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Modeling and Simulation of the Resistance of Bacteria toAntibiotics

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ABSTRACT

The unnecessary use of antibiotics has given rise to antibiotic resistance and for this reason is a cause of growing concern in contemporary health care contexts. Antibiotic resistance means that an antibiotic is losing or has lost the ability to kill a given bacteria and/or to prevent it from reproducing. The result: an increase in the number of patients suffering from and even dying of infections. Resistant bacteria continue to increase in number, as they survive the antibiotic designed and used to kill them. The disease induced by the bacteria lasts longer, therefore, than would have been the case were the bacteria not antibiotic resistant. Thus, prolonged treatment and/or even death results together with an increase in cost associated with these outcomes. The purpose of this study is to investigate the interactions among the bacteria, immune system cells, and antibiotics in a Repast Simphony 2.1 agent-based simulation environment modeled to observe the effects of the antibiotic resistance in the infection process. According to our results, increased antibiotic resistance constitutes a serious threat to the success of established methods used in the treatment of bacterial infections.

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1. Introduction

In the domain of biology, the process of bacterial evolution and the biology of bacterial population are the main ways to describe antibiotic resistance—a phenomenon that poses a significant threat to human health.Antibiotic resistance refers to case in which an antibiotic has lost or is losing its ability to kill or preventthe reproductionof a bacteria it was designed to combat. This phenomenon leads to an increase in the number of patients suffering from and even dying of infections given the failure of treatment. In this study, we explore the interactions among the bacteria, immune system cells, and antibiotics by mimicking a real biological environment and thereby observe the effects of antibiotic resistance on the infection process.In order to explore the dynamics of antibiotic resistance, we developed an agent-based model that includes immune system cells, bacteria as agents, and nutrient and antibiotic layers as the simulated environment's objects.

Agent-based modeling is a rule-based computational modeling approach that focuses on rules and interactions among the individuals or components of a real system. The aim is to generate a large set of interacting agents and simulate their interactions and behaviors in a represented environment.Using agent-based modeling, we can develop an understanding of the mechanisms of antibiotic resistance and the dynamics of microbiological systemsthat take place in the process of bacterial evolution. Given that the properties of the process of bacterial evolution and the biology of bacterial population such as collective behavior can be accounted for by

simple rules of operation, lack of central control, adaptation, and sophisticated information processing, agent-based modeling is the most suitable technique for modeling bacterial populations and antibiotic resistance.
There are several approaches to modeling the effects of antibiotic resistance, and research on this topic has been widely published in journals. A mathematical model of bacterial transmission in a hospital is elaborated in [1] to show the effects of measures designed to control the nosocomial transmission of bacteria and to decrease antibiotic resistance in nosocomial pathogens. In order to explore the efficacy of cycling programs, a mathematical model of antimicrobial cycling in a hospital is developed and explained in [2]. In [3], a mathematical model is used to identify the conditions in which resistant bacteria continue to exist in a hospital and conversely to define the conditions in which prevalent resistant bacteria can be removed completely from a hospital environment. A model of the transmission dynamics of infection in the presence of dual resistance to antibiotics is developed by tracking several patients in hospital settings to observe their colonization status [4].
In this paper, we present our model for bacterial resistance to antibiotics in detail. The principal way in which our study is distinguished from other research in this area is that the system we propose is relatively simple: we identify simple rules for interactions between elements without complex mathematical calculations. Our goal in this regard is to offer a system that is as simple as possible through a bottom-up modeling approach.
The remaining sections of this paper are organized as follows. Section 2 provides background information detailing the biology of bacterial population. Section 3 presents a brief explanation of the fundamentals of Agent-Based Modeling and Simulation (ABMS). Section 4 introduces the agent-based model. Section 5 illustrates the experimental model developed for the study, presents the data, our analysis, and a discussion of the approach we used. Section 6 presents a summary of this research study.

2. Bacteria

Populations of bacteria known as bacteria flora live on human skin and in the mouth and digestive tract. Most bacteria flora iscommensal or mutualist andare not harmful in the parts of the body where they ordinarily exist. However, they can be harmful if introduced to other parts of the body. If communalistic and mutualistic bacteria do find their way to these other parts of the body, however, a bacterial infection can develop in the host as a consequence.
Pathogens, which do not usually live in healthy human bodies and are transmitted to healthy individuals from infected people, can also cause infections. Antibiotic treatment is often effective against both infections caused by flora bacteria and those caused by pathogenic bacteria. Before the discovery of penicillin, it was very difficult to treat some infections such as ear infections and bacterial pneumonia. However, antibiotics changed this, as they significantly improved a patient's chances of recovering from numerous kinds of bacterial infections. At present, there are almost 100 different antibiotics in clinical use. Yet, hospital patients continue to develop infections that cannot be cured by antibiotics because of bacterial evolution.
As it provides substance for natural selection, the emergence of mutations is an important phenomenon underlying evolution. Bacterial cells invade a host and then each bacterial cell divides,thereby forming two daughter cells. Not all the cells in a bacterial population are genetically identical, as cell division sometimes results in mutations,some of which reduce a cell's ability to survive and reproduce independently of the environment. However, the effects of many mutations are dependent on the environment.
A random mutation can change a bacterial protein required for the antibiotic to enter bacteria cells. In this case, the antibiotic will not be able to enter a mutant cell and, therefore, will not be able to undermine the protein synthesis. Thus, the mutant cell will reproduce even though an antibiotic has been introduced. In contrast, antibiotic-sensitive cells either fail to reproduce and/or die in this case [5].

