

Antibiotic Resistance in Escherichia Coli: A Comparative Study of Antibiotic Susceptibility with and without Ampicillin Resistance Gene

Lili Bettencourt

Division of Infection and Immunity, Faculty of Medicine, University College London, London, UK
Email: lili.bettencourt.23@ucl.ac.uk

Abstract—*Escherichia coli* is a widely studied species of bacteria that has been known to exhibit antibiotic resistance, an up and coming issue of the 21st century. Plasmids, or circular DNA, are one method that such bacteria are able to confer resistance. Through the generation of growth curves and the completion of a disc diffusion assay, this study aims to analyze both growth and resistance characteristics of an *E. coli* strain containing the pGLO plasmid. These methods proved little difference between the rate of growth for wild type and transformed *E. coli* (containing the pGLO plasmid). In terms of conferred resistance, however, it is shown that the transformed *E. coli* results in greater susceptibility against Tetracycline, and diminished susceptibility towards Ampicillin (relative to the wild type *E. coli*). These findings are as expected and confirm that future studies should focus on the interactions between various antibiotics (so as to decrease interference).

Keywords—*E. coli*, plasmid, antibiotic resistance, antibiotic susceptibility, bacterial transformation, microbiology

I. INTRODUCTION

In recent years, it has become commonplace knowledge that antibiotic resistance is an impending issue that the clinical world faces today. In a report on public health challenges in the 21st century, the World Health Organization (WHO) lists antibiotic resistance as a formidable challenge [1]. Because of the rate at which more and more antibiotics are being rendered useless against infections, medical workers are running out of options to treat their patients. As explained by the WHO, though antibiotic resistance is naturally occurring, the rate at which it is conferred is sped up by the unnecessary use of antibiotics [2]. What is often overlooked, however, is just how severe the issue of antibiotic resistance is. Based on a report commissioned by the UK Prime Minister in 2014, economist Jim O'Neill predicted 10 million deaths per year attributed to antibiotic resistance in the year 2050 [3]. This figure was later found to be an underestimate as an approximated 4.95 million deaths in 2019 were associated with antibacterial resistance, 1.27 million of those as a direct result of antibiotic resistance [4]. In 2019

alone, half of O'Neill's original estimate for antibiotic resistance related deaths per year was already reached, demonstrating how critical a challenge it truly is.

In this study, we will examine two strains of *E. coli* – one with a plasmid, pGLO, and the other a wild type strain. Growth curves were performed in order to determine whether the addition of the plasmid conferred any fitness disadvantages (i.e., rate of growth). Additional antibiotic susceptibility tests were conducted to further compare the two strains and investigate if the plasmid might also confer resistance to various antibiotics.

II. LITERATURE REVIEW

Escherichia coli (*E. coli*) is a particular species of bacteria that has been thoroughly studied as well as known for conferring antibiotic resistance. *E. coli* was first identified by researcher Theodore Escherich in 1885. It is a Gram-negative bacterium that is of rod shape and can typically be found in the gastrointestinal tract of both humans and animals. Though it can exist safely in the intestinal tract, specific strains were found to have evolved into pathogenic *E. coli* [5]. Such strains have been identified by the WHO as among the most critical of priority pathogens [6]. These strains are known to cause Hemolytic Uraemic Syndrome (HUS), Urinary Tract Infections (UTI), *E. coli*-associated diarrheal disease, and bacteremia [7–9]. HUS is often a result of Shiga toxin-producing *E. coli* whose toxins travel into the bloodstream and begin destruction of red blood cells as well as kidney functions. A UTI is a result of bacteria that travels into the urinary system. From *E. coli*, most UTIs are in the bladder, urethra, or kidneys [7]. One particular strain, identified as O157:H7 has been commonly associated with diarrheal disease, and according to the CDC, has had numerous outbreaks in America every year since 2006 [10]. The symptoms of an O157:H7 infection include bloody diarrhea, abdominal cramps, fatigue, and nausea [11].

