Research Article

Escherichia Coli Adaptive Resistance to Clinical Antibiotics

Twinkle Patel¹ and Anastasia Levitin²*

¹*Genomics Department, University of California, Riverside, USA*
²*School of Applied Life Sciences, Keck Graduate Institute, USA*

Abstract

Escherichia coli are beneficial bacteria found in human intestinal tracts. However, certain *E. coli* strains are pathogenic and can cause serious infections, such as neonatal sepsis and meningitis. The goal of this work is to create an awareness of the phenomenon of bacterial adaptation to antibiotics. We demonstrate that the pre-exposure of *E. coli* to low levels of antimicrobial agents (inducers), including broad-spectrum agents such as chloramphenicol and piperacillin, results in a decreased sensitivity of *E. coli* to lethal doses of the same or another antibiotic (testers), including ceftriaxone and vancomycin. We suggest that the treatment options considered for a particular *E. coli* infection should be based upon a review of the patient's treatment history. Antibiotic treatments for prior infections might result in residual drug accumulation by the body acting as inducers and therefore, influence treatment effectiveness for a current infection. In this work, nearly six hundred combinations of two antibiotics were tested, and the combinations that resulted in an increase in the half maximal inhibitory concentration (IC_{50}) to the tester by an average of 1.5 folds or more were selected. The results of this work show that *E. coli* can transiently adapt to antibiotics and consequently become more resistant to lethal doses of treatment antibiotics.

INTRODUCTION

Antimicrobial resistance is a growing global concern. Overuse and misuse of antibiotics have resulted in an increasing number of resistant microorganisms that fail to respond to standard treatment measures. As naturally occurring antibiotics used for the treatment of infections become unavailable, more expensive and longer treatments must be used, which greatly increase health-care costs [1]. There are various types of antibiotic resistance, most broadly categorized as inherited and non-inherited. Inherited antibiotic resistance refers to a type of resistance that is passed genetically from one generation of bacteria to the next, or through horizontal gene transfer [2]. Conversely, non-inherited antibiotic resistance describes a form of transient resistance that occurs due to the organism's ability to adapt and phenotypically modify itself in order to develop resistance to certain stressors [3], such as heat [8], *etc.* where pre-exposure to antibiotics remains a major cause of future resistance [4]. This paper specifically focuses on a particular type of non-inherited antibiotic resistance, *i.e.* adaptive antibiotic resistance. Adaptive resistance is an ephemeral type of resistance that is characterized by a decreased bacterial sensitivity to inhibitory or lethal doses of one antibiotic (tester) due to a short pre-exposure to residual levels of the same or another antibiotic (inducer). Pre-exposure occurs when bacteria is introduced to a sub-inhibitory concentration of an antibiotic which does not hinder bacterial growth, but is capable of activating certain mechanisms resulting in increased resistance to same or different antimicrobial agents. Although, this phenomenon is transitory and the removal of an inducer restores normal sensitivity [Levitin, unpublished results], a longer pre-exposure to sub-inhibitory levels of antibiotics can result in the emergence of permanent mutations. Although the study of the mechanism of adaptive resistance lags behind, it has been shown that it involves down-regulation of bacterial growth after initial exposure to an antibiotic, described as a suppressed response of the antibiotic to the bacteria [4]. It has also been observed that the minimum inhibitory concentrations (MICs), which are defined by a visible change in bacterial growth in the presence of these antibiotics, also increase over time; this phenomenon is referred to as the "baseline creep" [5]. The study of the mechanism of adaptive resistance to gentamycin has been linked to an increase in mRNA and regulatory protein levels in *P. aeruginosa*, resulting in a change in the nitrite reductase denA involved in the control of the anaerobic respiratory pathway in response to the antibiotic [6]. Additionally, in some organisms, specifically methicillin-resistant *S. aureus* (MRSA), adaptive resistance has been associated with a thickening of the cell wall [7].