3. Agent-Based Modeling and Simulation

Agent-Based Modeling and Simulation (ABMS) is a powerful technique used in order to understand the mechanisms of systems and/or the system dynamics of complex phenomena in many domains, including in the social sciences [6], ecology [7], economics [8], and biomedicine [9]. ABMS consists of three main components: agents, a simulated environment, and a simulation environment.
Agents are actors that operate in the real system and influence and are influenced by the simulated environment. The agents are involved in the simulation model as model components that perform actions individually and interact with other agents and the simulated environment, thereby representing behaviors in the real system.

Agent-based models consist of dynamically interacting autonomous agents that act according to their local knowledge (rules, behaviors, and information) by taking account of the environment and reacting to changes within it. Agent-based modeling provides a bottom-up approach in order to model a range of application domains. Adopting the bottom-up approach to model a complex system reduces the complexity of the system by distributing the complexity to a large set of interacting individuals with simple behaviors. As a result of individual behaviors and interactions, the system accounts for complex or adaptive behaviors at a higher level [10]. Thus, ABMS provides asound opportunity to model complex systems that exhibit behaviors of this kind.
A simulated environment consists of components that cannot be represented as agents in the real system but that represent important components in regard to representingthe real system. Further, the simulated environment also represents the real environment in which agents live, operate, and interact with each other. The simulated environment is part of the simulation model. In a simulated environment, sources and state definitions are present and are often shared in the overall model by all the agents and contain information in some cases.
A simulation environment consists of agents and the simulated environment, enables the simulation model to operate, and represents the simulation infrastructure. Conceptually, the simulation environment is considered to be part of ABMS. The first reason for this is that if the simulation model is to represent the current system in real terms, the infrastructure required must be part of the real system. In ABMS, the concept of time is typically represented as time steps. In every time step, each agent in the model performs an operation, and/or interacts with other agents [11].

4. Agent-Based Modeling and Simulation (ABMS) of the Resistance of Bacterıa to Antibiotics

The agent-based modeling and simulation (ABMS) of the resistance of bacteria to antibiotics has three main components: agents, a simulated environment, and a simulation environment. The immune system cells and bacteria are the agents of the model. They have attributes and behavior rules and react to changes in the environment. The virulence factor, which is an attribute of the bacteria, defines the extent of the pathogenicity of a given pathogen. Whereas bacteria engage in intraspecies and interspecies competition, immune system cells fight bacteria.Theseinfluence interactions among agents of the model and between agents of the model and the simulated environment.
The simulated environment in the model consists of a grid with 100 × 100 grid cells to represent human tissue or a human organ.Each grid cell provides a suitable environmental layer with the nutritional resources needed for bacteria to live,grow, and reproduce. This layer is represented by tones of green color. Moreover, in order to observe the process whereby an antibiotic kills bacteria or whereby bacteria resist the introduction of anantibiotic, the model is designed so that each grid cell provides an antibiotic layer with a constant concentration level of antibiotics.
In this study, we used Repast Simphony 2.1 to create agents and system objects that cannot be implemented as agents [12]. In the Repast Simphony simulation environment, the agents and objects inthe simulated environment are written using Java programming language. After defining the agents and system objects in the simulated environment, it is necessary to describe the behaviors of the agents.

4.1. Bacterial Behavior

At the initialization, the programmer defines the number of bacteria to create for the simulation. The bacterial cells are created and randomly assigned virulence factors of between 1 and 4. For example, a bacterial cell assigned a virulence factor of 1reproduces in every time step of the simulation.
Each bacterial cell is randomly distributed on the simulation environment represented by a gridwith 100 × 100 grid cells. Each bacterial cell increases in cell size by consuming nutrients where it is located.Each grid cell provides a high level of nutrients to bacterial cells. The nutrients consumed by a bacterial cell are subtracted from the nutrient availability of its grid cell. At each time step, nutrient availability increases in accord with nutrient production. Under suitable conditions, each bacterial cell grows to a fixed size and then reproduces such that two identical daughter cells are produced.

4.2. Immune System Cell Behavior

At the initialization, the programmer defines the number of immune system cells to create for the simulation. Immune system cells are created and randomly distributed in the grid. An immune system cell kills one of the bacterial cells in the grid by looking at the neighboring 48 cells and locates in the dead bacterial cell's grid cell in every time step. If an immune system cell destroys 2 bacteria cells, this immune system cell signals an

immune system cell and then dies. If there are more than 40 bacterial cells in the neighborhood, an immune system cell also signals another immune system cell. If there are bacterial cells in the range of 2 to 15 in the neighborhood, then an immune system cell vanishes. If an immune system cell cannot kill a bacteria cell through 15 time steps after its creation, it then vanishes.

5. Experimental Study

We performed five experiments in order to observe the bacterial competition of the flora, interactions between the bacteria and the immune cells in the presence of an antibiotic, the resistance of bacteria to an antibiotic, the suppressed immune system in case the resistance of bacteria to an antibiotic exists, and the effect of the use of the second antibiotic in the treatment of the infection caused by the resistant bacteria.

