SIMULATION AND THEORY OF ANTIBIOTIC RESISTANT BACTERIA POPULATIONS

by

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A Proposal Submitted to the Honors Council

For Honors in the Department of Physics

September 19, 2016

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1 Introduction

The threat of antibiotic resistance, even amongst currently well-controlled diseases, is becoming increasingly prevalent. In order to combat this rising phenomenon, we must first understand the conditions that allow emerging antibiotic resistant bacteria populations to dominate antibiotic sensitive populations. A dominant mechanism of intercellular transmission of antibiotic resistance is through transfer of plasmids encoding for antibiotic resistance [1].

In our work we plan to focus on two main methods of plasmid transfer between cells, namely transformation and conjugation. Both processes involve plasmids, which are small, independently replicating pieces of genetic material. We specifically consider plasmids including DNA segments encoding for antibiotic resistance. Incorporating a plasmid's genetic material actually has a directly negative impact on the host cell, imposing a small fitness cost[2] that manifests as an increased reproduction time as a result of needing to transcribe extra genetic information during reproduction. The somewhat seemingly contradictory nature of this is sometimes referred to as the "plasmid paradox" [3]. However, when presented with selective pressure such as exposure to antibiotics, plasmids become hugely beneficial to their host cells, allowing them to survive in conditions that would otherwise kill off entire populations [3][2].

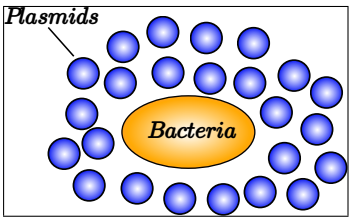
Through transformation, a sensitive cell acquires a plasmid from its environment, translating any encoded genes into its own DNA sequence. In conjugation, a resistant cell transfers genetic material to a sensitive cell through direct physical contact.

We plan to explore interactions between bacteria and plasmids in two significantly different environments. The well-mixed environment is analogous to a shaken mixture of bacteria suspended in a fluid in a flask, where cells are uniformly distributed and have equal access to plasmids. The spatially-structured case examines a situation like a biofilm, where bacteria have coalesced to form a two-dimensional, lattice-like layer. These present both the need for different simulation techniques, and vastly different results. Though we investigate applications to a biological system, we use mathematical and simulation frameworks which are also applicable in a wide range of physical and chemical contexts.

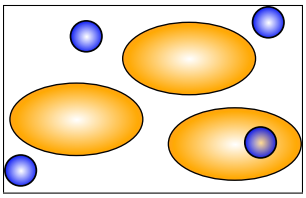
2 Project Description

2.1 Preliminary Investigation

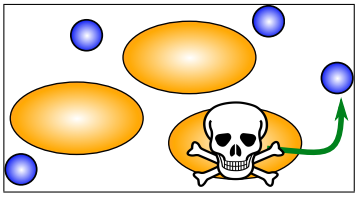
Work conducted in the summer of 2016 provides preliminary results which we plan to use as a starting point for more sophisticated simulation. We focused on transformation as the dominant method of plasmid transfer, and examined three primary regimes of different transformation rates α under which it occurs, demonstrated in Figure 1.



(a) Plasmid abundance effectively yields a constant rate of transformation, α.



(b) A fixed initial population of plasmids that are consumed by transformation yields the linear α case.



(c) A feedback mechanism where dead cells return free plasmids to the environment defines the recycled α case.

Figure 1: Transformation regimes explored in preliminary research.

2.1.1 Reactions

In our previous research, we defined four main reactions as follows:

Reactions		Equations	
$S \xrightarrow{b_S} 2S$	(1)	$\frac{dS}{dt} = b_S \left(1 - \frac{S+R}{K}\right) S - \alpha \left(\frac{P_f}{P}\right) S$	(5)
$S + P_{free} \xrightarrow{\alpha} R$	(2)	$\frac{dR}{dt} = b_R \left(1 - \frac{S+R}{K}\right) R + \alpha \left(\frac{P_f}{P}\right) S - \delta R$	(6)
$R \xrightarrow{b_R} 2R$	(3)	$\frac{dP_f}{dt} = -\alpha \left(\frac{P_f}{P}\right) S + \delta R$	(7)
$R \xrightarrow{\delta} \varnothing + P_{free}$	(4)	$\frac{dP}{dt} = b_R \left(1 - \frac{S+R}{K}\right) R$	(8)

Table 1: The reactions and associated differential equations modeled by our simulation. Highlighted in red and blue are terms specific to the linear and recycled α cases, respectively.

