Modeling the Effect of Bacteriophages on the Spread of Antibiotic Resistance

Grant Proposal

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Author Note

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Abstract

Antibiotic resistance is one of the biggest health threats right now, with 1.27 million people being directly killed by it in 2019. While bacteriophage therapy has emerged as an alternative to antibiotics, the dynamics between phages, bacteria, and antibiotics remain poorly understood. Current research often suffers significant limitations: math models often lack experimental validation, and empirical phage studies don't provide a quantitative and predictive framework. This project addresses these gaps by developing an integrated computational-experimental approach to create a mathematical model and validate it. We propose to create this mathematical model using ordinary differential equations to simulate the population dynamics of both susceptible and resistant bacteria when being pressured by both the phages and antibiotics. The model will be implemented in both a deterministic and stochastic framework, and parameter values for E. coli and T7-like phages will be taken from literature. The model will later be validated through experimental testing which will find the population of susceptible and resistant bacteria after a week in vitro with phages and antibiotics. This research represents a shift from traditional single-disciplinary approaches toward an integrated approach that combines both mathematical rigor and biological experimentation.

Keywords: Antibiotic resistance, bacteriophages, math modeling, antibiotics, experimental validation, deterministic, stochastic

Section I: Background

Modeling the Effect of Bacteriophages on the Spread of Antibiotic Resistance

Antibiotic resistance is a major global health threat, driven by biological and environmental interactions including antibiotic use, bacterial genetics, and ecological dynamics. At its core, antibiotic resistance arises when bacteria gain or evolve mechanisms that repel antibiotics, allowing them to reproduce the next generation of resistant bacteria. The development and spread of antibiotic resistance are caused by many factors, among them bacteriophage concentration and antibiotic concentration.

Antibiotic overuse: Antibiotic overuse is the biggest driver of antibiotic resistance, as bacteria that are exposed to antibiotics face selective pressure that favors resistant mutants. Although resistance mutations generally impose a fitness cost that reduces reproductive rate, compensatory evolution and horizontal gene transfer can offset these disadvantages, allowing resistant bacteria to thrive even after antibiotic use declines (Andersson & Hughes, 2010). Similarly, Knoppel et al. (2017) found that even after antibiotic use was stopped, resistant populations flourished due to mutations that increased the growth rate.

Bacteriophages: An under-looked factor in the spread of antibiotic resistance is bacteriophage activity. Bacteriophages, viruses that infect bacteria, can mediate gene transfer between multiple bacteria through a process called transduction. Calero-Caceres et al. (2019) demonstrated that bacteriophages often carry antibiotic resistant genes (ARGs) and facilitate their spread across bacterial populations. They can transfer ARGs rapidly and across different species, regardless of the antibiotic concentration. Beke et al. (2016) used a mathematical model to simulate and plot the growth of susceptible and resistant populations after phages were introduced, showing how resistant bacteria populations spread when affected by phages and other factors such as temperature and pH.

Math models: Mathematical models have been used when quantifying how resistance spreads under various conditions. Ibarguen-Mondragon et al. (2019) developed a deterministic model using ordinary differential equations to simulate susceptible populations, resistant populations, plasmid concentrations, and antibiotic concentrations. Setting the equations to 0 and solving them out, the researchers found that there were three states of equilibrium in this system, and that outside of these states of equilibrium the population numbers for both resistant and susceptible bacteria would oscillate. Stochastic approaches have also been used when modeling resistance. Merdan et al. (2017) compared deterministic and stochastic models and found that adding random fluctuation to the parameters produced a more realistic and variable model.

Machine learning: Machine learning methods have also been used to quantify the spread of resistance. Ali et al. (2020) used a U-Net convolutional neural network to classify E. coli strains as resistant or susceptible based on their morphology after exposure to antibiotics. The model had an area of 0.83 under the Receiving Operating Characteristic curve, showing that while there is some error, it is still overall a really accurate model. Image-based and ML models can complement mathematical modeling really well by quantifying resistance patterns and allowing for comparisons between real-life and simulation data.