5.1. Experiment: Bacterial Competition on Flora

In this experiment, we begin our consideration of competition in populations of bacteria with observations pertaining to bacterial populations and provide an introduction to how competition can affect interactions among the bacteria. Therefore, 4000 bacterial cells with virulence factors in the rangeof 2 to 4 were created in the Repast Simphony simulation environment. A nutrient layer was included in the simulation environment to enable the bacteria to live, grow, and reproduce.
At the initial time step, a settlement of4000 bacterial cells accrued thatranged in terms of virulence factor from 2 to 4 in the Repast Simphony simulation environment, as shown in Fig. 1(a). Bacterial cells with a virulence factor of 2are represented as yellow, those with a virulence factor of 3 are represented as red, and those with a virulence factor of 4 are represented as purple.

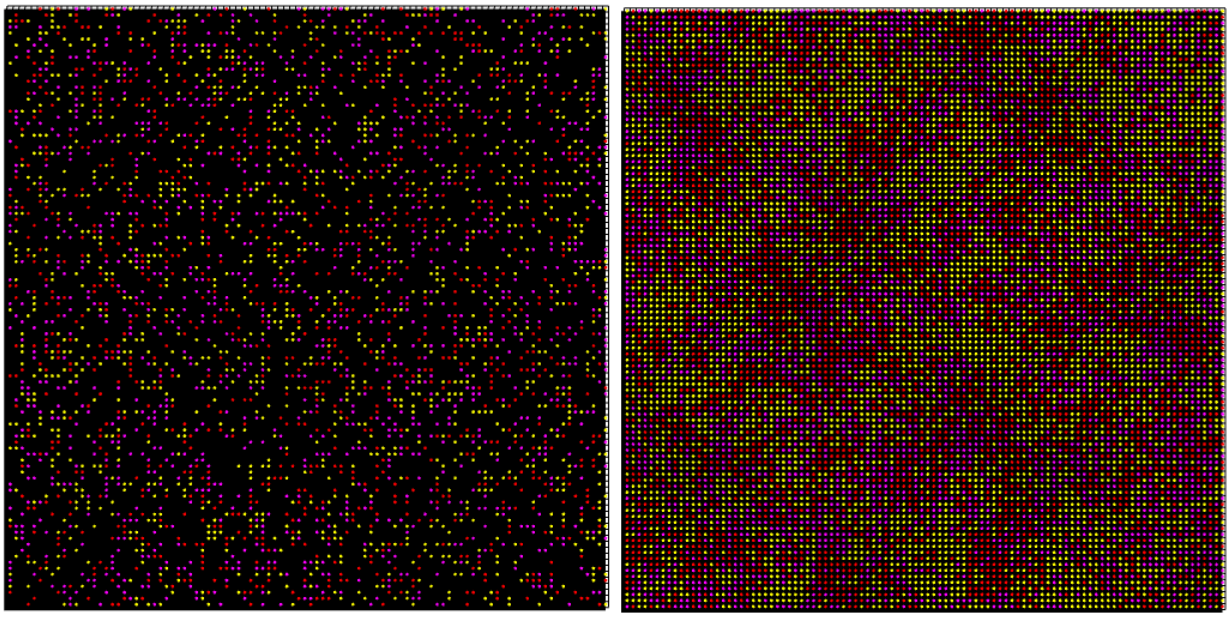

Figure 1. (a) Settlement of bacterial cells in the Repast Simphony simulation environmentat the initial time step, (b) Bacterial cells in the Repast Simphony simulation environmentat 1000thtime step

After 1000time steps, the bacterial cells are allocated to all the grid cells and there are no empty grid cells in the simulation environment, as shown in Fig.1(b). The number of bacterial cells increases given that they grow and divide rapidly under appropriate conditions. The bacteria with a low virulence factor reproduce very rapidly; therefore, the number of bacteria shown in yellow is higher than the number of bacteria shown in either red or purple. Indeed, bacteria compete with their neighbors for space and nutritional resources. Bacteria with similar nutritional requirements, such as members of the same population or different bacterial species, compete for nutrients as these become depleted by the growing population of bacteria. In our simulated environment, competition can lead to selection for variants that have low virulence factors.

5.2. Experiment: Antibiotic Usage

In this experiment, 100 immune system cells and 4000 bacterial cells with virulence factors ranging from 2 to 4 were created in the Repast Simphony simulation environment. The nutrient layer was included in the simulation environment for the bacteria to live, grow, and reproduce.An antibiotic layer was also included in the simulation environment.

At the initail time step, a settlement of 100 immune system cells and 4000 bacterial cells with a range of virulence factors accrues in the Repast Simphony simulation environment, as shown in Fig. 2(a). The bacteria cells are represented by yellow, red, and purple circles, and the immune system cells are representedby blue circles.

As shown in Fig. 2(b), the simulation environment includes only 9 immune system cells and the bacterial cells are removed from the environment after 664 time steps. In our simulation, each grid cell provides a constant concentration level of antibiotics, which kill the bacteria. In Fig. 3, a graph shows the number of bacteria alive at each time step: the number of bacteria decreases when the simulation starts,as the immune system cells kill most of them before they can begin to reproduce. The bacteria must grow in cell size before theycan reproduce. Then, the number of bacteria increases as they are in the simulation environment with a high level of nutrients as the simulation progresses. However, the increase in the population of bacterial cells cannot exceed the number given as the initial value (4000) because the antibiotic starts to kill some of the bacteria and helps the immune cells to handle the remaining bacterial cells. The bacterial population reaches its peak at the 155thand 305th time steps.Then, the simulation pauses at the 664th time step because there are no bacteria in the simulation environment. We expected this result as the antibiotic kills all the bacteria.

