	5	5	10	5	5	5	5	40	80	160	320	640	1280	2560	5120	5120	20480	40960	163840	163840
TMP-S-1	0.6	4.8	1.2	1.2	9.6	19.2	19.2	38.4	38.4	38.4	76.8	153	153	153	153	76	153	153	153	307	153
TMP-S-2	0.6	4.8	4.8	1.2	1.2	9.6	38.4	38.4	38.4	153.6	307	153.6	307	614	307	153	307	153	614	1228	614
TMP-M-1	0.6	1.6	2.4	4.8	9.6	19.2	9.6	9.6	9.6	9.6	38.4	38.4	38.4	38.4	38.4	9.6	38.4	76.8	153	307	307
TMP-M-2	0.6	1.6	2.4	4.8	9.6	19.2	4.8	4.8	19.2	38.4	38.4	38.4	38.4	76.2	76.2	153	307	614	614	153	307
AMP-S-1	2.5	5	5	10	20	10	10	10	20	20	40	40	40	40	20	20	40	40	40	40	40
AMP-S-2	2.5	5	5	10	20	10	10	10	20	40	40	40	40	40	40	40	40	40	40	40	40
AMP-M-1	2.5	2.5	2.5	5	5	10	20	10	10	20	20	10	10	10	10	10	10	10	10	10	10
AMP-M-2	2.5	2.5	2.5	5	5	10	20	10	10	20	20	10	10	10	10	10	10	10	10	10	10
AMK-S-1	2.5	5	20	40	40	40	80	80	80	80	40	80	40	80	160	160	80	80	160	160	640
AMK-S-2	2.5	5	20	40	40	40	80	80	160	80	80	80	160	160	320	320	320	320	320	320	320
AMK-M-1	2.5	5	2.5	10	10	5	10	40	40	40	40	40	80	40	40	80	80	80	80	160	80
AMK-M-2	2.5	5	2.5	10	10	5	10	40	40	40	40	40	80	40	40	80	80	80	80	160	40
CEF-S-1	1	2	4	16	16	16	16	32	128	128	64	32	128	256	256	1024	1024	2048	2048	2048	2048
CEF-S-2	1	2	4	16	16	16	16	32	64	32	64	32	128	128	128	128	128	128	128	512	1024
CEF-M-1	1	2	1	2	8	8	8	16	8	32	64	64	32	32	32	64	64	64	64	32	32
CEF-M-2	1	2	1	2	8	8	8	16	8	32	64	64	32	32	32	64	64	64	64	32	32
PIP-S-1	1	4	2	2	4	4	8	2	4	4	4	4	8	4	8	8	16	16	16	16	16
PIP-S-2	1	4	2	4	8	4	8	8	32	128	64	64	128	512	128	512	64	128	128	128	128
PIP-M-1	1	2	1	4	8	2	8	8	4	8	4	0.5	8	16	4	16	16	8	8	16	4
PIP-M-2	1	2	1	4	8	2	8	8	4	8	4	4	8	4	4	16	4	8	8	16	4
TOB-S-1	0.5	1	4	2	4	8	8	16	16	32	64	64	128	128	128	512	256	512	512	512	512
TOB-S-2	0.5	1	4	4	8	8	8	16	16	32	64	64	64	64	64	128	128	128	256	512	256
TOB-M-1	0.5	1	1	1	1	0.25	1	0.25	0.5	0.25	0.25	0.5	0.5	0.5	1	2	4	4	2	0.5	2
TOB-M-2	0.5	1	1	1	1	0.25	1	0.25	0.5	0.25	0.25	0.5	0.5	0.5	1	1	2	2	2	0.5	1
SPT-S-1	10	80	40	80	160	320	320	2560	5120	20480	20480	40960	40960	40960	40960	40960	40960	40960	40960	40960	40960
SPT-S-2	10	80	40	80	80	160	320	2560	5120	20480	20480	40960	40960	40960	40960	40960	40960	40960	40960	40960	40960
SPT-M-1	10	40	40	80	80	80	80	80	80	80	80	80	80	80	80	80	160	160	160	80	160
SPT-M-2	10	40	40	80	80	80	80	80	80	80	80	80	80	80	80	80	160	160	160	80	160
FUS-S-1	100	800	6400	6400	3200	3200	3200	1600	1600	3200	3200	1600	3200	3200	3200	1600	3200	3200	3200	3200	3200
FUS-S-2	100	800	6400	6400	3200	3200	3200	1600	3200	1600	3200	1600	3200	1600	1600	3200	3200	3200	3200	3200	3200
FUS-M-1	100	400	800	800	3200	3200	3200	1600	1600	1600	3200	1600	1600	3200	3200	3200	3200	3200	3200	3200	3200
FUS-M-2	100	400	800	800	3200	3200	3200	1600	1600	1600	3200	1600	1600	1600	3200	3200	3200	3200	3200	3200	3200

7.3 Appendix C

MIC values of all evolved strains in 22 antibiotics.