Conclusion: Together, these studies indicate that resistance emerges from the interaction of selective pressure and genetic exchange. This project aims to model how these forces interact to influence the evolution of resistance, providing insight into what recommendations can be made to curb its spread.

Section II: Specific Aims

This proposal's objective is to develop a mathematical model that quantifies how bacteriophages affect the spread of antibiotic resistance in a bacteria population. The model will assess a phage's beneficial role as a bacterial predator and harmful role as a mediator for the bacterial acquisition of ARGs (antibiotic resistance genes). Once finished, this model can generate insights on the development of phage therapies that can be used in conjunction with antibiotics.

Specific Aim 1: Develop a mathematical model that quantifies the effect of phages on the spread of antibiotic resistance

Specific Aim 2: Perform Monte Carlo simulations on a stochastic version of the model

Specific Aim 3: Conduct wet-lab testing and confirm/improve both the deterministic and stochastic models

The expected outcome of this project is a mathematical model that can predict the population of susceptible and resistant bacteria and identify optimal initial phage populations that minimize resistance levels. Specifically, the model will be able to forecast optimal phage populations and susceptible/resistant bacterial populations over a one-week period with less than 5% error compared to experimental measurements.

Section III: Project Goals and Methodology

Relevance/Significance: Antimicrobial resistance is one of the biggest issues in medicine in the 21st century, with an estimated 1.27 million deaths directly caused by it in 2019 (World Health Organization, 2023), and $1 trillion to $3.4 trillion in losses per year by 2030 (World Health Organization, 2023). In 2050, a projected 10 million people will die due to the antibiotic resistance crisis (O'Neill, 2016). Antibiotic resistance has already severely undermined modern medicine, and will continue to do so in the future if preventative measures are not taken. Additionally, very few antibiotics are coming into the

market (Theuretzbacher et al., 2020), giving bacteria more time to gain resistance to current antibiotics. However, bacteriophages can potentially fill the void left by reduced drug development, as they are known to kill bacteria without harming humans. Additionally, it is nearly impossible for bacteria to become resistant to both antibiotics and bacteriophages, meaning that if either treatment plan fails, the other can be used. Currently, research on the dynamics between phages and antibiotic resistance is very limited. This project is significant as it develops a predictive framework for understanding how phages and antibiotics influence the spread of resistance.

Innovation: Extensive research has been conducted on antibiotics and how to curb the rising issue of antibiotic resistance. However, very little research has gone into using either mathematical tools or bacteriophages, and even less research has been done involving both. Additionally, research involving math models often excludes wet lab testing to confirm the data from the model, raising the possibility that the model's predictions are incorrect. My project aims to combine a math model to make predictions on the populations of susceptible and resistant bacteria, bacteriophages as an under-looked method to slow the spread of resistance, and wet lab testing to ensure that the model works.

Methodology: Assumptions will be made about the populations/concentrations of susceptible bacteria, resistant bacteria, phages, and antibiotics. Equations will then be made for the four populations listed in the first sentence. The values for the parameters will be specific to a strain of bacteria and a phage strain. The model will then be built using Python. For the wet lab, I will isolate the bacteria and phages in a petri dish and record their growth. After the initial lab testing and preliminary data collection, I will adjust my model so that it aligns with the preliminary data. I will then record population growth in a small animal population (mice) to better understand how antibiotic-resistant bacteria move through a human population. I will then adjust the model again by adding extra parameters to account for animals and making sure the model aligns with recorded data.

Specific Aim #1:

The first aim of this project is to develop a mathematical model that quantifies the effect of phages on the spread of antibiotic resistance. The methodology is to first develop equations measuring the populations of susceptible bacteria, resistant bacteria, and phages, and the concentration of antibiotics. Parameter values will then be taken from literature, and the model will be implemented using Python's NumPy, SciPy, and matplotlib/seaborn libraries.