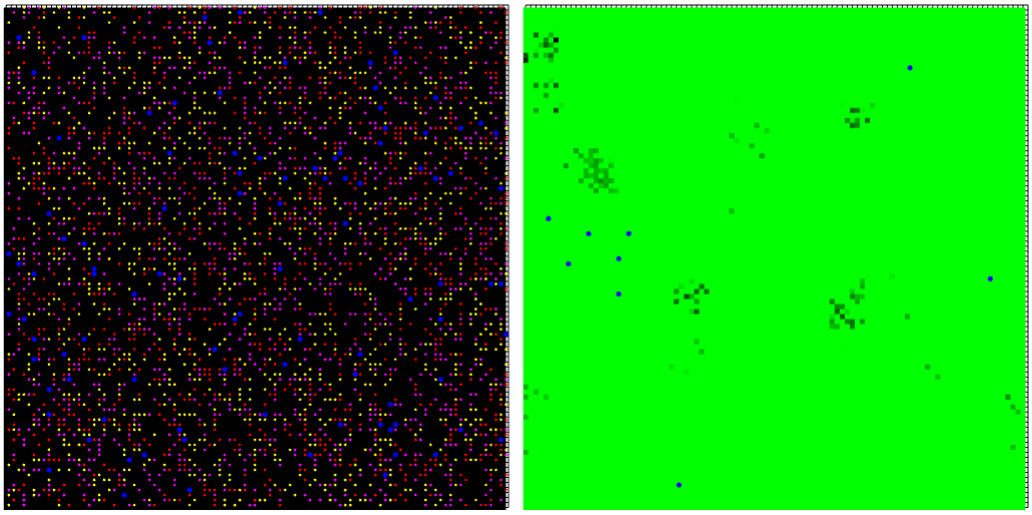

Figure 2. (a) Immune system cells and bacterial cells in the Repast Simphony simulation environment at the initial time step, (b) Immune system cells and bacterial cells in the Repast Simphony simulation environment at 664thtime step

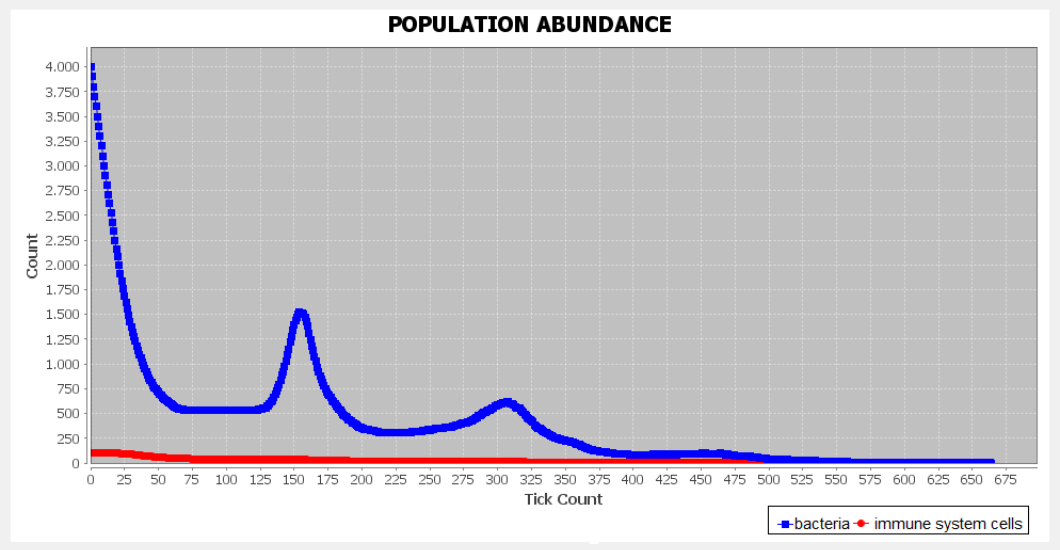

Figure 3.Populations at 664th time step

5.3. Experiment: Antibiotic Resistance

In this experiment, 100 immune system cells and 4000 bacterial cells with virulence factors ranging from 1 to 4 were created in the Repast Simphony simulation environment. Bacterial cells shown in white (virulencefactor=1) represent mutant cells, i.e., cells that have become resistant to antibiotics. The nutrient layer was included in the simulation environment for the bacteria to live, grow, and reproduce. An antibiotic layer was also included in the simulation environment for this experiment.

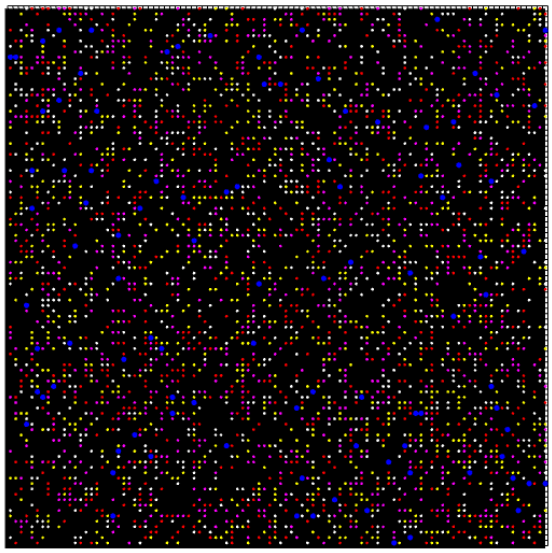

Figure 4.Settlement of 100 immune system cells and 4000 bacterial cells in the Repast Simphony simulation environment

At the initialtime step, a settlement of 100 immune system cells and 4000 bacterial cells with a range of virulence factors accrues in the Repast Simphony simulation environment, are shown in Fig. 4:The bacteria cells are represented as white, yellow, red, and purple circles, and the immune cells are represented as blue circles. In this experiment, the bacteria shown in white are antibiotic-resistant such that the antibioticcannot kill them.