Though resistance to antibiotics may differ among strains of *E. coli*, it has been found to be consistently resistant to certain drugs like tetracyclines, erythromycin, amoxicillin, fluoroquinolones, and others [12–14]. Tetracyclines are a class of antibiotics that are most commonly used for treatment of bacterial infections and infectious diseases [15]. First reported in 1948,

Tetracyclines were released in the following years because of their success in the clinical stage [16]. For this class of drug, the method of action is protein synthesis inhibition through the 30s ribosomal subunit [15]. Erythromycin, first discovered in 1952, is also used to treat a wide variety of illnesses, both bacterial and not. Similar to tetracyclines, erythromycin targets bacteria through inhibition of protein synthesis (only now in the 50s subunit) [17]. Amoxicillin was first discovered in 1972 following the waves of resistance against penicillin [18]. Falling within the Beta-lactams class of antibiotics, however, amoxicillin's method of action is through inhibition of cell wall synthesis [19]. First developed in 1962, Fluoroquinolones are another class of broad spectrum antibiotics that are commonly used to treat respiratory and urinary tract infections. This class of antibiotics operates by inhibiting mRNA transcription and DNA replication [20, 21].

III. MATERIALS AND METHODS

Liquid Broth Media Preparation: The liquid media was prepared previously by combining 8 grams of Invitrogen Luria Broth Base (12795-027) and 1 liter of deionized water in a 1 liter glass Pyrex lab bottle and shaken until the solution was homogenous. The media was then autoclaved in an All American 25x electric autoclave at 20 psi for one hour.

Agar Plate Preparation: Agar plates were made by mixing 5g of Bio-Rad LB Nutrient Agar Powder (1660472) and 200mL of sterile water in a 250mL glass lab bottle. After being shaken, the agar solution was autoclaved at 20 psi for one hour. The bottle was left to cool until the temperature reached around 65°C (or manageable to handle). Plates were poured by measuring 20mL of agar with a 50mL falcon tube before pouring into 94mm Greiner Bio-one standard sterile plates. Plates were left next to a Bunsen burner to completely solidify before being covered with a lid and stored upside down at 4°C.

Streaking for Single Colonies: To streak for single colonies, a single-use, wrapped 10uL inoculation loop was dipped inside the liquid overnight culture and streaked onto the previously prepared agar plates. Plates were placed upside down in an incubator at 37°C and left overnight. For long-term storage, plates with colonies were kept at 4°C.

Overnight Liquid Culture: Once single colonies were achieved, to prepare overnight cultures, an inoculation loop was used to pick up a colony and place it into a 15mL falcon tube containing 3–5mL of nutrient broth media. This was then incubated overnight in the shaking incubator at 37°C and 90 rpm.

Glycerol Stock: Because the culture started from a lyophilized stock, for the sake of the study, a frozen *E. coli* stock had to be made. An overnight culture was prepared the day before as well as a 50% glycerin stock. This was done by mixing 30mL of Flinn Scientific Glycerin (G0007) and 30mL of sterile water in a lab bottle, shaking, and then autoclaving at 20 psi for one hour before leaving at room temperature. The next day, 500µl of the 50% glycerol stock was put into each of two sterile 1.5mL

Eppendorf tubes. Once that was completed, 500µl of an overnight culture was also added to the tubes so that there was a final concentration of 25% glycerin in the stock. Tubes were labeled and stored at –20°C.

Growth Curve: In order to determine at what point in a day culture the *E. coli* strain would be in the exponential (growth) phase, a growth curve was performed. This started by measuring the OD600 of an overnight culture, first diluting to 1:10 by using 100µl of the culture and 900µl of fresh media in a 400µl cuvette. To read, a Thermo Scientific Spectronic 200 was blanked with a 1:10 dilution of liquid broth media to deionized water. Once the initial reading was complete, the proper volume was calculated so that the OD600 would read 0.05 for a subculture with a final volume of 15mL. The overnight culture was diluted with the calculated amount of fresh media in a 50mL falcon tube and left in a shaking incubator. Samples were taken over 24 hours to generate a growth curve and OD was read as described previously. Readings were stopped at the end of the 24 hours. A growth curve was also performed using three biological replicates, meaning that three overnight cultures were started from three different colonies. Readings were taken for each of the three cultures at the same time over 24 hours (using the methods described above).

