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Simulating Bacterial-Antibiotic Interactions through Agent-Based Modeling

Hanna Qasim [1] and Talhah Peerbhai [2]

[1] Carnegie Mellon University; hqasim@andrew.cmu.edu
[2] Carnegie Mellon University; tpeerbha@andrew.cmu.edu

Abstract: Agent-based models are stochastic models constructed from the bottom up, meaning that individual agents are assigned certain attributes. They are computer simulations used to study interactions between people, things, places, and time (*Agent-Based Modeling*, 2022). In our context, we used agent-based modeling to build a simulation model of bacterial growth and antibiotic resistance to understand the complex interactions between bacteria and antibiotics with each other and their environment. This approach offers a logical framework for deducing low-level biochemical details about the individual molecular components to high-level pharmacodynamic parameters, like an antibiotic's MIC (Minimum Inhibitory Concentration). However, note that this simulation of the bacteria and antibiotics shown is for the general populations of bacteria and antibiotics in an environment with predefined parameters and is only concerned with vertical transfer mechanisms of antibiotic resistance genes. By applying the computational methods our work has developed, we aim to give researchers a more efficient way to discover new ways to fight against antibiotic resistance.

Keywords: Bacteria, antibiotics, enzymes, antibiotic-resistant bacteria, simulation, agent-based modeling

1. Introduction

Antibiotic-resistant bacteria are becoming a major problem worldwide, endangering human health. To tackle this threat effectively, we need to understand how resistance develops over time, however, the usual lab experiments are slow and costly, which restricts our ability to study all the different ways resistance can evolve. The primary inquiry of this study is to explain how antibiotic resistance emerges and propagates across different scenarios where antibiotics are utilized. Building upon the framework established by Murphy and Walshe (2010) and Park et al. (2018), our study seeks to explain the mechanisms underlying antibiotic resistance evolution. Current literature reflects a diverse array of methodologies employed to tackle the challenge of simulating antibiotic resistance. Agent-based modeling, characterized by its bottom-up approach, offers a powerful framework for dissecting the complexities of resistance evolution. By simulating individual agents, this methodology captures emergent behaviors and facilitates a nuanced exploration of the impact of the introduction of antibiotics on bacterial populations. This study employs visualization techniques via model simulation, to observe the interactions between bacteria and antibiotics, as well as their interactions with the surrounding environment. We do this by modeling the interactions between bacteria and their environment in a 2-dimensional grid, using *Mesa* (a Python library for agent-based modeling), where each bacterium is an agent that can interact with the environment and other agents. The model simulates the growth and behavior of the bacteria under varying conditions, including exposure to antibiotics. The parameters governing bacterial behavior, such as growth rate and nutrient uptake, are derived from existing literature and experimental

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Received: date
Revised: date
Accepted: date
Published: date

data. These parameters are crucial for accurately simulating bacterial responses to antibiotic exposure and environmental stimuli. For modeling enzyme activity and antibiotic degradation, we employ Michaelis-Menten kinetics equations, drawing on established biochemical principles to simulate the enzymatic processes involved in antibiotic resistance. In modeling resistance transfer, we focus primarily on vertical gene transfer occurring during bacterial replication. While horizontal gene transfer plays a significant role in real-world resistance dynamics, our simulation simplifies this aspect by focusing on a single bacterial strain. Overall, our methodology leverages agent-based modeling techniques and established biochemical frameworks, through which we aim to explain the complex interplay between antibiotics and bacterial populations and aid researchers in discovering new ways to combat antibiotic resistance.

2. Materials and Methods

Our study does not require any specialized datasets, as our developed program is designed for generalizability. It relies on experimentally derived values pertaining to specific bacterial strains, antibiotic kinetics, and enzyme kinetics. Therefore, these values can be obtained from existing research or through experimental determination.