Justification and Feasibility:

Mathematical models have been widely used to better understand the dynamics between antibiotics and bacteria. Ibarguen-Mondragen et al. (2019) successfully developed a system of ordinary differential equations to model populations of susceptible bacteria, resistant bacteria, plasmids, and antibiotic concentrations, identifying states of equilibrium in the system. Similarly, Beke et al. (2016) developed a model for phage-bacteria interactions, and integrated under-looked factors such as temperature and pH. Multiple graphs of Beke's model are shown in Figure 1, where she alters the temperature and pH in each one to show how much they affect the susceptible and resistant bacteria

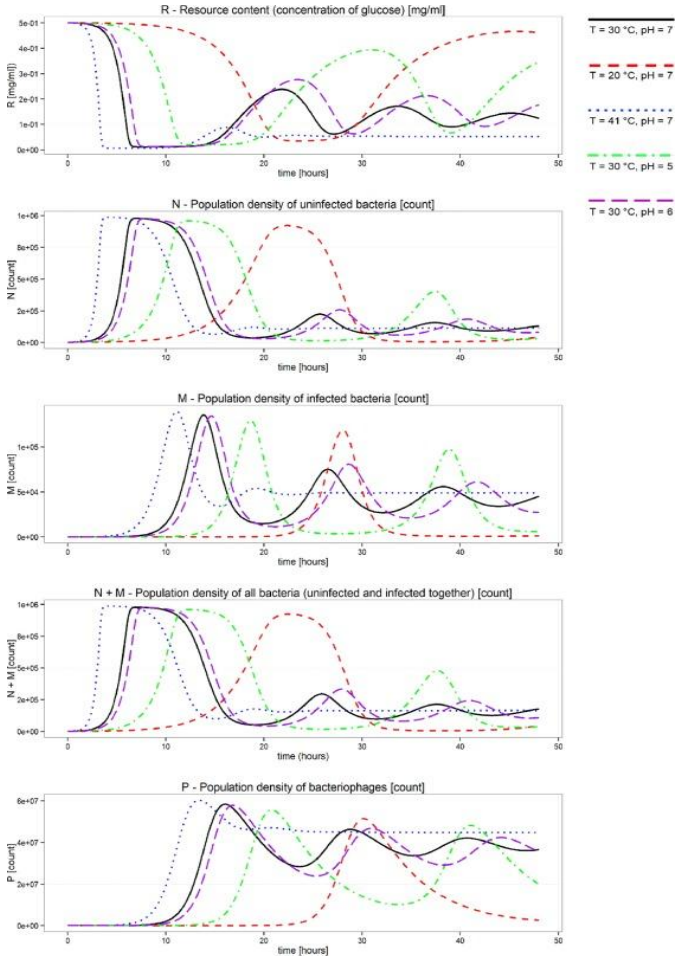

Figure 1: Graphs of bacteria and phage populations over time when affected by different temperatures and pH.

populations. A critical knowledge gap that was found in the literature is the integration of bacteriophages as both predators of bacteria and vectors of resistance genes. Calero-Caceres et al. (2019) highlighted that phages are reservoirs of resistance genes and facilitate their spread via transduction, a process not commonly captured in existing resistance models. Therefore, building upon these established models by including phage populations and their dual roles is justified by both current research gaps and feasible given the available methodological precedents.

Summary of Preliminary Data. The structure for our model is derived from the ODE system made by Ibarguen-Mondragon et al. (2019), where S represents susceptible bacteria population, R represents resistant bacteria population, P represents plasmid population, and C represents antibiotic concentration. Our model will use this framework, but factor in bacteriophage dynamics instead of plasmids. Some key parameters for phage burst size and absorption rate will be taken from the parameters Beke et al. (2016) found from other literature sources.

$$\begin{aligned}
\frac{dS}{dt} &= \beta_s S\left(1-\frac{S+R}{K}\right)-(\overline{q}+\overline{\alpha}_S)\,CS-\overline{\delta}PS-\gamma S-\mu_s S\\
\frac{dR}{dt} &= \beta_r R\left(1-\frac{S+R}{K}\right)+\overline{q}CS+\overline{\delta}PS-\gamma R-\mu_r R\\
\frac{dP}{dt} &= \sigma_p R-\mu_p P\\
\frac{dC}{dt} &= \Lambda-\mu_c C.
\end{aligned}$$