Adaptive resistance has been studied in a variety of gram-

positive and gram-negative organisms, including *P. aeruginosa*, *S. aureus*, and *E. coli* [6, 7, 12]. A number of studies discuss adaptive resistance between antibiotics belonging to the aminoglycosides group [4, 9]. It has also been shown that adaptive resistance lasts temporarily after an initial exposure to sub-inhibitors and increases greatly with constant exposure [10]. This knowledge is vital to patient care establishments when designing the most effective regimen to fight infections [3]. Recently, it has been shown that the dosage of antibiotics is also of eminent importance [11]. Currently, there are a number of combinations known to result in adaptation in *E. coli*. These include triclosan, a synthetic antimicrobial chemical, whose pre-incubation results in an adaptation to imipenem, tetracycline, trimethoprim, *etc.* [12]. Pre-exposure to kanamycin has also been shown to cause adaptation to ampicillin, streptomycin, tetracycline, *etc.* [13]. One of the proposed mechanisms of adaptive resistance is the divergent expression of the genes encoding efflux pumps, which are responsible for expelling antibiotics from the cell [Levitin, unpublished results, 14].

This paper focuses on the identification of combinations of antibiotics resulting in *E. coli* adaptive resistance. *E. coli* is a human pathogen that is responsible for a wide array of bacterial infections in humans, most commonly intestinal infections and urinary tract infections [15,16]. Both antibiotics that are prescribed to treat broad spectrum infections and antibiotics that are very specific to *E. coli* infections are tested in this work. Although other treatment methods exist, antibiotics still remain significant players in the regulation of *E. coli* infections [17]. While mild *E. coli* infections do not require any treatment, more serious infections such as neonatal pneumonia might entail dialysis, blood transfusions, and the replacement of bodily fluids [17,18]. In this work, we have tested an assortment of commonly used clinical antibiotics, including vancomycin, amoxicillin, and ciprofloxacin. We have found that many combinations of the tested antibiotics result in *E. coli* adaptation and an increase in resistance to the lethal doses of certain antibiotics. Therefore, we suggest that a review of a patient's antibiotic history be a part of the prescription practice to allow the best treatment outcome for patients with *E. coli* infections.

We postulate that residual amounts of antimicrobial agents accumulated by the body from previous treatment regiments might act as inducers and consequently result in bacterial adaptation and an increased resistance of *E. coli* to the same or different antibiotics, thus abolishing the effectiveness of the treatment. We believe that providing the healthcare practitioners with a database of antibiotic combinations that result in adaptive resistance, in conjunction with the knowledge of a patient's previous treatment history, will allow them to make educated choices when prescribing an efficient treatment for patients with *E. coli* infections.

MATERIALS AND METHODS

Bacteria and media

All experiments were done on an *E. coli* bacterial strain C600 (F- tonA21 thi-1 thr-1 leuB6 lacY1 glnV44 rfbC1 fhuA1 λ-, CGSC# 3004) and used lysogeny broth (LB) media. The *E. coli* was initially grown on a LB agar plate at 37°C. Subsequently,

the liquid bacterial culture was grown from a single colony by shaking at 200 RPM at 37°C for a period of 16 hours prior to the beginning of the experiment.

Antibiotics

A total of twenty-six antibiotics were used in this project, and were tested in combinations of two. The antibiotics were dissolved in either water or dimethyl sulfoxide (DMSO) and diluted in LB medium to initial concentrations ranging from 2.5 µg/ml to 200 mg/ml.