Our approach aims to develop a simulation focusing on bacteria that produce beta-lactamase enzymes and interact with beta-lactam antibiotics, such as penicillin-class antibiotics. Employing an agent-based modeling approach, each bacterium is individually modeled with its own set of rules, facilitating the emergence of behaviors reflective of real-world scenarios (Murphy & Walshe, 2010).

However, it is important to acknowledge certain considerations inherent in simulating a complex analog world with myriad conditions and variables. To streamline the scope and address computational limitations, we will prioritize the inclusion of essential conditions while omitting factors such as temperature, acidity, light availability, etc. Additionally, the computational demands posed by exponential bacterial growth necessitate constraints on the model's scale. Thus, we will focus on modeling a single strain of bacteria and implement discrete time steps to manage computational complexity. Furthermore, to simplify the model, we will exclusively simulate vertical gene transfer, a consequence of modeling a single bacterial strain.

The model comprises four primary components: the environment, bacteria, antibiotics, and enzymes. The environment serves as the backdrop, housing bacteria, antibiotics, enzymes, and essential nutrients necessary for bacterial growth and interaction.

2.1. Environment

The environment is represented as a discrete 2-dimensional grid, a choice driven by resource constraints that prevented a 3-dimensional simulation. While this limitation may marginally affect the realism of our results, we anticipate that it will not substantially compromise the validity of our findings.

To adhere to standard conventions and avoid confusion with bacterial cells, each unit within the grid is referred to as a "patch." Each patch in the simulation encompasses three layers: antibiotic concentrations, nutrient units, and enzyme concentration levels specific to that patch. The diffusion of these layers is facilitated by a discretized implementation of Fick's First Law of diffusion, realized through a diffusion algorithm (Gisbert et al., 2002). Note, that the grid we used is non-toroidal, hence the boundary conditions reflect any diffusing molecules back into the cell. The diffusion process is governed by the following equation: the concentration difference between adjacent patches and the current one is multiplied by a diffusion coefficient. For diagonally adjacent patches, the same computation is performed, but the result is multiplied by a factor of $\frac{1}{\sqrt{2}}$. The environment is initialized with the following parameters:

Table 1. Environment Initialization.

Parameters	Values
Width	1000
Height	1000
Nutrient diffusion coefficient	0.01
Maximum nutrients	300
Minimum nutrients	200
Nutrient distribution type	Random

[1] In these parameters, we have two types of nutrient distribution. The first type is "random" distribution, which places nutrient biomass units randomly across the grid within a range between the minimum and maximum nutrient levels. The second type of distribution is "uniform," where all patches are assigned the maximum nutrient level.

2.2. Bacterial-Agents

The bacteria agent class concerns itself with the behavior and attributes of the individual bacteria agents. Each bacterium agent possesses properties such as biomass, nutrient intake rate, resistance to antibiotics, and growth phase characteristics. The agents then interact with their environment by consuming nutrients, responding to antibiotic exposure, reproducing, and moving within the 2D grid based on nutrient availability and overcrowding conditions and eventually death. For this, we had the following parameters:

Table 2. Bacterial-Agent Initialization.

Parameters	Values
Number of initial agents	1
Nutrient intake (b.u $loop^{-1}$)	6
Biomass threshold before splitting (b.u)	6
Initial biomass (b.u)	2
Minimum biomass before death (b.u)	2
Survival cost (b.u $loop^{-1}$)	1
Lag phase (loop)	25
Beta-Lactamase production rate (uM $loop^{-1}$)	25
Maximum number bacteria in patch	4
Resistant	True

2.2.1. Growth

The agents grow by consuming nutrients in their grid cells. The rate of growth is influenced by factors such as nutrient intake rate and metabolic parameters, however, note that growth may be restricted in certain conditions, such as exposure to antibiotics or a lack of nutrients available. Additionally, there is a survival cost that influences the growth rate; at each step, some biomass is removed to account for metabolic processes within the cell. These values must be experimentally determined, and the maximum number of bacteria in a patch will depend on the estimated diameter of the bacterial cells and the determined size of each patch. Bacterial agents also have a minimum biomass, and once they reach this threshold, they are unable to grow and will die.