Figure 2: The ODE system developed by Ibarguen-Mondragon et al. (2019)

Symbol	Definition	Default value
R	Concentration of glucose in the system	user defined [mg.ml^{-1}]
N	Population density of bacteria	user defined [count]
M	Population density of infected bacteria	user defined [count]
P	Population density of bacteriophages	user defined [count]
R_0	Glucose concentration in the reservoir	0.5 mg.ml^{-1}
ω	Flow rate	0.2 h^{-1}
ε	Growth efficiency	2×10^6 mg
μ_{max}	Maximal growth rate	calculated* [h^{-1}]
K	Glucose concentration at which the bacteria grow at one-half μ_{max}	0.0727 mg.ml^{-1}
Δ	Adsorption rate of bacteriophage on bacteria	2×10^7 phage.cell^{-1}.ml^{-1}.h^{-1}
B	Burst size	98 viruses per bacterial cell infected
τ	Latent period time	0.5 h
t	Run time of the experiment	user defined [h]
T	Experimental temperature	user defined [°C]
T_{min}	Minimal temperature	3.06°C
T_{opt}	Optimal temperature	41.10°C
T_{max}	Maximal temperature	45.06°C
pH	Experimental pH	user defined
pH_{min}	Minimal pH	3.88
pH_{opt}	Optimal pH	7.2
pH_{max}	Maximal pH	12.17
μ_{opt}	Optimal growth rate	2.635 h^{-1}

Figure 3: Parameters used in the model by Beke et al. (2016)

Expected Outcomes. The outcome of this aim is to develop a deterministic, ODE-based model that simulates the dynamics of susceptible bacteria, resistant bacteria, bacteriophages, and antibiotics. This model will generate predictions about

how phage therapy might alter the balance between susceptible and resistant strains. This model will serve as the core for the stochastic simulations that will take place in specific aim 2.

Potential Pitfalls and Alternative Strategies: While deterministic models are a simple and effective way to predict population numbers, nothing in the real world follows the steady patterns found in a deterministic model. Oftentimes, there is an element of randomness in bacterial models, which stochastic models can capture very well unlike their deterministic counterparts.

Specific Aim #2:

The second aim of this project is to develop a stochastic version of the model and perform Monte Carlo simulations on it. To turn the model from deterministic to stochastic, the parameters will be converted from constants to normally distributed random variables. In other words, instead of the parameters being set values for every simulation run, the parameters will be selected from an interval of values. For the Monte-Carlo simulation, 1000 simulations will be run, and for each simulation the final value for susceptible, resistant, and phage populations will be recorded and plotted on a bar graph. The average, median, and standard deviation for each population will also be calculated.

Justification and Feasibility. Deterministic models assume average behavior and can't capture the randomness in biological systems, such as different burst sizes per bacteriophage or tiny fluctuations in antibiotic concentrations. Merdan et al. (2017) directly compared deterministic, random, and stochastic models for resistant bacteria populations, and found that deviations in the resistant bacteria population in the stochastic and random populations reached up to 22.9%. Figure 4

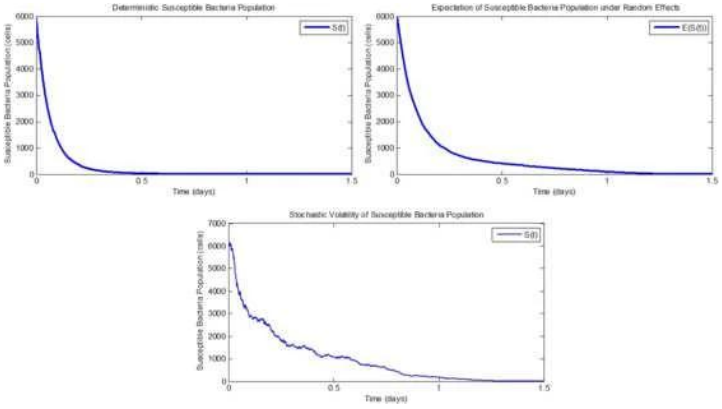

Figure 4: Graphs developed by Merdan et al. (2017) that show the difference between how a deterministic, random, and stochastic model predict susceptible bacteria populations over time