ug/ml	DXL-S-1	DXL-S-2	DXL-M-1	DXL-M-2	CLI-S-1	CLI-S-2	CLI-M-1	CLI-M-2	ERY-S-1	ERY-S-2	ERY-M-1	ERY-M-2	SPR-S-1	SPR-S-2	SPR-M-1	SPR-M-2	FUS-S-1	FUS-S-2	FUS-M-1	FUS-M-2	AMK-S-1	AMK-S-2	AMK-M-1	AMK-M-2	TOB-S-1
Chloramphenicol	224.558	182.477	65.345	28.358	2.057	5.837	6.220	6.067	5.968	5.938	5.955	2.399	20.154	6.375	17.718	20.985	6.016	6.049	6.308	5.828	4.468	4.670	6.290	5.445	52.472
Clindamycin	348.528	328.821	941.862	844.455	1878.254	1878.975	354.689	358.155	96.910	98.805	322.002	185.168	359.303	369.054	958.789	892.439	103.214	365.961	100.471	108.843	358.954	95.955	110.978	313.292	98.005
Erythromycin	200.262	340.717	192.048	76.482	77.998	68.014	68.700	65.886	72.654	2000.000	588.739	220.545	1011.804	1953.140	282.981	610.694	74.676	84.452	66.431	63.391	70.365	19.015	73.024	90.349	21.166
Spiramycin	676.865	612.179	585.621	267.724	574.687	214.932	553.647	264.243	262.233	761.497	1827.990	761.026	5274.044	4388.610	1974.815	1890.514	238.583	220.676	281.210	187.337	69.474	183.790	216.724	63.000	132.632
Fusidic Acid	1784.812	2151.438	1728.396	1850.708	704.592	711.729	767.059	837.845	881.415	599.362	341.578	462.190	750.171	607.808	1048.770	1499.485	6000.000	6100.000	6100.000	6100.000	377.526	646.607	725.871	341.604	333.781
Amikacin	4.701	13.939	15.040	14.351	15.073	14.471	14.855	15.115	15.013	14.907	6.143	14.881	15.107	14.660	14.933	15.101	14.757	15.292	15.217	17.673	476.923	1451.759	150.449	152.068	1560.000
Tobramycin	0.938	0.914	2.969	3.062	1.092	0.964	2.916	2.980	1.002	1.093	1.018	0.972	1.120	2.695	2.963	2.885	2.957	2.969	0.395	9.515	98.827	306.295	9.956	32.104	905.962
Streptomycin	4.727	14.407	15.143	15.407	14.882	14.850	15.255	15.228	14.937	15.208	15.194	15.131	14.884	14.896	42.188	15.180	15.346	16.203	14.831	15.002	147.648	439.313	149.943	144.117	1586.145
Kanamycin	3.771	12.959	12.335	16.978	11.571	11.311	11.525	15.527	11.515	11.717	11.752	11.521	11.879	11.541	35.379	11.872	12.279	36.283	91.577	44.249	361.181	1129.486	138.985	163.471	3693.578
Tetracycline	0.570	3.865	3.594	2.579	1.235	1.242	1.264	1.240	1.293	1.238	0.398	2.750	1.231	2.626	1.121	2.531	1.723	1.778	1.760	1.823	0.554	0.368	1.355	0.425	1.187
Doxycycline	9.581	6.968	3.877	3.515	1.157	1.173	1.559	1.767	1.648	2.166	0.777	3.070	2.463	2.417	1.522	2.354	1.864	1.713	1.493	1.782	0.642	0.570	0.774	0.551	0.762
Spectinomycin	65.804	54.530	88.855	76.049	72.678	72.029	164.572	162.096	59.831	58.541	63.495	58.614	61.443	65.375	70.166	81.192	62.672	197.218	64.342	56.815	61.732	62.534	59.982	58.478	47.507