2.2.2. Reproduction

Cell division occurs once the lag phase has elapsed, and the bacteria have adapted to the environment. Once this period has elapsed, another requirement for division is that the agents must have reached a certain biomass threshold to split. Vertical gene transfer is implemented, meaning that daughter cells will inherit any traits of the mother cell, including resistance.

2.2.3. Movement

Bacteria move either randomly or toward a higher concentration of nutrients if they are at most one patch away from a location with higher nutrient levels than their current cell. If no nutrients are present, they will continue moving around in search of nutrients until they eventually die if none are found. The bacteria also move around while taking into account the possibility of cells being overcrowded. This aspect will be further addressed in the next part about modeling.

2.2.4. Overcrowding

The patches have constrained size and capacities and can fit only a limited number of bacteria in them. If a patch becomes overcrowded, bacteria are relocated to adjacent patches; the probability of moving to neighboring patches is calculated inversely proportional to the relative masses. The adjacent cell with the highest probability is selected for the current agent to move to.

2.2.5. Interaction with antibiotics

In our model, we will consider antibiotic responses when the concentration reaches the MIC (Minimum Inhibitory Concentration). At this point, bacteria will immediately stop growing and begin to lose their biomass. If the bacteria show resistance, they will start producing beta-lactamase enzymes to degrade the antibiotics.

2.3. Enzymes

The enzymes help in degrading the antibiotics, with the parameters for degradation determined experimentally through kinetic values for both the enzyme and antibiotics. We utilized the standard Michaelis-Menten equation, $V_0 = V_{max} * \frac{[S]}{K_m + [S]}$, to calculate the degradation rate, where [S] represents the antibiotic concentration. In addition to this interaction, the enzymes also have a half-life, leading to gradual decay over time.

2.3. Antibiotics

Antibiotics are modeled under two conditions: either when a specific bacterial population threshold is reached or after a predetermined duration of time has elapsed. The concentration of antibiotics added can be specified, and diffusion can be enabled or disabled for their distribution. The antibiotics can also either be randomly distributed or placed towards areas of highest bacterial concentration, but, due to computing constraints, the density calculation may not be as precise as desired; each simulation run would require approximately an hour, which is not feasible within current time constraints. However, future studies could explore the possibility of improving the accuracy of density calculations.

2.3. Simulation Model

The simulation model class orchestrates the simulation process by initializing the grid environment by placing the bacterial agents within the grid and advancing the simulation over discrete time steps. It also deals with other crucial components of the simulation model such as diffusing the nutrient and antibiotic concentrations and the population dynamics of the bacterial agents.

2.4. Evaluation Metrics

To comprehensively assess our simulation of bacterial-antibiotic interactions, we will employ a comparative and sensitivity analysis on our model. Conducting a comparative analysis allows us to assess the models performance relative to alternative modeling

approaches such as the Murphy and Walshe (2010) and Park et al. (2018) papers that we are modifying and reimplementing. Thus, comparing outcomes with these papers facilitates our understanding over the strengths and limitations of our model in capturing the complexities of bacterial-antibiotic interactions. Further, assessing the robustness of our model's parameters is also essential, as sensitivity analysis helps in the identification of critical parameters that drives model behavior and reveals sources of uncertainty or variability in the results, all of which that will help us to refine our model's assumptions or parameter estimates.

3. Results

In this study, the simulations were divided into two experimental groups. The first group focused on implementing and observing bacterial growth, while the second group aimed to predict the consequences of antibiotic addition to multiple bacterial groups.