shows how stochastic models better capture external noise found in a real environment instead of the ideal environments found in deterministic and random models. Merdan's work justifies the need for stochastic methods to produce more realistic results and assess the risk of extreme outcomes. Their methodology of setting the parameters to a random variable in a confidence interval, adding noise throughout the simulation, and running Monte-Carlo simulations to capture all possible outcomes provides a clear, feasible template for this aim.

Summary of Preliminary Data. Merdan et al. (2017) developed a deterministic model like that of Ibarguen-Mondragon et al. (2019), where he sets S as the susceptible bacteria population, R as the resistant bacteria population, B as the immune cell population, and A as the antibiotic concentration. Just like Ibarguen-Mondragon's model, the immune cell dynamics will be replaced with bacteriophage dynamics, although they both serve similar roles as predators of bacteria. Marden also builds a stochastic model based on the deterministic equations, which will serve as the framework for our stochastic equations. The parameters from Figure 7 were used in both equations.

$$\begin{aligned}
\frac{dS}{dt} &= \beta_S S\left(1-\frac{S+R}{T}\right) - \bar{\eta}SB - S\frac{E_{\max}A}{E_{50}+A} - \mu SA - \sigma SR,\\
\frac{dR}{dt} &= (1-c)\beta_S R\left(1-\frac{S+R}{T}\right) - \bar{\eta}RB + \mu SA + \sigma SR,\\
\frac{dB}{dt} &= \beta_B B\left(1-\frac{B}{\Lambda}\right) - \lambda B(S+R),\\
\frac{dA}{dt} &= -\alpha A.
\end{aligned}$$

Figure 5: Deterministic equations developed by Merdan et al. (2017)

$$\begin{aligned}
\frac{dS}{dt} &= \left(\beta_S S\left(1-\frac{S+R}{T}\right) - \bar{\eta}SB - S\frac{E_{\max}A}{E_{50}+A} - \mu SA - \sigma SR\right)dt + \gamma_1 S(t)\,dW_1 t,\\
\frac{dR}{dt} &= \left((1-c)\beta_S R\left(1-\frac{S+R}{T}\right) - \bar{\eta}RB + \mu SA + \sigma SR\right)dt + \gamma_2 R(t)\,dW_2 t,\\
\frac{dB}{dt} &= \left(\beta_B B\left(1-\frac{B}{\Lambda}\right) - \lambda B(S+R)\right)dt + \gamma_3 B(t)\,dW_3 t,\\
\frac{dA}{dt} &= (-\alpha A)\,dt + \gamma_4 A(t)\,dW_4 t,
\end{aligned}$$

Figure 6: Stochastic equations developed by Merdan et al. (2017)

Parameter	Description	Value
β_S	Birth rate of sensitive bacteria	$0.8\ \text{day}^{-1}$
T	Carrying capacity of bacterial population	10^9 cells
c	Fitness cost	0.5 (dimensionless)
$\bar{\eta}$	Death rate of sensitive and resistant bacteria	$0.3\ \text{day}^{-1}$
μ	Sensitive bac. mutation rate by exposure to antibiotic	10^{-6} mut × gen
σ	Conjugation rate of bacteria	$10^{-5}\ \text{day}^{-1}$
$E_{\max}$	Maximum killing rate of antibiotic	$26.4\ \text{day}^{-1}$
E_{50}	Antibiotic concentration for half max. kill rate	$5\ \mu\text{g/ml}$
β_B	Recruitment rate of immune cells	$3\ \text{day}^{-1}$
Λ	Carrying capacity of immune cells	1.8×10^5 cells
λ	Loss rate of immune cells by apoptosis	$6 \times 10^{-6}\ \text{cells}^{-1}\ \text{days}^{-1}$
α	Dose of antibiotic administration	$5\ \text{mg/kg/day}$

Figure 7: Parameters Merdan et al. (2017) used in their mathematical models.