Piperacillin	5.670	5.430	5.682	3.260	2.719	2.700	2.670	2.685	1.917	2.074	1.300	2.174	2.023	1.915	1.390	1.874	2.698	2.708	2.757	1.571	1.390	1.390	1.390	1.390	2.690
Ampicillin	14.351	13.948	27.055	6.870	9.813	8.801	6.798	6.760	4.658	9.268	2.355	16.489	9.558	4.725	2.363	9.519	13.289	15.200	4.873	6.925	1.667	2.690	5.235	3.245	9.367
Cefoxitin	17.482	7.353	7.525	1.897	1.844	1.876	1.903	1.928	1.922	1.918	2.181	1.838	5.658	16.195	1.857	7.709	1.895	1.898	1.903	50.842	177.503	175.410	4.853	1.832	5.931
Nalidixic acid	71.486	69.667	7.798	7.645	25.140	7.637	7.676	7.738	7.732	8.033	7.338	8.289	237.213	205.594	23.215	7.719	7.635	7.682	8.262	7.774	7.200	7.428	7.685	7.706	7.377
Lomefloxacin	0.767	0.414	0.313	0.104	0.948	0.105	0.278	0.106	0.302	0.293	0.100	1.140	0.308	0.398	0.940	0.308	0.304	0.295	0.298	0.307	0.099	0.316	0.104	0.097	0.292
Ciprofloxacin	0.817	0.818	0.947	0.816	0.982	0.814	0.815	0.815	0.815	0.814	0.805	0.805	0.805	0.815	0.815	0.815	0.815	0.815	0.815	0.815	0.805	0.814	0.805	0.805	0.815
Sulfamonomethoxine	1.771	1.813	1.749	1.355	0.912	0.941	1.365	1.410	1.347	1.658	0.911	1.161	1.780	1.720	1.551	1.783	1.409	1.444	1.408	1.638	0.912	0.485	1.165	0.457	0.309
Trimethoprim	15.848	10.855	14.690	14.040	12.473	8.280	10.826	15.403	15.428	18.545	4.255	8.440	16.932	13.745	12.992	16.091	4.861	6.860	6.366	5.767	8.504	4.377	5.980	1.340	1.193
Sulfamethoxazol	0.772	0.888	2.068	1.805	2.168	2.456	2.374	2.325	2.263	2.598	2.436	2.388	2.963	2.983	2.434	2.493	2.345	2.347	2.231	2.437	0.893	2.191	2.630	2.354	0.790
Nitrofurantoin	3.788	2.670	4.644	4.746	4.794	4.773	4.871	4.885	4.804	4.622	4.735	4.670	4.654	14.072	4.672	5.647	4.664	4.730	4.659	4.506	4.510	4.278	5.579	4.553	1.763

ug/ml	PIP(M(1	PIP(M(2	AMP(S(1	AMP(S(2	AMP(M(1	AMP(M(2	CEF(S(1	CEF(S(2	CEF(M(1	CEF(M(2	NAL(S(1	NAL(S(2	NAL(M(1	NAL(M(2	LOM(S(1	LOM(S(2	LOM(M(1	LOM(M(2	CIP(S(1	CIP(S(2	CIP(M(1	CIP(M(2	SMO(S(1	SMO(S(2	SMO(M(1
Chloramphenicol	6.667	20.307	23.501	27.882	20.226	6.967	58.800	56.626	20.442	18.488	6.137	5.974	5.850	6.112	57.366	178.632	6.066	6.145	9.128	21.882	19.691	6.110	6.104	5.968	5.964
Clindamycin	347.775	318.113	99.897	99.578	103.921	104.430	89.268	114.298	357.811	309.778	99.583	100.906	99.854	99.507	384.900	970.427	103.872	100.765	299.902	101.972	330.471	97.516	97.859	89.303	98.889
Erythromycin	174.461	183.882	62.445	61.311	61.136	65.290	58.663	62.661	60.504	59.760	71.138	65.413	73.530	71.898	226.625	590.691	76.320	81.164	72.473	183.120	67.036	66.724	76.777	91.983	73.044