Initial Kill-Curve Experiments

This set of experiments was aimed at determining the optimal sub-inhibitory, inhibitory and lethal concentrations (no visible change in bacterial growth, visible adverse effects on growth, and no proliferation of *E. coli* observed at optical density of 600nm (OD_{600}) as compared to control cultures, respectively). A set of twelve different concentrations at a two-fold concentration gradient was used during testing. The overnight grown culture was diluted in fresh LB medium to 0.05 OD_{600} and and aliquoted in 100µl into a 96-well plate, followed by the addition of an array of tester antibiotic concentrations in volumes of 5µl. The same volumes of LB media were added to control wells. The plates were grown with constant shaking at 37°C and the absorbance was measured by a SpectraMax Plus Microplate Reader (Molecular Devices) every 1000 seconds for a period of 6 hours. The concentrations of antibiotics that did not result in a detrimental change in the visible growth of the bacterial culture were chosen as sub-inhibitory concentrations. The concentrations that reduced bacterial growth, as well as the concentrations that inhibited growth, were used as tester concentrations in subsequent experiments.

Combination Kill-Curve Experiments

The antibiotics were tested in combinations of two with one antibiotic added at a sub-inhibitory concentration (inducer) and another added as a gradient of inhibitory/lethal concentrations (tester) to study whether such combinations resulted in adaptive resistance. The overnight bacterial culture was diluted in fresh LB media to OD600 of 0.05 and grown at 37°C for one hour to acclimatize the cells to specific growth conditions. The culture was aliquoted in 100µl into a 96-well plate, followed by the addition of an inducer in a volume of 5µl. The same volumes of LB media with corresponding concentration of the solvent used to prepare the inducer solution (water or DMSO (< 0.1%)) was added to control wells. The plate was incubated for 1 hr at 37°C with constant shaking. Next, a gradient of five different inhibitory and lethal concentrations of the tester antibiotic were added in volumes of 5 µl in order to create a widely spread kill-curve with a control well containing the same amount of LB media with corresponding concentration of the solvent used to prepare the inducer solution. The effect of the sub-inhibitory level of the inducer on bacterial growth was compared to the growth rate of un-induced cells exposed to the same concentrations of the tester antibiotics.

RESULTS

Initial Kill-Curve Experiments

The antibiotics used in this paper are representative of broad-

Patel et al. (2014) (2014)
Email: Anastasia_Levitin@kgi.edu

○SciMedCentral

spectrum antibiotics commonly prescribed as part of empiric therapy (e.g. chloramphenicol, amoxicillin, piperacillin, *etc*.), as well as specific antibiotics routinely prescribed to treat *E. coli* infections (e.g. ceftriaxone, vancomycin, ciptofloxacin, *etc*.). Each of the antibiotics used in this work was tested as both an inducer and a tester. The *E. coli* strain C600 proved to be insensitive to the tested concentrations of three out of the twenty-six antibiotics used in this work. Therefore, these antibiotics could not be used as testers, but were assessed as inducers. Those antibiotics were novobiocin, bacitracin, and oleandomycin. As a result, twenty-six inducers and twenty-three testers and a total of 598 possible combinations were tested.

Combination Kill-Curve Experiments

An *E. coli* strain C600 was pre-incubated with a sub-inhibitory level of an inducer antibiotic and subsequently grown in the presence of lethal doses of tester antibiotics. IC50 values of tester antibiotics in control cultures with no pre-incubation with an inducer are indicated in the Solvents section. IC50 values of tester antibiotics in cultures pre-exposed to sub-inhibitory levels of inducer antibiotics are listed in the Inducers section. Antibiotics were diluted in either water (highlighted in gray), or in DMSO (indicated in white). The IC50 values shown are averages of 3 independent experiments.

Out of 598 total antibiotic combination kill-curve experiments performed, 30 antibiotic combinations displayed the phenomenon of adaptive resistance. The results of the combinations are shown in (Table 1), with inducers listed horizontally and testers listed vertically with the half maximal inhibitory concentration (IC_{50}) shown for each combination resulting in adaptive resistance.

E. coli was pre-exposed to a sub-inhibitory amount of ciprofloxacin (0.008 μg/ml) for one hour and was subsequently grown with various concentrations of phosphomycin for a period of 6 hours. The half maximal inhibitory concentration (IC50) to phosphomycin increased from 0.05 μg/ml to 0.1 μg/ml. The error bars from 3 independent experiments are shown.