These reactions describe S cell birth, transformation, R cell birth, and R cell death respectively. The additional colored terms are illustrative of the three different transformation regimes. The added P_{free} term in Equation 2 defines the linear α case shown in Figure 1b, where a free plasmid from the environment must be consumed for transformation. Additionally, adding a free plasmid to the products of Equation 4 describes the recycled α case.

On the equation side, the constant α case is described by just two equations, Eqn. 5 and Eqn. 6, which describe the S and R population growth rates. In order to describe the linear case it is necessary to now track not only the number of free plasmids, but also the number of total available plasmids, which will now be increasing as a result of what we refer to as symmetric reproduction. Essentially, when Equation 3 occurs, an additional R cell is created without consuming a free plasmid, and so the total number of plasmids in the system increases by one. An equation for the recycled case is obtained by simply incrementing free plasmid count proportionally with R cell death.

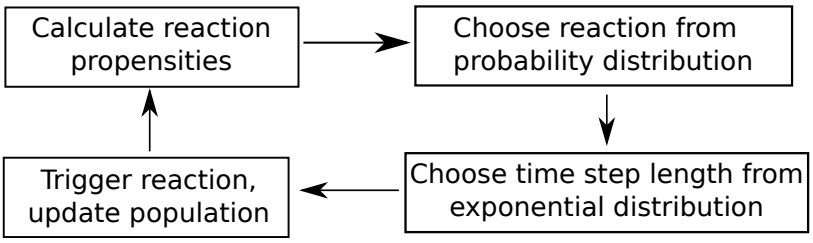


Figure 2: Basic diagram of Gillespie Algorithm. Reactions are randomly selected based on propensities, which are calculated using their rates of reaction. Time step length is chosen from an exponential distribution such that it adjusts itself based on the number of particles being simulated. More details are available in Appendix A.

2.1.2 Simulation overview

We implementated the Kinetic Monte Carlo (K.M.C.) method (or Gillespie Algorithm) to perform our simulations. This is an algorithm that was initially developed for simulating stochastic chemical processes. However, the K.M.C. method is agnostic to the actual quantities being simulated, relying only on having a set of reactions that occur with given propensities. This means that our simulation methods are not specific to what we're simulating, just to the reactions and population sizes, and therefore can be generalized to a wide range of systems. Furthermore, K.M.C. randomly samples time step length from an exponential distribution. Since one reaction occurs per time step, adjusting the time step length allows the algorithm to adjust for a larger number of particles, which would have reactions occuring more frequently, and conversely to a smaller number of particles, which would have less frequent reactions.

2.1.3 Preliminary results

We obtained results for all three transformation mechanisms in the well-mixed case. Most notably, we discovered a transition point where for certain ranges of birth rate and α values, population dominance switches from the S to R population. We found that this transition point is heavily dependent on the transformation mechanism, and very minimally sensitive to the actual value of α. Furthermore, the linear case tends to population extinction, with the most dynamic behavior of the three.

2.2 Proposed Research

We plan to continue this research by expanding our simulation to model the populations more accurately. Specifically, we plan to accomplish this by incorporating three main new elements, and studying their impacts.

	Transformation	Conjugation	Diffusion	Antibiotic Exposure
Well-Mixed	✓	$O\square$	×	
Lattice	?	×	×	

Table 2: This shows aspects of our research we either have completed or will study. Well studied regimes are denoted by a checkmark, an empty circle indicates an area we will explore, and a shaded circle represents an area we have begun to explore, but have not fully analyzed.

2.2.1 Conjugation

Firstly, we plan to introduce a new reaction for cell-to-cell conjugation as in Equation 9.

$$S + R \rightarrow 2R \tag{9}$$

Presently, we consider transformation as the exclusive mechanism of plasmid transport. However, conjugation is also a common process, and may introduce interesting system dynamics as populations of R cells bordering groups of S cells now have a mechanism to directly transfer plasmids encoding antibiotic resistance to them.
To successfully study the effects of conjugation on our system, we will also incorporate it into our mathematical model to obtain predictions for behavior. To do this, we will need to add an additional term to Equations 5 and 6.