We observed that the bacteria shown in yellow, red, and purple were removed from the simulation environment until time step 664, as described in Section 5.2. Some were killed by the antibiotic whereas the rest were killed by the immune system cells. As shown in Fig. 5, at the 454th time step, only immune system cells and antibiotic-resistant bacteria remain in the simulation environment. The number of immune system

cells is very high in this experiment,as the resistant bacterial cells divide rapidly (virulence factor=1) and the number of bacterial cells increases, which causes the immune cells to increase in the number as well.

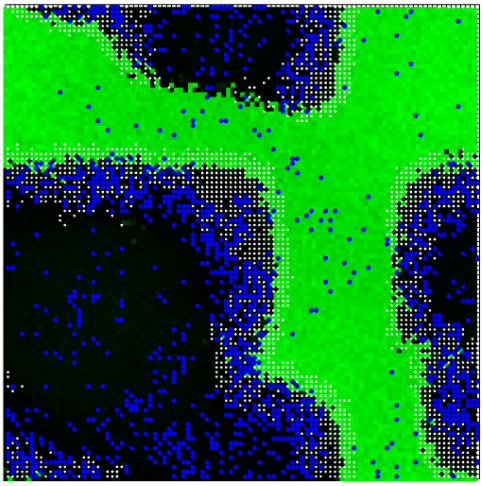

Figure 5. Immune system cells and bacterial cells in the Repast Simphony simulation environment at 454thtime step

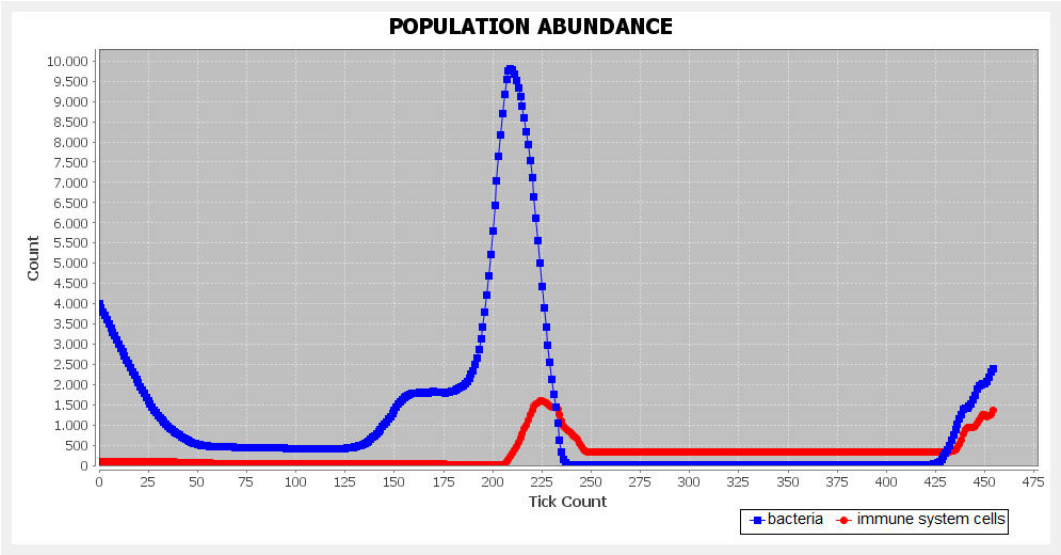

Figure 6. Populations graph of immune system cells and bacterial cells at 454th time step

Fig. 6 shows a graph of the number of bacteria alive at each time step: the number of bacteria decreases when the simulation startsbecause some are killed by the immune system cells and they cannot start reproducing immediately. The bacteria must grow in size before they can reproduce. The population of bacteria reaches it maximum value at the 200thtime step whereas the immune cell population reaches its maximum value at the 225th time step to fight the bacterial cells.The bacterial population reaches almost 10000 at the 200thtime step. After the 200thtime step, the antibiotic helps the immune system cells and both remove the bacteria shown in yellow, red, and purple. At the 454thtime step, a new bacterial attack is initiated by the antibiotic-resistant bacteria.

The simulation is paused at the 1000thtime step, at which point the simulation environment contains 28 antibiotic-resistant bacteria and 450 immune system cells, as shown in Fig.7.The body's immune system alleviates the bacterial attacks and works to maintainthe body's tissues in balance. The bacterial attack is also shown in Fig.8 before the simulationis paused. After the 454th time step, the bacteria attack several times. The attacks will continue as long as these bacteria encounter an environment that supports their ability to live, grow, and reproduce.