As an example of adaptive resistance, Figure 1 shows the effect of a 1-hour pre-exposure of *E. coli* to a sub-inhibitory dose of ciprofloxacin which decreased sensitivity of the bacterial culture to inhibitory doses of phosphomycin as compared to the control culture that was not pre-exposed to ciprofloxacin. The two samples initially started out at the same optical density (OD) level; however, the pre-exposed *E. coli* showed a maximal 2-fold decrease in sensitivity in response to inhibitory levels of phosphomycin as opposed to the control culture. The lethal concentration of phosphomycin for *E. coli* also increased as a result of pre-exposure to ciprofloxacin (from 0.12 to 0.25 μg/ml). Additionally, the MIC of phosphomycin increased from 0.03 to 0.06 μg/ml in the pre-exposed *E. coli* culture.

DISCUSSION

Adaptive resistance is a phenomenon whereby pre-exposure of an organism to sub-inhibitory levels of one antibiotic results in transient [Levitin, unpublished results] resistance of bacteria to lethal doses of the same or other antibiotics to which the organism was sensitive prior to the pre-exposure. The mechanisms underlying such adaptation were shown to involve efflux pumps, and components involved in control of the anaerobic respiratory pathway, thickening of the cell wall, etc. [Levitin, unpublished results, 6, 7, 14].

Table 1: Inducer-tester combinations that give rise to a reduced sensitivity of *E. coli* to lethal levels of tester antibiotics as a result of pre-exposure to sub-inhibitory doses of inducer antibiotics.

		Solvents		Inducers															
		H₂O	DMSO	QC	NM	CQ	FM	NB	CX	CC	PP	OF	BT	CF	SF	CA	CT	VM	OM
Testers	NA	31 ± 1.8	30 ± 1.6		50 ± 3.6				55 ± 5.1	62 ± 3.8	61 ± 2.9		44 ± 1.4						
	QC	608 ± 33	615 ± 37				1954 ± 59				1841 ± 25	1319 ± 51	2189 ± 37			1644 ± 73			2767 ± 136
	NM	8.3 ± 0.6	7.9 ± 0.7			12 ± 1.2													
	FM	0.05 ± 0.01	0.04 ± 0.003			0.17 ± 0.03								0.1 ± 0.02	0.15 ± 0.04		0.15 ± 0.02		0.17 ± 0.01
	CC	87 ± 7.4	91 ± 6.3					154 ± 21											
	AM	7.2 ± 0.5	7.3 ± 0.9											22 ± 1.4					
	AC	0.8 ± 0.1	3.1 ± 0.3			1.8 ± 0.4													
	CS	1.2 ± 0.6	14 ± 0.6	25 ± 1.6															
	CA	8.2 ± 1.2	8.1 ± 0.9														12 ± 1.1		
	CT	0.32 ± 0.01	0.36 ± 0.03	0.65 ± 0.07		1.2 ± 0.09						8.1 ± 1.3	0.69 ± 0.04	5.4 ± 1.1					
	VM	174 ± 14	179 ± 12	1096 ± 38			362 ± 18												
	RM	1.3 ± 0.3	1.4 ± 0.3					3.3 ± 0.2											

