The dose-dependent effect of Ampicillin on the growth of mixed resistant and non-resistant *Escherichia coli*

Annabelle Marsh
Saint Olaf College, Northfield, Minnesota 55057

This project studied the relationship between antibiotic dose (in this case, Ampicillin) and *Escherichia coli* growth. Antibiotic behavior is vital to study because of antibiotic resistance and. We grew *E. coli* with different amounts of ampicillin for 6 hours and measured *E. coli* concentrations. We then modeled this growth based on assumptions around resistant bacteria and death rates. We used our model to investigate our hypothesis that the relationship between antibiotic dose and *E. coli* death is linear and that there is a negative relationship between the number of resistant bacteria and antibiotic concentration. Our findings reflect both of these relationships.

Introduction

After the discovery of penicillin in the 1920s, penicillin derivatives and other antibiotics have saved countless lives. However, in recent years, antibiotic resistance has led to decreased efficacy of these same antibiotics. “Each year in the U.S., at least 2.8 million people are infected with antibiotic-resistant bacteria or fungi, and more than 35,000 people die as a result”(CDC). The better scientists understand how antibiotics function and how resistance works, they can develop new antibiotics that bacteria, such as *E. coli,* do not have resistance to.

Penicillin and penicillin derivatives like Ampicillin are some of the oldest and most commonly used antibiotics. They function by binding to receptors called membrane-bound penicillin-binding proteins (PBPs) in susceptible cells and then preventing the formation of cell walls during cell division (Tomasz). Some resistant bacteria are resistant because they use efflux pumps to pump the ampicillin out before the antibiotic binds to the PBPs (Teelucksingh), while others have modified PBPs to which the antibiotic cannot bind (Horikawa).

We used mathematical modeling to determine the portion of bacteria that are initially resistant to each dose of antibiotic and the rates at which the antibiotic causes cell death. In the future, we can use this to compare antibiotic doses and the efficacy of different antibiotics.

Materials and Methods

Laboratory Methods

We chose to study *E. coli* because of its easy availability in our lab. Ampicillin was our antibiotic of choice because of its simple mechanism of action and availability.

The lab TA added 1/4mL of overnight *E. coli* culture to 5 flasks, creating 50mL of culture. Then, she placed the flasks in an incubator at 37C. This incubator shook the bacteria continuously, leading to a well-mixed culture that aerated for optimal growth conditions. We set our spectrophotometer to 600nm and blanked it using sterile broth.

We started with a vial of Ampicillin diluted at 2,500 ug/mL. We added it into the flasks every 2 minutes with the following volumes and concentrations and measured initial absorbance values.

Added Volume	Final Concentration
2 uL	0.1 ug/mL
10 uL	0.5 ug/mL

20 uL	1 ug/mL
60 uL	3 ug/mL

Table 1: Chart showing the amount of ampicillin solution added to each flask.

Every 20 minutes from the start of each flask, we pipetted 1mL of that flask into a cuvette. Then, we took a spectrophotometer reading. We recorded these spectrophotometer readings in a spreadsheet along with timestamps. Next, we imported this data into R and used the OD600 to cells/mL conversion from page 84 of the textbook to convert our data from absorbance to cells/mL. The ratio we used is 8×10^8 cells/mL × OD600.

Mathematical Model

We used the following compartmental model to inform our mathematical choices.

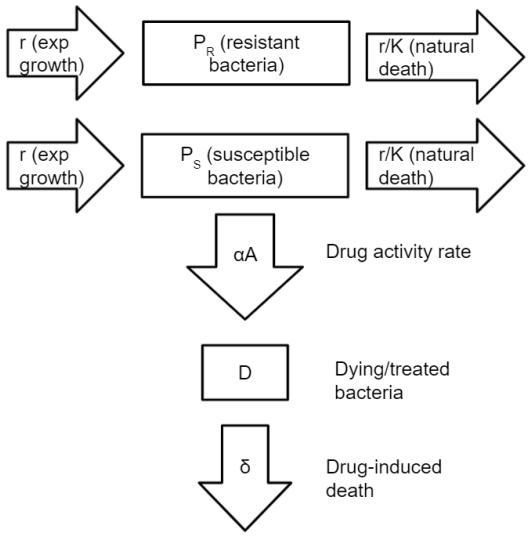

Figure 1: Compartmental model showing our hypothesized states and parameters.