Expected Outcomes. The overall outcome of this aim is to generate a probability distribution of future bacterial and phage populations under various treatment scenarios. We will not obtain just a single prediction but a 9% confidence interval for the size of resistant and susceptible populations over time. This knowledge will be used to quantify uncertainty in the model's predictions and identify intervention scenarios such as increased phage populations that can work regardless of biological randomness.

Potential Pitfalls and Alternative Strategies. We expect that the assumption that there is a normal distribution for each parameter will likely fail for certain processes. An alternative strategy for this is to either use Poisson distributions or log-normal distributions. Additionally, running thousands of simulations for every model run has a high computational expense, which can be mitigated by optimizing the simulation code. There is also no way to validate the model with our current methods. A way we can verify our model is by running wet-lab tests, which will be explained more in depth in Specific Aim #3.

Specific Aim #3:

The third aim of this project is to validate and improve the models based on experimental data found through wet-lab testing. This will be done by growing a bacteria population in a petri dish along with phages for a week. After the week is over, I will measure the populations for susceptible/resistant bacteria and phages. I will do this multiple times over, varying the initial bacteria and phage populations each time. After I collect this data, I will compare it with the predicted population from my model. If the average error is greater than 5%, I will improve my model so it matches the values from the experimental data.

Justification and Feasibility. A major limitation in many current math models is the lack of empirical validation. This aim is justified by the need to verify our theoretical work with experimental data to ensure accurate predictions. We will adopt a two-tier experimental approach. First, in vitro

experiments in petri dishes will allow us to track population dynamics of a model bacteria, such as E. coli, and a specific phage under defined antibiotic concentrations, like how most models are set up. Second, in vivo validation is crucial as the environment in a host is vastly different compared to the predetermined conditions of an in vivo experiment. Xu et al. (2020) used metagenomic sequencing in mice to track gut microbiome shifts and ARG abundance, and recorded each bacteria's abundance compared to their initial population. Their work demonstrates the feasibility of obtaining data from an animal model to create model parameters related to immune response.

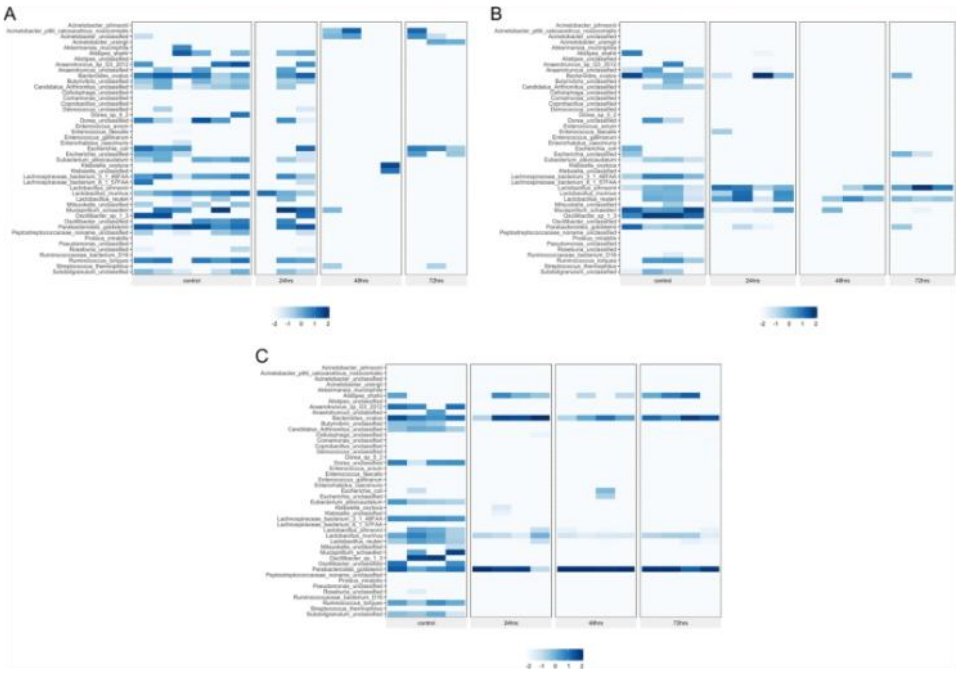

Figure 8: The relative abundance of 45 different bacteria species after being treated with Ampicillin (A), Ciprofloxacin (B), and Fosfomycin (C).