3.1. Simulation of bacterial growth

We initially examined the bacterial growth across 200-time steps, which revealed an exponential increase in the bacterial population, ultimately depleting all available nutrients within the grid and reaching an approximate count of 40,000 agents. While parameter selection was somewhat arbitrary, efforts were made to align them as closely with real-world conditions. The results proved to be as expected (Figure 1), the proliferation of the bacterial agents illustrated exponential growth until the 75th time step, after which it gradually began to taper off. By the 130th time step, a significant portion of the bacteria began to perish due to nutrient exhaustion, leading to stress-induced mortality; given additional time, the population would have gone down to zero. In Figure 2, we plot an analysis of the overall number of agents produced. Although the graph's resolution may prevent immediate insights, a visible trend is seen to emerge after the 25th time step, indicating a progressive increase in the bacterial population from the initial 1 to eventually a peak of approximately 40,000 agents. Note, that the initial flat line preceding the 25th time step corresponds to the lag phase, during which the bacteria are still adapting and adjusting to the environment.

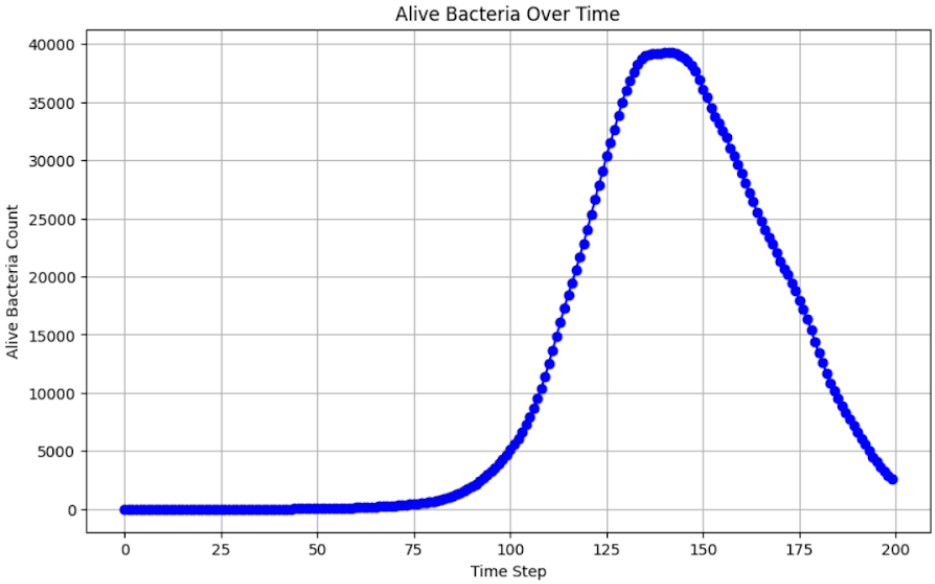

Figure 1. Simulation of bacterial growth results. Alive bacteria over time.