Spiramycin	624.260	624.333	245.563	251.136	245.322	232.372	190.412	195.363	254.633	170.625	275.913	255.745	244.256	242.507	657.961	612.716	282.309	269.433	229.006	601.920	597.329	634.942	277.421	253.989	242.345
FusidicIAcid	1107.103	1057.580	609.960	586.701	468.086	459.864	264.316	273.835	516.756	505.422	527.236	545.454	915.795	866.241	1707.203	1237.359	1060.437	935.379	1554.821	1727.025	991.876	917.700	782.868	571.191	491.998
Amikacin	14.817	15.119	14.902	4.565	14.568	4.878	14.600	19.364	14.997	14.579	14.751	14.398	14.822	14.056	5.804	4.570	14.384	15.050	15.462	14.894	14.983	14.993	5.713	14.659	13.321
Tobramycin	2.842	2.713	2.974	0.961	2.906	2.711	2.909	3.109	2.995	2.967	0.999	1.036	1.236	1.205	0.962	0.961	2.824	2.771	2.784	1.072	2.936	2.968	0.975	1.177	1.145
Streptomycin	15.435	14.864	14.485	4.793	14.748	14.208	16.128	44.541	44.500	14.838	14.919	14.872	14.810	15.163	14.562	14.003	15.018	15.006	15.009	14.891	15.519	15.082	14.968	14.826	14.776
Kanamycin	11.599	11.517	11.733	3.761	11.740	35.403	12.665	35.789	36.503	31.986	11.578	11.615	11.334	13.144	11.349	10.227	11.747	11.559	11.517	11.581	12.738	11.575	11.363	11.678	11.600
Tetracycline	2.533	3.530	3.505	3.449	2.600	2.479	7.719	10.385	3.460	3.580	1.220	1.227	1.736	1.216	3.619	3.572	1.307	1.298	3.548	3.569	2.534	3.454	1.812	1.296	1.200
Doxycycline	2.339	2.292	0.822	0.804	0.787	0.773	4.225	6.988	1.177	1.606	1.456	1.642	1.598	1.651	7.326	4.792	2.027	2.084	3.446	3.667	1.785	2.007	2.598	1.197	1.168
Spectinomycin	73.249	69.687	63200.000	57.049	63200.000	87.885	58.405	70.825	1043.225	157.023	59.374	59.558	58.700	58.271	59.857	54.345	59.337	60.026	65.368	60.199	170.037	162.346	58.866	61.464	60.710
Piperacillin	3.929	5.525	11.988	5.970	3.954	5.531	2.930	7.677	5.489	5.453	2.079	1.841	2.210	2.364	9.180	11.884	2.761	2.707	5.320	5.526	4.071	5.406	2.860	1.761	1.390
Ampicillin	9.735	19.823	82.476	82.502	30.347	27.792	115.130	120.000	56.532	39.559	13.713	4.791	4.864	3.673	14.202	30.968	28.129	28.429	7.266	15.406	13.696	13.417	6.386	6.732	6.464
Cefoxitin	5.910	18.351	58.029	58.069	207.310	18.089	4256.058	1758.138	222.509	519.306	6.287	105.702	7.083	16.242	17.126	23.817	1.901	2.594	22.852	16.322	15.210	34.762	1.971	1.932	1.873
Nalidixicacid	7.647	7.689	7.770	7.821	8.033	7.700	214.130	221.810	7.624	7.810	790.000	790.000	790.000	790.000	790.000	250.067	790.000	790.000	790.000	790.000	790.000	234.348	31.873	73.636	74.698
Lomefloxacin	0.311	0.310	0.308	0.294	0.299	0.318	0.346	0.367	0.222	0.886	3.151	3.193	3.209	3.000	9.687	9.527	3.346	3.255	9.618	9.875	3.261	3.154	0.342	0.312	0.299