Summary of Preliminary Data. Bootsma et al. (2012) conducted experimental procedures finding the MICs of an E. coli strain over time when given a varying amount of antibiotics, and then graphed them. After that, the researchers built a system of ordinary differential equations, where E is the concentration of efflux pumps and A is the concentration of antibiotics.

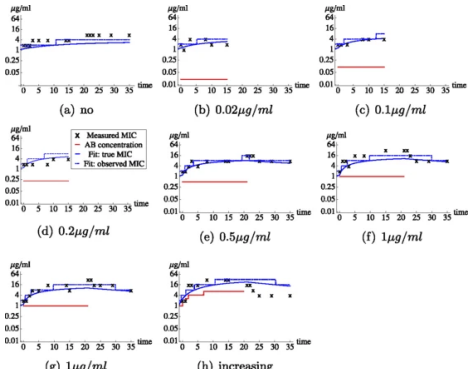

Figure 9: MIC values for an E. coli population over time when treated with varying levels of antibiotics

$$\frac{d}{dt}E = -\lambda E + c_0 + \frac{c_M A}{A_h + A}$$

$$\frac{d}{dt}A = \xi(A_0 - A) - \nu AE.$$

Figure 10: Deterministic equations developed by Bootsma et al. (2012)

While the data we will collect won't be the same, the overall methodology will be the same as Bootsma's apart from the fact we're building my model first and conducting experimental tests later.

Expected Outcomes. The overall outcome of this aim is a rigorously validated model. The experimental data will be used to adjust certain model parameters and model structure to minimize errors between simulated and observed population dynamics. The final, validated model will be a powerful tool for predicting the effectiveness of phage-antibiotic combination therapies, and will be used to propose specific, data-backed protocols for using phages to suppress antibiotic resistance.

Potential Pitfalls and Alternative Strategies. We expect that a phage-bacteria system will behave very differently in an animal model than in vitro due to the introduction of an immune system. We will address this by adding animal-specific parameters to the model such as an immune system parameter. Another pitfall is that handling mice in a lab environment requires extensive training and approval from various scientific organizations. An alternative solution to this is using a non-animal multicellular organism, such as fruit flies.

Section IV: Resources/Equipment

For the first part of my project where I build the model, the only things I need are a computer, Python, and data from various articles. For the wet lab portion of my project, I will need bacteria, bacteriophages, antibiotics, petri dishes for preliminary data, mice for further testing, and an environment to contain the mice (if there is time).

Section V: Ethical Considerations

For the computer simulation side of my project, there are no safety or ethical considerations. For the lab portion however, there are many safety and ethical considerations. For the bacteria, I need to wear protective clothing that cover my entire body and handle them and the antibiotics in a crossfire hood. After I'm finished with my experimentation, the bacteria will be treated with a 10% bleach solution for

30 minutes, and then they will be autoclaved. For the mice if I have time, I will use as few as possible to achieve the goals for my research. I will make sure they are provided with bedding, food, water, nesting material, tunnels/shelters to allow for natural behaviors, proper temperature/humidity/light, and anesthesia if they are to go through any painful procedures.

Section VI: Timeline

From November until December 19, I will continue reading articles and patents to learn more about my project. In early November, I will finish making my assumptions, building my base equations, and implementing my Python simulation. From late November to early December, I will perform my preliminary experiments (measuring susceptible and resistant population growth). From early December until December Fair, I will adjust my model so it aligns with the recorded data from the preliminary experiment. I will conduct experiments in a small animal ecosystem from early to mid-January, and will adjust the model again by early February. I will then prepare everything I need for February Fair.

Section VII: Appendix

Section VIII: References

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