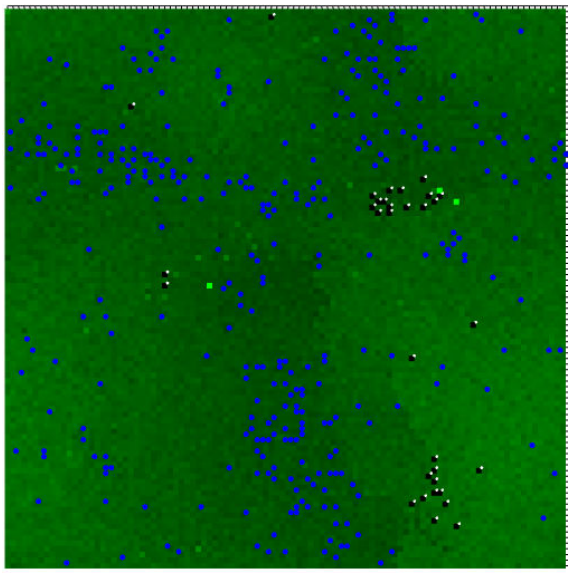

Figure 7.Immune system cells and bacterial cells in the Repast Simphony simulation environment at 1000thtime step

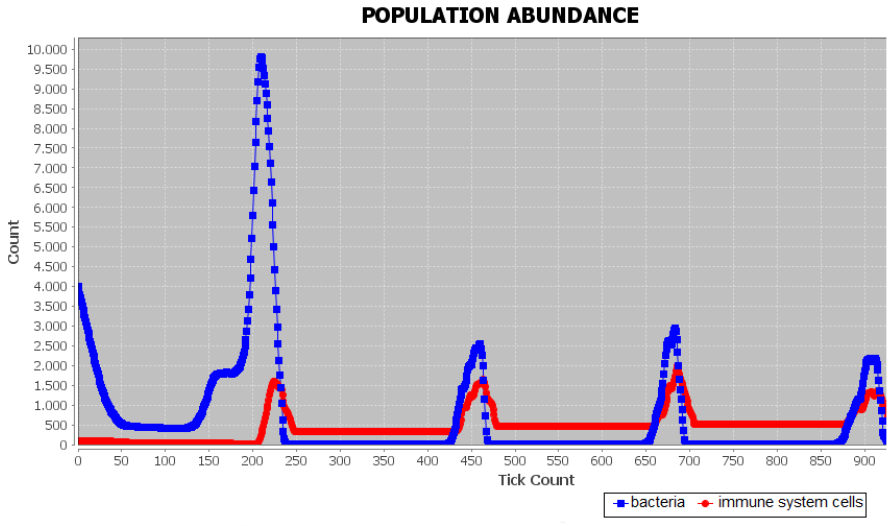

Figure 8. Populations graph of immune system cells and bacterial cells at 1000th time step

5.4. Experiment: Antibiotic Resistance and the Suppressed Immune System

Cortisol is a hormone that is secretedfrom the adrenal cortexin response to stress. It has a strong effect in the body on blood sugar regulation, blood pressure regulation, immune system suppression, and in the reduction of inflammation. The use of hydrocortisone (a synthetic form of cortisol) during infections prevents an over-activation of the inflammatory response by weakening the activity of the immune system. In order to study the effect of cortisol on the immune system cells, a similar experimental setup as described in Section 5.3 was performed. For this simulation, 100 immune system cells and 4000 bacterial cells with arbitrary virulence factors ranging from 1 to 4 were created in the Repast Simphony simulation environment.A nutrient layer and an antibiotic layer werealso included in the simulation environment for this experiment. The behavior of immune system cells in the simulation program was modified in order to fight the bacterial cells slowly since cortisol suppresses the immune system. Thus, an immune system cell kills one of the bacterial cells in the grid by looking at the neighboring 48 cells and moves intothe dead bacteria's grid cell in every fourth time step. Immune system cell dies afterkilling 2 bacterial cells, and causes immune system to recruit another immune cell into the simulation environment.

At the initial time step, a settlement of 100 immune system cells and 4000 bacterial cells with a range of virulence factors accrues in the Repast Simphony simulation environment, are shown in Fig.9:The bacterial

cells are represented as white, yellow, red, and purple circles, and the immune system cells are represented as blue circles. In this experiment, the bacteria shown in white are antibiotic-resistant.

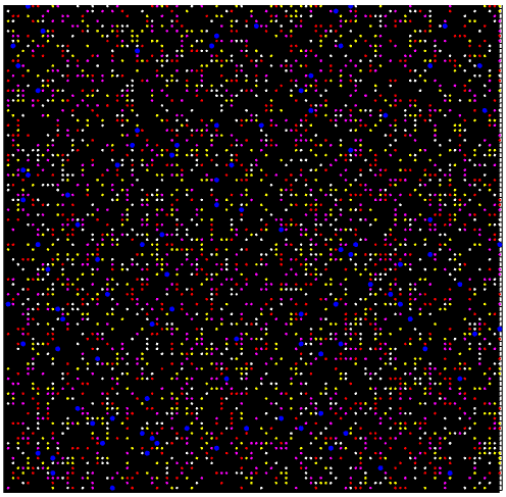

Figure 9.Settlement of 100 immune system cells and 4000 bacterial cells in the Repast Simphony simulation environment

Fig. 10 shows a graph of the number of bacteria alive at each time step: the number of bacteria decreases when the simulation startsbecause some are killed by the immune cells and they cannot start reproducing immediately. The bacteria must grow in size before they can reproduce. The population of bacteria reaches almost 7000 at the 225thtime step whereas the immune system cell population reaches its maximum value at the 225thtime step to fight the bacterial cells. We observed that the antibiotic helped the immune system cells and both removed the bacteria shown in yellow, red, and purple until the 400th time step. As shown in Fig. 11, at the 400th time step, only immune system cells and antibiotic-resistant bacteria remain in the simulation environment.