Generation of Competent Cells: Before transforming the *E. coli* cells, they first needed to be washed with CaCl₂ buffers. Three were prepared: a stock CaCl₂ solution of 1M, a working CaCl₂ solution of 0.1M, and a working CaCl₂ + 15% glycerol solution of 0.1M. For the stock solution, 11.1g of anhydrous CaCl₂ was weighed and added to 80mL of deionized water in a glass lab bottle. This was then shaken to combine before adding another 20mL of deionized water (totaling to a final volume of 100mL of stock solution). The bottle was autoclaved at 20 psi for one hour before being left at 4°C overnight. The following day, a working CaCl₂ solution of 0.1M was prepared by diluting the stock 1:10 with deionized water. This was completed by combining 5mL of stock solution and 45mL of water in a 50mL falcon tube and repeated so that two tubes were prepared. For the CaCl₂ + 15% glycerol solution, 6mL 1M CaCl₂, 9mL sterile glycerol, and 45mL deionized H₂O were mixed and then poured into two 50mL falcon tubes. All four tubes were then autoclaved at 20 psi for one hour and then left at 4°C. A subculture was then started from an overnight culture by calculating the needed volume of overnight to add to fresh media so that the OD600 would read 0.05 (at a final volume of 10mL). This was left for 2–3 hours in the shaking incubator at 37°C and 200rpm. The culture was then centrifuged at 4°C and 4000 rpm for 10 minutes in a Thermo Scientific Sorvall ST 8. The supernatant was poured out and the pellet was resuspended using a micropipette and 10mL 0.1M CaCl₂ (that had been stored at 4°C). This was left to incubate on ice for thirty minutes before centrifuging again. Once the supernatant had been discarded again, the pellet was resuspended with 5mL CaCl₂ + 15% glycerol solution and then the contents from the tube were divided into 5 Eppendorf tubes and stored at –20°C.

Heat-Shock Transformation of *E. coli*: In order to transform the competent cells with the pGLO plasmid, one Eppendorf tube of *E. coli* wild type competent cells was first thawed on ice. To the thawed tube, 10 μ l of the plasmid stock was added and pipetted to mix. The tube was left on ice for 30 minutes before being placed on a Labnet D1100 AccuBlock Digital Dry Bath (previously set to 42°C) for 30 seconds. The tube was then transferred back on ice for another two minutes. Following this, 1mL of fresh LB was added to the tube before incubating in the shaking incubator at 37°C and 200 rpm for 1–2 hours. After incubating, 100 μ l was taken from the tube, plated on a LA containing Ampicillin at 100 μ g/mL, and then placed in a static incubator at 37°C overnight. The next day the plate was taken out of the incubator and stored at 4°C.

Disc Diffusion Assay: One form of antibiotic susceptibility testing performed was a Disc Diffusion assay, where Biogram antibiotic discs were used. A single colony of *E. coli* containing pGLO plasmid was first cultured overnight in a shaking incubator at 37°C and 200 rpm in 3mL of LB containing Ampicillin at a concentration of 100 μ g/mL. The same was done with the wild type *E. coli*, except in normal LB. Then, the next day, the OD600 of each of the two cultures was taken and used to calculate a dilution so that a subculture in fresh LB could be started with an OD600 of 0.05. Cultures were left to grow at 37°C and 200 rpm shaker until they reached OD600 of 0.15. From each subculture, 1mL was added on top of one LA plate and spread around the entire surface of each plate with a loop. The plates were then left to dry for 30 minutes next to a Bunsen burner. Once the surfaces of the plates were completely dry, various antibiotic discs were placed with even spacing from each other and the plate edges with flame-sterilized tweezers. The antibiotic discs used (and their concentrations) were Erythromycin at 15 μ g, Tetracycline at 30 μ g, Penicillin at 10 units, Streptomycin at 10 μ g, Gentamicin at 10 μ g, and Ampicillin at 10 μ g. Once the discs had been placed, the plates were left to incubate overnight in a static incubator at 37°C. The next day, the zone of inhibition surrounding each disc was analyzed in comparison to the type of antibiotic and the strain of *E. coli*.

IV. RESULT AND DISCUSSION

The results from Fig. 1 show that the pGLO plasmid has not conferred any growth defects, and almost improves the rate of growth. Ultimately, the addition of the pGLO plasmid creates no decrease in ecological fitness (specifically in terms of growth). It has been previously suggested that the acquisition of plasmids by *E. coli* may lead to the increased need of ATP which might negatively impact its growth [22]. However, our data in Fig. 1 suggests that this is not the case for the pGLO plasmid.

The curves graphed in Fig. 1 illustrate the increase in optical density over time as the *E. coli* incubated in a shaking incubator at 37°C and 200 rpm. There is a slight increase in the *E. coli* with pGLO plasmid curve, compared to the wild type, however, the error bars indicate that the difference is most likely not statistically significant.

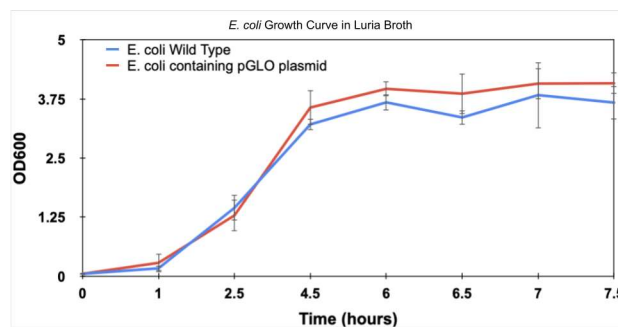


Fig. 1. Growth curves graphing optical density at 600 nm of *E. coli* cultures against time.