2.2.2 Diffusion

Presently, in the spatially-structured case we do not include any model of cellular motion, instead assuming that a cell stays at its initial lattice site for its entire lifetime. As a result, populations can only spread out by reproduction, which randomly places daughter cells at adjacent lattice sites. In reality, bacterial cells are capable of individual motion. We plan to simulate this using a simple diffusion model, where populations will attempt to slowly spread out of high concentration areas into lower

concentration areas. This will involve adding a new reaction which will move a random cell. This is specific to the lattice case, so we will not be able to mathematically model it.

2.2.3 Antibiotic Exposure

The crux of this research is determining which conditions lead to and which conditions mitigate antibiotic-resistant bacteria population dominance. An important consideration in this is if a population is dosed with antibiotics early on when each population numbers relatively few, this could lead to an early extinction of sensitive cells, even in situations that would otherwise tend to sensitive cell dominance. In order to better understand this potential effect, we plan to simulate different regimes of antibiotic exposure to see how they affect population dominance over time.

3 Contribution

My role in this project will involve work on both the computational and theoretical aspects of our research. In our work from the summer of 2016, I developed two computer simulations we used to study the populations, one for the well-mixed case, and one for the spatially-structured case. I was responsible not only for programming the simulation, but for implementing a number of optimizations without which the simulation would have taken too long to run to gather meaningful data. These were exceptionally useful in the spatially-structured case due to the hugely increased computational complexity. I have acquired experience in software development and simulation through a number of projects outside of my research at Bucknell, including work on parallelized multiprocessing and simulation development. As our project becomes increasingly technically demanding, I will continue to apply my unique experience in this area to further develop our simulation and add the more detailed and accurate features mentioned above to it. These improved simulations will allow my advisor to conduct more in-depth study for her own research on bacterial population dynamics.

Additionally, in our summer research I helped develop the differential equations that characterize the populations. I proposed and developed the equations for the free plasmid and total plasmid numbers, which were necessary for both the linear and recycled case. I also used these models to generate and analyze predicted population trajectories[5], which I verified using my simulation. I plan to apply similar techniques as we expand our simulation, and believe this will be increasingly useful as our population dynamics become more complex.

4 Significance

Antibiotic resistance is a rising epidemic the World Health Organization describes as "so serious that it threatens the achievements of modern medicine."[6] Antibiotic resistant mutations of currently well-contained diseases such as tuberculosis and bacteria like E. coli present us with strains of these familiar ailments that can be completely nonresponsive to contemporary treatments. Startlingly, the WHO also states that study on antibiotic resistance is "neither coordinated nor harmonized", leaving us with "many gaps in information on bacteria of major public health importance."[6] Our next steps will, among other things, give us deeper insight into how antibiotic dosing affects the dynamics of population growth. This will allow us to compare relative effectiveness of different antibiotic dosing regiments currently being used for treatment.

An in-depth understanding of this phenomenon is an essential first step towards being able to combat it. Our research provides a means by which we can begin to model how these populations develop, and what factors are most critical in whether sensitive or resistant bacteria dominate the population. By making a more detailed, refined model, we improve our ability to be able to hone in on certain factors. Already we have obtained significant, meaningful results, such as finding that in the parameter ranges we have considered, the fitness cost imposed by the plasmids on the cell is only a second-order effect, and does not significantly affect dominance.

As I go forward into graduate school and more research, this project will give me invaluable experience in the research process, and in conducting simulation and modelling of complex problems with real-world consequences.

References

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Gillespie Algorithm details

1. Initialize simulation
2. Calculate propensity a for each reaction
3. Choose reaction μ according to the distribution

$$\mathrm{P}(\text{reaction } \mu) = a_\mu / \sum_i a_i$$

4. Choose time step length τ according to the distribution

$$\mathrm{P}(\tau) = \left(\sum_i a_i \right) \cdot \exp\left(-\tau \sum_i ai \right)$$

5. Update populations with results of reaction
6. Go to Step 2

See Gillespie (1976) for more details.[4]