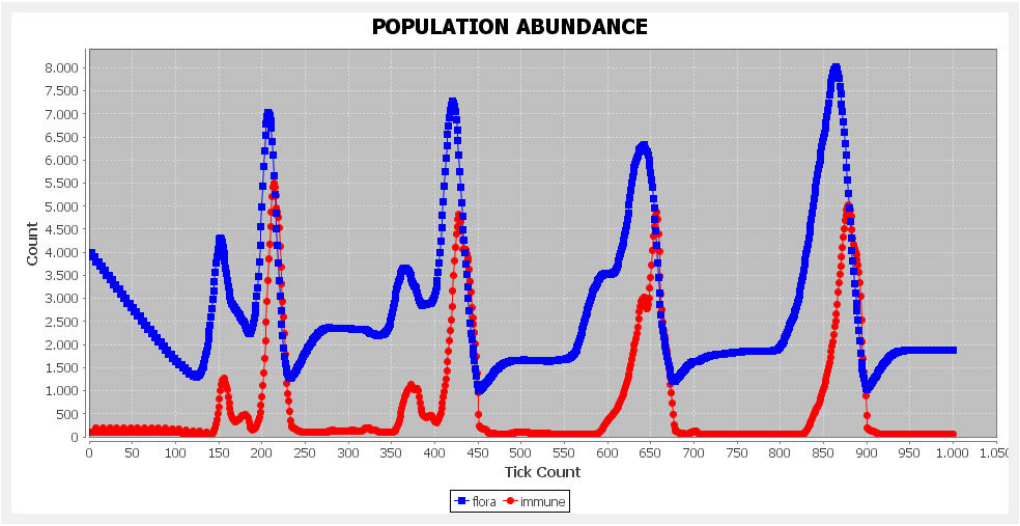

Figure 10. Populations graph of immune system cells and bacterial cells at 1000th time step

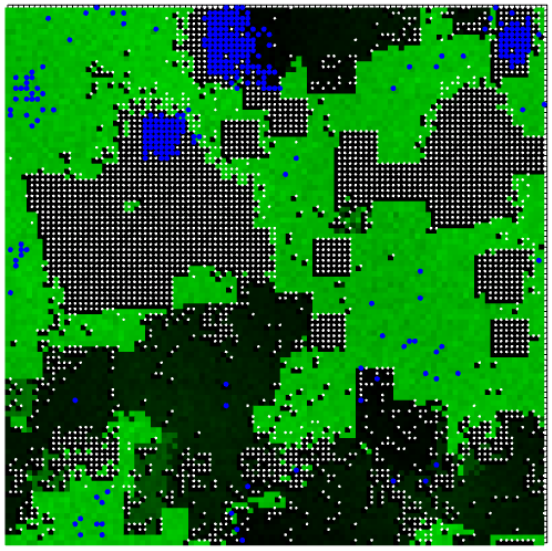

Figure 11.Immune system cells and bacterial cells in the Repast Simphony simulation environment at 400thtime step

The number of the bacterial cells is very high during this experiment. As the number of bacterial cells increases, it causes the immune system cells to increase in the number as well. As shown in Fig. 10, after 400 time steps, several bacterial attacks are initiated by theresistant bacteria. However, the immune system cells cannot totally remove the antibiotic resistant bacterial cells or decrease the number of bacterial cells to a small number such that the immune system can alleviate the infection; because, the immune system cells are too slow to defend the body against the bacterial attacks. The simulation is paused at the 1000thtime step, at which point the simulation environment contains almost 1800 antibiotic-resistant bacteria and 40 immune system cells, as shown in Fig.12.Cortisol suppresses the immune system andthe body is at increased risk of infection.

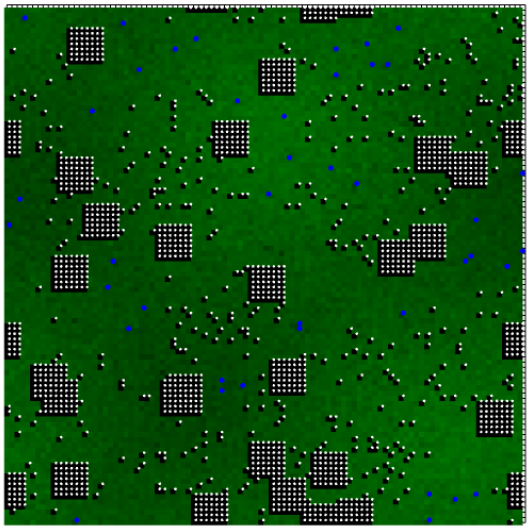

Figure 12.Immune system cells and bacterial cells in the Repast Simphony simulation environment at 1000thtime step

5.5. Experiment: Usage of a Second Antibiotic

A second antibiotic course can be justified only if infection with the resistant bacteria is suspected. In order to study the effect of the use of a second antibiotic on the resistant bacteria, a similar experimental setup as described in Section 5.4 was performed.Thus 100 immune system cells and 4000 bacterial cells with virulence factors ranging from 1 to 4 were created in the Repast Simphony simulation environment.The nutrient layer, antibiotic layer and antibiotic2 layer werealso included in the simulation environment for this experiment. Antibiotic2 layer represented the use of second antibiotic for the treatment of the infection caused by the

antibiotic resistant bacteria and it was activated at 400th time step. The behavior of immune system cells in the simulation program was modified in order to fight the bacterial cells slowly since cortisol suppresses the immune system. Thus, an immune system cell kills one of the bacterial cells in the grid by looking at the neighboring 48 cells and moves intothe dead bacteria's grid cell in every fourth time step. Immune system cell dies afterkilling 2 bacterial cells, and causes immune system to recruit another immune cell into the simulation environment.