Abbreviations: Nalidixic acid, NA; Quinacrine, QC; Neomycin, NM; Chloroquine, CQ; Phosphomycin, FM Novobiocin, NB; Ceftriaxone, CX; Cloxacillin, CC; Azithromycin, AM; Piperacillin, PP; Amoxicillin, AC; Ofloxacin, OF; Bacitracin, BT; Ciprofloxacin, CF; Cefsulodin, CS; Sparfloxacin, SF; Chloramphenicol, CA; Cefotaxime, CT; Vancomycin, VM; Oleandomycin, OM.
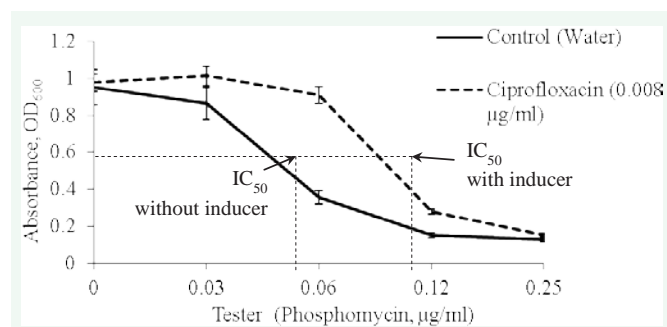
Figure 1 Pre-incubation of *E. coli* with sub-inhibitory levels of ciprofloxacin resulted in a decreased sensitivity of the bacteria to lethal levels of phosphomycin.

From the 598 combinations of clinically relevant antibiotics, 5% of all combinations tested expressed adaptive resistance. Each combination that showed evidence of adaptive resistance by displaying an increase in IC50 was recorded in [Table 1] and represents the averages of the IC50 values obtained from three independent experiments. For example, pre-incubation with a low dose of ofloxacin commonly used to treat chronic bronchitis, pneumonia, and urinary tract infections results in an increase in IC50 to cefotaxime, an antibiotic used to treat numerous infections including bacteremia/septicemia, lower respiratory tract, and gynecologic infections caused by *E. coli*.

Based on our observations done on gram-positive bacteria, the degree of adaptive resistance is dependent on the length of pre-incubation with the inducer. We found that the increase in the length of pre-treatment from zero minutes to 1 hour is directly proportional to the increase in the magnitude of adaptive resistance to the tester antibiotic. However, pre-incubation for periods longer than 1 hour resulted in only a modest further change in adaptive resistance. Concentration of an inducer was also observed to directly affect adaptation to a tester antibiotic [Levitin, unpublished results].

Although adaptive resistance is both transient and reversible, the occurrence of this phenomenon can result in the inability to control the infection and the emergence of antibiotic resistant strains. Many of combinations with a potential to result in adaptive resistance consist of broad-spectrum antibiotics as inducers and narrow-spectrum antibiotics as testers, such as phosphomycin (inducer) and vancomycin (tester), a combination which might be prescribed to treat endocarditis and *E. coli* infections. Certain inducers in combinations resulting in adaptive resistance are broad-spectrum antibiotics that treat a number of bacterial infections, such as ciprofloxacin, ceftriaxone, and vancomycin.

The prescription of broad-spectrum antibiotics prior to the identification of the cause of an infection might give rise to bacterial adaptation to *E. coli*-specific antibiotics, resulting in an ineffective treatment or an exacerbated infection. Various organs of the body accumulate residual levels of treatment antibiotics, and depending on antibiotic half-life, migh serve as an inducer and affect subsequent treatment regiments. As a consequence, treatment for *E. coli* infections initiated with broad-spectrum antibiotics (inducers) as part of empiric therapy, and followed by treatment with narrow-spectrum antibiotics (testers), such as amoxicillin and chloramphenicol, would be inefficient due to the phenomenon of adaptive resistance in *E.coli*.

CONCLUSION

This work offers a record of antibiotic combinations that are capable of exhibiting adaptive resistance. More testing of various antibiotics combinations should be done to create an extensive database of antibiotic combinations that give rise to adaptive resistance. This database would allow healthcare professionals to make wise and well thought-out decisions when prescribing medication in order to quickly and effectively treat patients.

The mechanism for adaptive resistance is still largely unknown; however, the results of this screening may assist in further discovering what cellular components play a part in this phenomenon.

REFERENCES

1. WHO Media Centre. Antimicrobial resistance. World Health Organization. 2013.

2. Courvalin P. Transfer of antibiotic resistance genes between gram-positive and gram-negative bacteria. Antimicrob Agents Chemother. 1994; 38: 1447-1451.