Ciprofloxacin	0.015	0.047	0.044	0.040	0.046	0.047	0.040	0.039	0.013	0.015	1.308	1.324	1.258	0.508	1.600	1.536	1.383	1.359	1.600	1.600	1.513	1.513	0.016	0.015	0.017
Sulfamonomethoxine	1.711	1.723	1.680	1.639	1.654	0.924	25.472	17.135	21.944	3.984	1.443	1.522	1.388	1.517	10.436	5.824	1.741	1.484	1.806	1.776	1.708	1.761	7.612	8.656	4.184
Trimethoprim	358.287	4.462	4.001	3.781	3.654	3.681	404.208	31.329	363.161	4.244	7.234	4.840	7.409	6.642	41.146	13.556	5.258	13.620	19.685	22.071	7.326	14.705	15.200	5.252	12.416
Sulfamethoxazol	2.470	2.407	1.890	1.856	1.662	1.560	20.541	2.976	9.519	3.030	2.362	2.279	2.175	2.247	2.678	2.607	2.374	2.366	2.367	2.397	2.461	2.994	2.967	2.706	2.667
Nitrofurantoin	5.083	12.491	4.642	4.548	4.574	4.492	4.859	13.462	13.836	15.297	4.673	4.664	4.644	4.671	12.093	12.829	4.699	4.932	17.000	13.519	14.405	14.790	5.275	14.200	12.718

ug/ml	TOB-S-2	TOB-M-1	TOB-M-2	STR-S-1	STR-S-2	STR-M-1	STR-M-2	KAN-S-1	KAN-S-2	KAN-M-1	KAN-M-2	TET-S-1	TET-S-2	TET-M-1	TET-M-2	DOX-S-1	DOX-S-2	DOX-M-1	DOX-M-2	SPT-S-1	SPT-S-2	SPT-M-1	SPT-M-2	PIP-S-1	PIP-S-2
Chloramphenicol	0.630	6.578	6.151	5.684	2.358	5.969	5.916	5.767	1.679	5.727	6.346	5.834	5.548	6.891	6.526	23.447	58.292	58.607	6.360	5.971	6.019	6.108	6.181	9.208	5.989
Clindamycin	36.861	116.548	358.226	98.142	88.751	798.252	366.609	96.210	30.694	876.632	286.855	17.442	31.608	342.494	367.800	174.755	492.306	317.180	98.578	959.547	882.860	372.078	316.995	98.207	98.872
Erythromycin	21.029	69.039	66.239	55.130	30.821	66.261	65.208	24.408	21.814	61.711	171.003	19.367	18.338	169.171	80.630	70.116	181.808	621.144	77.729	99.318	62.651	60.687	57.573	71.337	64.982
Spiramycin	58.380	245.485	582.985	228.537	212.685	222.073	594.504	150.315	63.000	249.548	689.054	566.002	63.000	634.516	620.661	324.510	638.899	1808.481	358.853	197.920	152.585	212.840	197.416	574.681	266.235
Fusidic Acid	239.146	800.064	731.093	768.950	84.008	498.237	462.756	575.362	167.323	1326.571	774.661	1219.816	455.995	1350.019	994.363	1635.261	1483.376	1651.021	773.604	338.803	157.843	501.071	357.728	508.852	498.237
Amikacin	1545.927	47.303	15.086	28.188	15.448	48.047	14.842	613.190	1481.112	49.778	48.838	5.643	13.724	14.476	14.618	5.276	13.602	14.984	14.424	15.420	14.760	14.986	14.810	14.173	14.482
Tobramycin	932.559	3.195	2.943	3.184	9.388	9.284	3.051	285.208	382.959	10.763	11.223	0.972	1.832	2.904	2.931	0.964	0.952	2.985	2.786	3.084	2.918	2.871	0.972	2.789	1.132