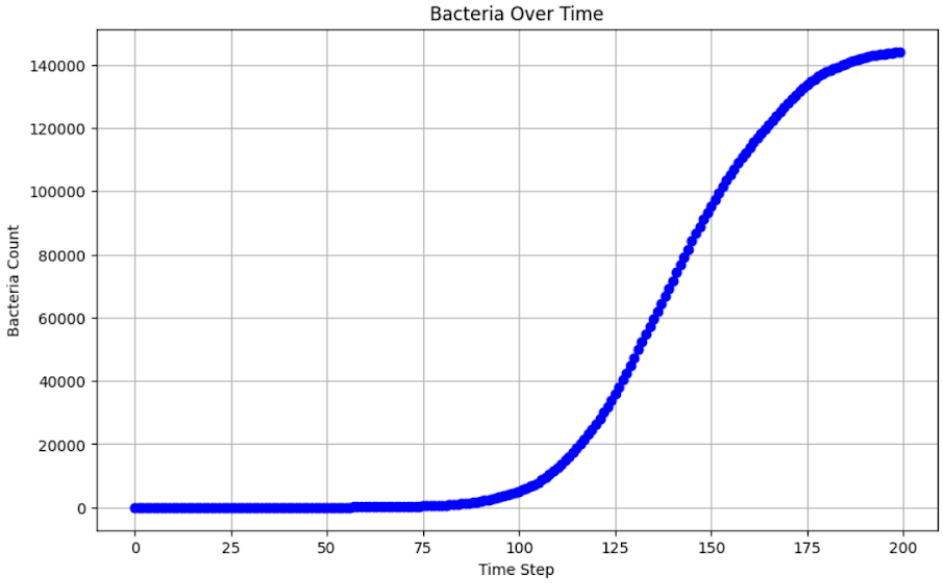

Figure 2. Simulation of bacterial growth results. Bacteria over time.

3.2. Simulation of antibiotic-bacterial interactionl and resistance

With a comprehensive understanding of bacterial growth dynamics, we will investigate how the addition of beta-lactam antibiotics impacts the bacteria's growth. The bacterial strain under examination in our simulation exhibits resistance to the antibiotics and initiates the production of enzymes targeting the beta-lactam ring, thus facilitating antibiotic degradation. Multiple doses of antibiotics are introduced, starting at the 50th time step with another at the 60th time step. These early introductions explain the significantly reduced number of bacterial agents observed compared to the ones in the bacterial growth simulation. In Figure 3, we model the number of bacteria alive over time. Initially, exponential growth is evident, but following the antibiotic doses, many agents cease growth, leading to gradual cell wall degradation and subsequent decline in number of bacteria as they die. By the 150th time step, resistance becomes apparent as the enzymes produced degrade the antibiotics, resulting in an exponential resurgence of bacterial agents. The subsequent decrease in bacteria around the 180th time step is due to the scarcity of available nutrients in the immediate area of the bacteria. Additionally, the presence of antibiotics from the earlier doses has caused the nutrient concentrations to be unevenly distributed, restricting bacterial movement across the grid. As a result, bacterial proliferation is hindered in areas where nutrient availability is limited. In Figure 4, we depict the bacterial growth over time, inclusive of both visible and non-visible agents. A distinct lag phase is observed around the 25th step, followed by a pause in bacteria growth after the 50th step due to the antibiotics's effects, before resuming growth around the 150th step.

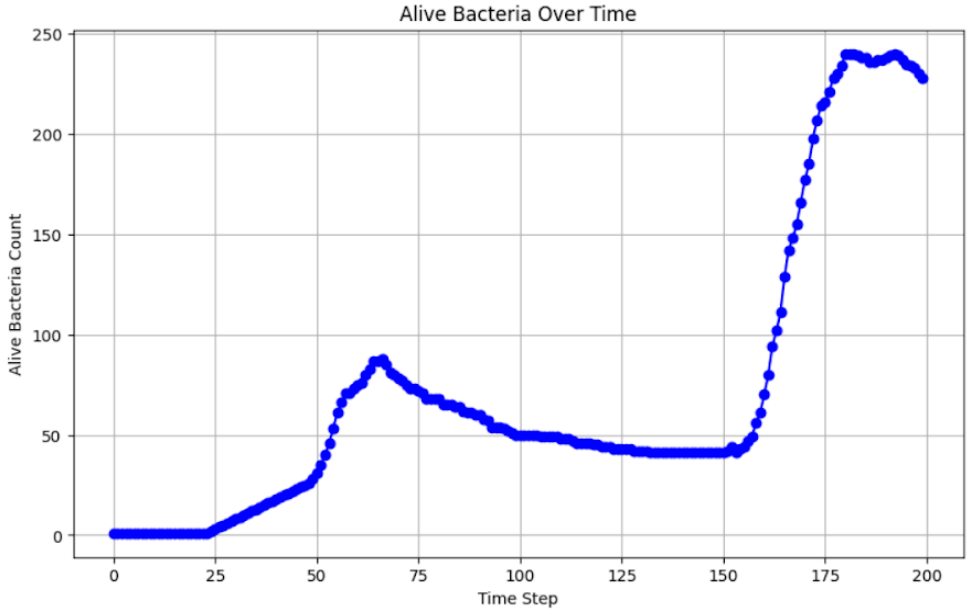

Figure 3. Simulation of antibiotic-bacterial interaction and resistance. Alive bacteria over time.