This model assumes that there are two kinds of bacteria in the culture; those that are resistant to that level of ampicillin, denoted by P_R, and those susceptible to it, P_S. Then, we assume that without the antibiotic, these two strains will grow logistically at the same rate, *r*, and with the same combined carrying capacity, *K*. This assumption is based on the fact that the two strains of bacteria eat the same kinds of food and are in the same environment. A logistic model has the following form:

$$\frac{dP}{dt} = rP\left(1 - \frac{P}{K}\right)$$

Figure 2: Differential equations representing a logistic model

Logistic models are commonly used to model population growth when carrying capacity is a consideration.

However, a simple logistic model does not account for the effects of ampicillin on the bacteria population. The ampicillin binds to receptors called membrane-bound penicillin-binding proteins (PBPs) in susceptible cells (Tomasz), which move into a dying stage, D. Our model assumes that this occurs at a rate αA, where α is a constant and A is the antibiotic concentration in ug/mL. We also assume that no bacteria are in the dying stage when the experiment first starts.

Next, as these dying cells attempt to divide, the PBP receptors prevent the cell walls from forming correctly, causing lysis and cell death (Tomasz). Our model represents this as cells leaving from the dying phase D at a constant rate, δ.

$$\frac{dP_r}{dt} = rP_r\left(1 - \frac{P_r + P_s}{K}\right)$$

$$\frac{dP_s}{dt} = rP_s\left(1 - \frac{P_r + P_s}{K}\right) - \alpha A P_s$$

$$\frac{dD}{dt} = \alpha A P_s - \delta D$$

Figure 3: Differential equations representing our model.

Note that if the amount of antibiotic, A, is 0 and D starts as 0, as we assumed, this reduces to a logistic model. All parameters are assumed to be positive.

Parameter	Meaning	Units
P_R	resistant bacteria	cells/mL
P_S	susceptible bacteria	cells/mL
D	dying bacteria	cells/mL

A	ampicillin concentration	ug/mL
t	time	minutes
r	growth rate	1/minutes
K	carrying capacity	cells/mL
α	antibiotic activity rate constant	mL/(minutes *ug)
δ	antibiotic death rate constant	1/minutes

Results

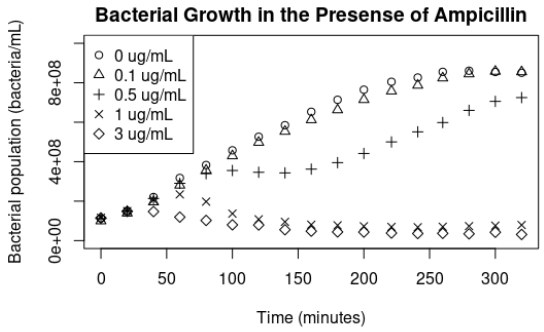

Figure 4: Data from the experiment.

The data from the experiment shows a clear negative correlation between the ampicillin dose and the growth of bacteria. We began fitting our model by fitting a logistic model to our control data. The following image shows our control data and a logistic model, which is an excellent fit and produced similar values to our previous *E. coli* fits from earlier in the class. We then used the growth rate, *r,* and carrying capacity, *K*, values from this fit to fit the rest of the data.

K	8.688585e+08
r	1.967001e-02

Table 3: Parameters from the logistic model from the control data

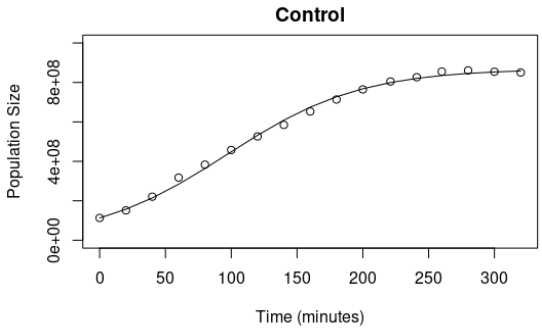

Figure 4: Control data with the logistic model.

Using the fixed *r* and *K* values from the logistic model, we simultaneously fit the four experimental data sets to determine the constants α and δ.

Parameter	Value
α	0.01947317
δ	0.02006167

Table 4: Parameters determined by fitting all of the data sets

Then, we fit each dataset individually to determine the portion of bacteria resistant to each dose of ampicillin.