Streptomycin	481.291	47.239	15.345	158000.000	66863.847	18126.771	158000.000	141.376	467.283	47.503	46.787	14.745	14.887	15.527	15.057	15.848	14.541	16.024	15.336	15.576	14.966	15.081	15.265	13.469	14.817
Kanamycin	3781.216	37.362	13.720	48.682	40.226	37.847	98.185	1180.894	3611.278	119.991	126.555	10.913	11.804	11.488	11.747	11.471	11.024	36.507	11.964	36.627	33.017	15.748	10.976	32.135	12.413
Tetracycline	1.179	1.204	1.783	0.881	0.381	1.197	1.117	0.860	0.308	1.103	1.245	3.727	3.555	3.265	3.443	3.776	10.908	2.505	1.216	1.198	1.706	1.176	1.185	1.298	1.129
Doxycycline	0.801	1.427	1.447	0.769	0.288	1.264	0.812	0.683	0.288	0.834	1.213	0.794	0.715	2.324	2.384	10.253	13.523	3.734	2.352	0.820	1.287	0.844	0.819	2.551	1.580
Spectinomycin	22.544	63.425	167.240	961.076	61200.000	184.755	61.622	59.877	53.658	59.336	72.127	58.895	61.554	166.384	186.908	60.265	53.676	362.155	61.886	61200.000	61200.000	61200.000	61200.000	56.607	58.845
Piperacillin	2.682	1.326	2.179	1.380	1.208	1.916	1.753	1.390	1.390	1.597	2.726	2.648	2.742	4.053	5.298	5.217	5.843	4.122	1.329	1.803	1.898	2.739	2.725	15.482	5.329
Ampicillin	12.564	3.618	6.885	18.333	6.728	4.718	6.479	2.883	2.264	6.475	5.468	7.005	8.252	9.743	9.836	7.890	13.585	4.759	3.332	6.733	6.637	9.525	6.750	40.345	82.331
Cefoxitin	0.600	2.595	1.934	1.954	1.868	5.457	1.916	5.961	1.845	1.899	1.969	5.708	5.801	16.086	5.977	5.937	18.472	2.009	2.581	1.928	1.925	5.106	1.997	18.253	5.988
Nalidixic acid	0.790	7.547	7.660	7.741	7.497	7.626	7.709	7.615	0.798	7.567	7.756	6.468	6.406	7.726	7.881	86.177	83.658	7.838	38.235	7.378	7.969	7.627	7.717	8.576	7.981
Lomefloxacin	0.095	0.099	0.104	0.191	0.111	0.103	0.106	0.249	0.091	0.101	0.108	0.108	0.107	0.107	0.112	0.953	0.971	0.104	0.103	0.113	0.292	0.120	0.104	0.100	0.103
Ciprofloxacin	0.806	0.805	0.815	0.814	0.816	0.812	0.813	0.813	0.806	0.814	0.809	0.849	0.846	0.816	0.815	0.816	0.848	0.813	0.816	0.815	0.815	0.814	0.814	0.848	0.815
Sulfonamomethoxine	0.390	1.724	1.489	1.170	0.203	1.202	1.201	1.200	0.150	0.909	1.677	1.389	1.407	1.750	1.725	1.831	2.471	4.376	0.987	1.676	1.664	1.642	1.649	1.405	1.577
Trimethoprim	0.811	5.463	6.487	4.299	1.274	4.470	4.241	1.867	0.900	4.373	12.435	4.127	4.303	14.057	13.621	13.530	12.364	15.710	18.681	4.858	4.549	4.761	5.065	1.838	5.731
Sulfamethoxazol	0.790	2.387	2.258	2.495	0.798	2.201	2.633	2.838	0.532	2.555	2.521	2.760	2.639	2.441	2.564	1.687	2.762	2.547	2.828	2.377	2.269	2.690	2.380	2.209	2.248
Nitrofurantoin	1.580	4.642	4.887	3.060	3.388	4.734	4.684	4.299	1.588	4.676	4.841	4.672	4.743	5.341	5.941	1.580	4.003	4.700	4.389	4.676	4.722	4.708	4.642	4.575	4.633