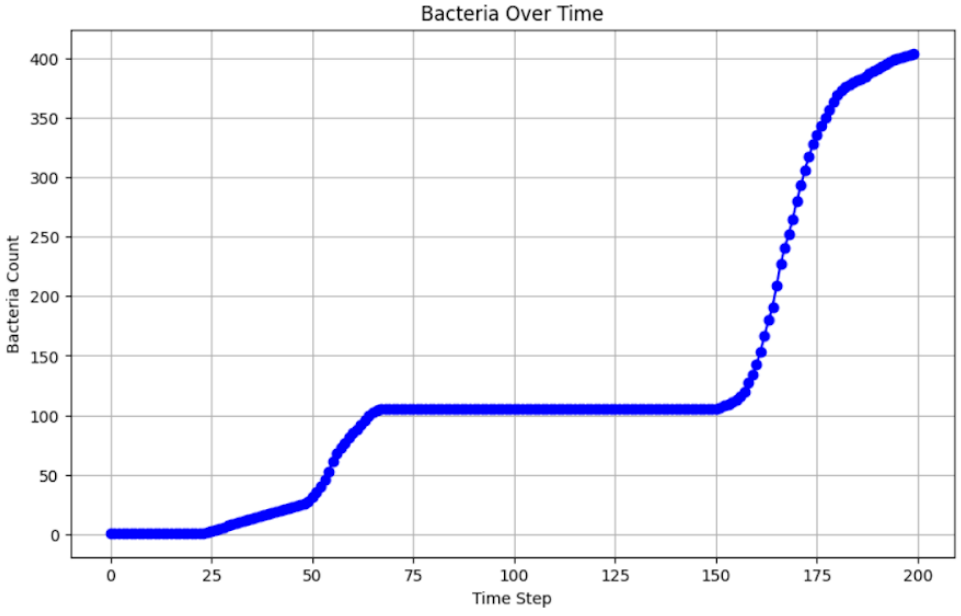

Figure 4. Simulation of antibiotic-bacterial interaction and resistance. Bacteria over time.

3.2. Simulation of antibiotic-bacterial interactionl and no-resistance

In the final simulation round, we investigate the impact of antibiotics on a bacterial strain that lacks resistance to these medications. The results are as expected, in Figure 5, the plot illustrates the population of viable bacteria over time. Initially, bacterial growth follows an exponential pattern; however, following the introduction of antibiotics around the 50th time step, there is an abrupt decline in growth. Subsequently, we observe a gradual decrease in the population of alive bacteria. This decline in the number of bacteria is attributed to the stress imposed by the antibiotics, which limits bacterial growth. For the remaining alive bacterial agents, their capacity for cell division is compromised, leading them to rely on the available nutrients until their cell walls degrade completely, leading to their eventual demise.