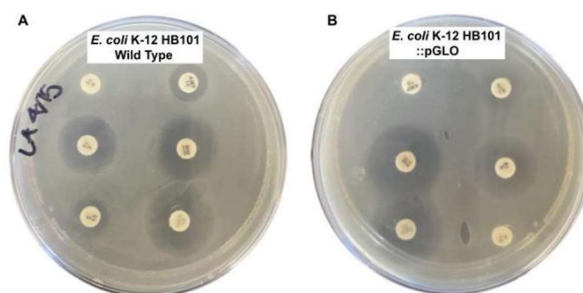


Fig. 2. Disk diffusion assay results. (A) is the plate containing the wild type *E. coli* strain, (B) is the plate containing the *E. coli* strain with the pGLO plasmid.

As for the disk diffusion assay (Fig. 2), the addition of the pGLO plasmid does allow for the *E. coli* strain to grow in the presence of Ampicillin, which is to be expected. This resistance to Ampicillin, however, does not also confer resistance to Penicillin. Such is a peculiar result as both antibiotics fall into the same class, β -lactams. Resistance towards β -lactam antibiotics typically occurs through three main methods: diminished access of β -lactams to their receptor proteins, decreased binding efficiency of β -lactams to their receptor proteins, and decomposition of the β -lactam antibiotic through the expression of β -lactamase genes [23]. Bacteria often demonstrate resistance to Ampicillin and Penicillin through the use of β -lactamases, but it is possible that the difference in resistance is a result of different, specific β -lactamase (for example, it was found that an OXA-1 β -lactamase was responsible for Ampicillin resistance, but perhaps this β -lactamase is not effective towards Penicillin resistance) [24]. One other possible explanation for the resulting differences between the resistance to Ampicillin and Penicillin is the age of the antibiotic disks, which is unclear. This would usually be ameliorated by repeating the assay with new disks. However, due to time and budget limitations, this was not possible during this study. Besides Penicillin, the data in Table I shows that the addition of the pGLO plasmid (which confers Ampicillin resistance) also results in decreased Tetracycline resistance, suggesting an antagonistic relationship between the two antibiotics. This is in agreement with the pharmacodynamic antagonism that occurs when both Ampicillin and Tetracycline are present. A resulting decrease in the expression of resistance genes is often the case [25, 26]. Similar to β -lactams, the common method of Tetracycline resistance is

reduced access of the antibiotic to its receptor proteins, sometimes caused by alterations in the outer cell membrane's permeability [27].

The plates in Fig. 2 allow for visual qualification of the effects of each antibiotic on a wild type strain as well as a transformed strain. Between the two plates, changes in the susceptibility of each strain to the various antibiotic disks are clear.

TABLE I. DIAMETER OF ZONES OF INHIBITION (MEASURED IN MILLIMETERS)

| Antibiotic | Wild Type <i>E. coli</i> | <i>E. coli</i> with pGLO plasmid |
|--------------|--------------------------|----------------------------------|
| Erythromycin | ~10 | ~10 |
| Streptomycin | ~18 | ~18 |
| Tetracycline | ~15 | ~22 |
| Gentamicin | ~22 | ~22 |
| Ampicillin | ~12 | 0 |
| Penicillin | 0 | 0 |

Disk diffusion assay results as measured in the diameter of the zone of inhibition surrounding each antibiotic. The zone of inhibition, or the space around a paper antibiotic disk where the bacteria does not grow, reflects the degree to which a strain on bacteria is resistant to an antibiotic (at a certain concentration). The larger the zone of inhibition, the more susceptible a strain may be to an antibiotic. By this reasoning, and as proven by the data in Table I, the transformed *E. coli* strain is more resistant (than the wild type) to tetracycline.

V. CONCLUSION

Through this study, it has been determined that the transformation of K-12 HB101 *E. coli* with the pGLO plasmid confers resistance to ampicillin without affecting the growth rate. However, we have also shown that the increased resistance to ampicillin is accompanied by decreased resistance to tetracycline, thereby highlighting an antagonistic relationship between the two antibiotics. Future research in the field of microbiology should explore why such antagonism exists, and between which antibiotics, so that treatment plans may be devised for suffering patients.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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