Ampicilin Concentration	Fraction of *E. coli* that are resistant
0.1 ug/mL	0.5341217
0.5 ug/mL	0.05384803
1 ug/mL	0.002118969
3 ug/mL	0.001247795

Table 5: Chart showing resistance in comparison to ampicillin dosing

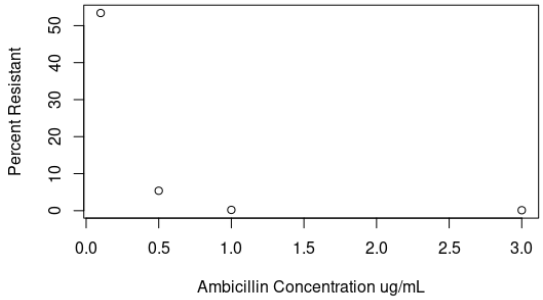

Figure 5: Graph showing the portion of *E. coli* that are resistant to each level of antibiotic dose.

This chart and graph show an expected correlation between the portion of bacteria resistant to a dose of ampicillin and the dose of ampicillin itself. The percentage of resistant bacteria decreases with antibiotics dose.

Next, we graphed the final model and overlaid it on the data.

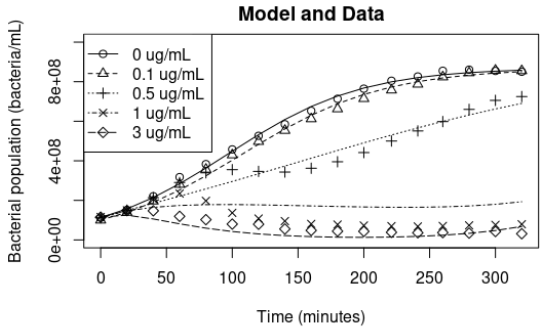

Figure 6: Final model with data

This graph shows that our methods achieved an excellent fit to the data, considering that only one unknown parameter varies for each dataset.

Finally, we graphed each dataset with its model, with the resistant, susceptible, and dying subgroups displayed. We include the 3ug/mL graph below.

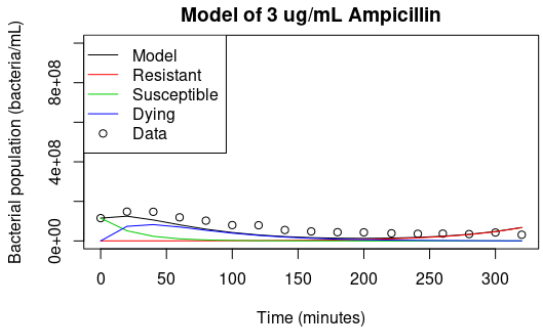

Figure 7: Final model with data for 3ug/mL Ampicillin, with model lines showing each subgroup of the model.

These graphs validate the fitted model as they all showed the expected behavior of each subgroup (resistant, susceptible, dying) of bacteria.

Discussion/Conclusion

This experiment validates our model and shows a dose-dependent relationship between Ampicillin concentration and growth rates of *E. coli*. We found an excellent fit between our model and the data. The fit implies that our assumptions are likely to be valid; however, there is more room for improvement in our model. In particular, there is a little "wiggle" in the data for 0.5 ug/mL Ampicillin that this model does not reflect.

We chose to fit the same α and δ parameters to all the datasets simultaneously Varying these parameters with each dataset has the potential to lead to a better fit. However, with limited data, a complicated fit leads to parameters losing their meaning.

This model helps us understand the behavior of *E. coli* and ampicillin. We have determined rates at which ampicillin kills *E. coli*. In addition, we have determined estimates for the percent of bacteria initially resistant to each ampicillin dose. These results are helpful for future research with other antibiotics and other strains of *E. Coli*. Using the same model, we can compare antibiotic resistance across strains and antibiotics.

References

Centers for Disease Control and Prevention. "About Antimicrobial Resistance." Centers for Disease Control and Prevention, 10 Sept. 2018, www.cdc.gov/drugresistance/about.html.

Horikawa S, Ogawara H. Membrane-bound penicillin-binding proteins of Escherichia coli. Comparison of a strain carrying an R-factor and the parent strain. J Antibiot (Tokyo). 1978 Dec;31(12):1283-91. doi: 10.7164/antibiotics.31.1283. PMID: 367999.

Teelucksingh T, Thompson LK, Cox G. The Evolutionary Conservation of Escherichia coli Drug Efflux Pumps Supports Physiological Functions. J Bacteriol. 2020 Oct 22;202(22):e00367-20. doi: 10.1128/JB.00367-20. PMID: 32839176; PMCID: PMC7585057.

Tomasz A. Penicillin-binding proteins in bacteria. Ann Intern Med. 1982 Apr;96(4):502-4. doi: 10.7326/0003-4819-96-4-502. PMID: 7039448.