ug/ml	SMO-M-2	TMP-S-1	TMP-S-2	TMP-M-1	TMP-M-2	SUL-S-1	SUL-S-2	SUL-M-1	SUL-M-2	NIT-S-1	NIT-S-2	NIT-M-1	NIT-M-2	WILD TYPE
Chloramphenicol	8.743	5.874	6.249	6.025	6.063	6.041	5.905	6.063	5.976	22.998	18.132	6.054	6.012	6.104
Clindamycin	95.705	89.139	101.352	105.769	106.660	99.323	96.696	97.987	96.586	292.381	95.332	347.428	363.380	97.577
Erythromycin	79.480	67.298	76.628	69.150	68.995	76.615	71.686	66.668	69.236	71.951	73.739	65.999	65.103	65.833
Spiramycin	347.048	221.378	300.593	249.526	249.593	272.020	265.958	256.061	270.768	200.985	299.346	567.022	549.837	259.084
Fusidic Acid	736.191	924.455	1017.620	756.651	510.760	1036.316	787.967	757.838	246.644	680.077	621.189	812.106	581.156	647.427
Amikacin	13.120	6.255	14.640	14.974	14.880	17.043	15.306	18.351	14.005	14.505	14.774	15.284	15.058	14.360
Tobramycin	1.045	0.999	1.019	1.029	1.006	3.139	4.122	1.252	4.532	1.008	1.119	2.915	2.888	1.144
Streptomycin	14.781	14.536	14.649	15.091	15.142	15.113	14.689	14.942	5.254	14.841	14.642	15.194	15.070	15.160
Kanamycin	11.428	11.394	11.530	11.601	15.489	63.425	14.392	12.721	12.215	11.552	11.599	11.616	32.905	11.696
Tetracycline	3.499	1.230	2.631	1.225	1.231	1.245	1.240	1.243	1.115	3.574	2.540	1.237	1.237	1.232
Doxycycline	2.892	0.776	2.379	1.769	1.710	1.760	1.553	1.518	0.772	2.530	2.066	1.348	1.618	1.710
Spectinomycin	57.391	57.346	57.402	59.357	59.336	60.743	59.668	60.104	58.350	64.759	58.828	168.075	171.067	61.209
Piperacillin	2.036	1.753	5.470	2.832	1.913	2.677	2.677	2.683	1.960	2.782	1.782	2.054	1.899	1.885
Ampicillin	10.273	3.512	7.245	4.938	4.615	3.833	4.913	5.061	9.641	10.232	7.276	9.489	5.382	4.409
Cefoxitin	53.592	1.850	5.391	1.896	71.358	1.922	1.903	1.925	2.180	57.204	19.067	2.894	1.905	1.916
Nalidixic acid	211.650	7.789	62.018	7.823	8.119	7.889	7.851	7.837	8.128	75.951	68.940	7.707	7.767	7.928
Lomefloxacin	0.340	0.393	0.323	0.305	0.317	0.302	0.300	0.299	0.317	0.333	0.306	0.103	0.493	0.303
Ciprofloxacin	0.051	0.016	0.016	0.015	0.016	0.015	0.015	0.015	0.015	0.049	0.047	0.015	0.015	0.015
Sulfamonomethoxine	24.282	1.821	7.353	1.831	1.148	6.774	9.854	7.194	1.651	10.991	16.301	1.425	1.661	1.397
Trimethoprim	14.578	345.655	1273.319	1174.949	1201.848	7.358	5.727	8.661	1.200	13.127	8.052	7.051	6.145	4.836
Sulfamethoxazol	25.338	2.214	2.553	2.495	2.248	26.710	26.228	26.205	107.699	10.282	9.028	2.308	2.352	2.451
Nitrofurantoin	14.125	4.584	4.742	4.703	4.607	4.734	4.731	4.648	3.156	158.344	139.563	4.821	4.824	4.755