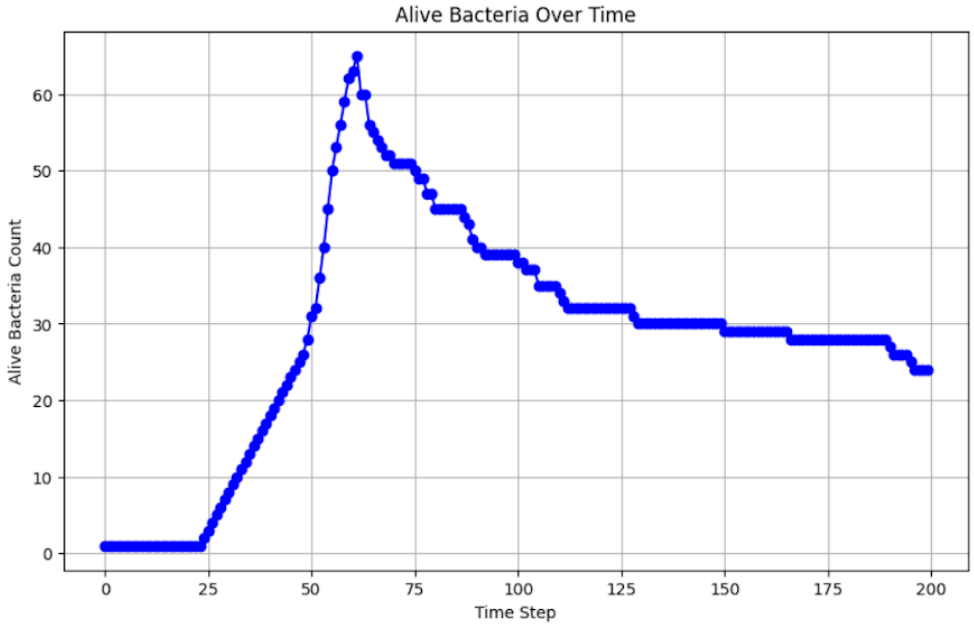

Figure 5. Simulation of antibiotic-bacterial interaction and no-resistance. Bacteria over time.

4. Conclusion

Overall, the main purpose of this study was to design realistic models of bacterial growth and antibiotic-bacterial interactions, to explain how antibiotic resistance emerges and propagates across different scenarios where antibiotics are utilized. We designed a bacterial growth model and verified its validity by comparing the results to the four phases of bacterial growth, namely: lag, exponential, stationary, and death. We also designed an antibiotic-bacterial interaction and resistance model which explored the actions and interactions carried out by the bacteria, enzymes, and antibiotics with each other and the environment.

Accurately, simulating resistance evolution helps us gain a deeper understanding of bacterial populations and the selection pressure they face; and through such insights and simulations, we can get closer to understanding the origins of resistance and develop more effective antibiotics. This is of utmost importance as according to the Centers for Disease Control and Prevention (CDC), at least 2.8 million people contract antibiotic-resistant infections annually, resulting in over 35,000 deaths (National Infection & Death Estimates for AR, 2022). Thus, enhancing our understanding of bacterial evolution could save numerous lives by providing new ways to fight against antibiotic resistance.

Future studies, as computing resources become more abundant, can advance modeling techniques to optimize hardware utilization and enhance accuracy. This can be achieved by incorporating a three-dimensional grid and accounting for additional environmental factors such as temperature and acidity. Expanding the model size to better emulate the spatial dimensions of a petri dish would further enhance representativeness. However, it's important to acknowledge that the choice of language and frameworks for this study are a limitation due to Python's garbage collection mechanism, which resulted in significant overhead and time loss.

An interesting avenue for future exploration would be to implement machine learning algorithms for each of the individual agents, allowing them to formulate their own rules and strains, thereby providing a more accurate simulation of evolution and offering insights into gene divergence and resistance mechanisms. In addition to this, future studies should also consider investigating a broader range of bacterial strains, including strains with varying degrees of resistance. This approach would yield a better understanding of antibiotic effectiveness and the diverse growth dynamics shown by the different strains. Similarly, strains other than the beta-lactamase-producing strains should

be explored for a comprehensive understanding of antibiotic efficacy across various bacterial strains as well.

Acknowledgments: This work has been carried out as part of an academic undergraduate program at Carnegie Mellon University Qatar, and was supposed by grant from Qatar Foundation. We thank Professor Madhavi Ganapathiraju for guidance and support during this process.

Author Contributions: Conceptualization, methodology, implementation and visualization: HQ TP. Data analysis and interpretation: HQ TP. Wrote the paper: HQ TP. Developed software: HQ TP.

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