3. Barclay ML, Begg EJ. Aminoglycoside adaptive resistance: importance for effective dosage regimens. Drugs. 2001; 61: 713-721.

4. Daikos GL, Jackson GG, Lolans VT, Livermore DM. Adaptive resistance to aminoglycoside antibiotics from first-exposure down-regulation. J Infect Dis. 1990; 162: 414-420.

5. Fernández L, Breidenstein EB, Hancock RE. Creeping baselines and adaptive resistance to antibiotics. Drug Resist Updat. 2011; 14: 1-21.

6. Karlowsky JA, Hoban DJ, Zelenitsky SA, Zhanel GG. Altered denA and anr gene expression in aminoglycoside adaptive resistance in Pseudomonas aeruginosa. J Antimicrob Chemother. 1997; 40: 371-376.

7. Yuan W, Hu Q, Cheng H, Shang W, Liu N, Hua Z, et al. Cell wall thickening is associated with adaptive resistance to amikacin in methicillin-resistant Staphylococcus aureus clinical isolates. J Antimicrob Chemother. 2013; 68: 1089-1096.

8. Schenk MF, de Visser JA. Predicting the evolution of antibiotic resistance. BMC Biol. 2013; 11: 14.

9. Mohamed AF, Nielsen EI, Cars O, Friberg LE. Pharmacokinetic-pharmacodynamic model for gentamicin and its adaptive resistance with predictions of dosing schedules in newborn infants. Antimicrob Agents Chemother. 2012; 56: 179-188.

10. Barclay ML, Begg EJ, Chambers ST. Adaptive resistance following single doses of gentamicin in a dynamic in vitro model. Antimicrob Agents Chemother. 1992; 36: 1951-1957.

11. Xiong YQ, Caillon J, Kergueris MF, Drugeon H, Baron D, Potel G, et al. Adaptive resistance of Pseudomonas aeruginosa induced by aminoglycosides and killing kinetics in a rabbit endocarditis model. Antimicrob Agents Chemother. 1997; 41: 823-826.

12. Braoudaki M, Hilton AC. Adaptive resistance to biocides in Salmonella enterica and Escherichia coli O157 and cross-resistance to antimicrobial agents. J Clin Microbiol. 2004; 42: 73-78.

13. Sidhu K, Talbot M, Van Mil K, Verstraete M. Treatment with sub-inhibitory kanamycin induces adaptive resistance to aminoglycoside antibiotics via the AcrD multidrug efflux pump in Escherichia coli K-12. JEMI. 2012; 16: 11-16.

14. Karlowsky JA, Saunders MH, Harding GA, Hoban DJ, Zhanel GG. In vitro characterization of aminoglycoside adaptive resistance in Pseudomonas aeruginosa. Antimicrob Agents Chemother. 1996; 40: 1387-1393.

15. Kennedy RP, Plorde JJ, Petersdorf RG. Studies on the epidemiology of Escherichia coli infections. IV. Evidence for a nosocomial flora. J Clin Invest. 1965; 44(2): 193-201.

16. Totsika M, Moriel DG, Idris A, Rogers BA, Wurpel DJ, Phan MD, et al. Uropathogenic Escherichia coli mediated urinary tract infection. Curr Drug Targets. 2012; 13: 1386-1399.

17. Nitschke M, Sayk F, Härtel C, Roseland RT, Hauswaldt S, Steinhoff J, et al. Association between azithromycin therapy and duration of bacterial shedding among patients with Shiga toxin-producing enteroaggregative Escherichia coli O104:H4. JAMA. 2012; 307: 1046-1052.

18. Gerber A, Karch H, Allerberger F, Verweyen HM, Zimmerhackl LB. Clinical course and the role of shiga toxin-producing Escherichia coli infection in the hemolytic-uremic syndrome in pediatric patients, 1997-2000, in Germany and Austria: a prospective study. J Infect Dis. 2002; 186: 493-500.

Cite this article