At the initialtime step, a settlement of 100 immune system cells and 4000 bacterial cells with a range of virulence factors accrues in the Repast Simphony simulation environment are shown in Fig.13:The bacteria cells are represented as white, yellow, red, and purple circles and the immune cells are represented as blue circles. In this experiment, the bacteria shown in white are antibiotic-resistant.

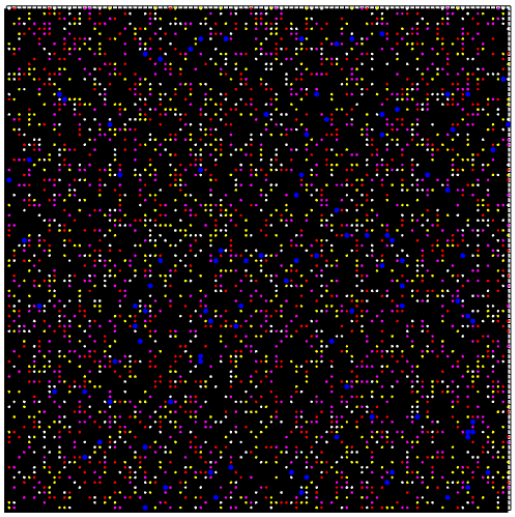

Figure 13.Settlement of 100 immune system cells and 4000 bacterial cells in the Repast Simphony simulation environment

At the 350th time step, we observed that the bacteria shown in yellow, red, and purple still existed in the simulation environment, as shown in Fig. 14(a) and they were removed from the simulation environment until the 400th time step. Some were killed by the antibiotic whereas the rest were killed by the immune system cells. As shown in Fig. 14(b), at the 400th time step, only immune system cells and antibiotic-resistant bacteria remain in the simulation environment. The numbers of immune system cells and antibiotic resistant bacterial cells are very high in this experiment,as the resistant bacterial cells divide rapidly (virulence factor=1) and the number of bacterial cells increases, which causes the immune cells to increase in the number as well. In order to remove the resistant bacteria from the simulation environment, Antibiotic2 layer was activated at the 400th time step. Thus, "antibiotic2" was able to alleviate the chronic infection caused by the resistant bacteria since the resistant bacteria shown in white were sensitive to "antibiotic2".

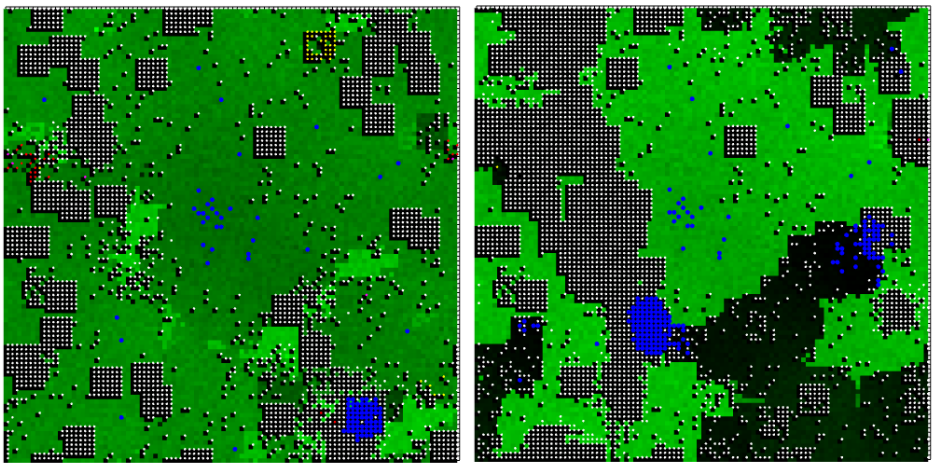

Figure 14. (a) Immune system cells and bacterial cells in the Repast Simphony simulation environment at the 350thtime step, (b) Immune system cells and bacterial cells in the Repast Simphony simulation environment at the 400thtime step

The simulation pauses at the 753rdtime step because there are no bacteria in the simulation environment.As shown in Fig. 15, the simulation environment includes only 72 immune system cells and the resistant bacterial cells shown in white are removed from the environment since each grid cell provides a constant concentration level of "antibiotics2", which kill the resistant bacteria after 400 time steps. Fig. 16 shows a graph of the number of bacteria alive at each time step: At the 425th time step, the number of bacteria increases and the population of the resistant bacteria reaches almost7000 whereas the immune system cell population reaches its peak to fight the resistant bacterial cells. After 550 time steps, the resistant bacteria attempt to attack one more time; however, the "antibiotic2" helps the immune system cells and both remove the resistant bacteria until 753rd time step.

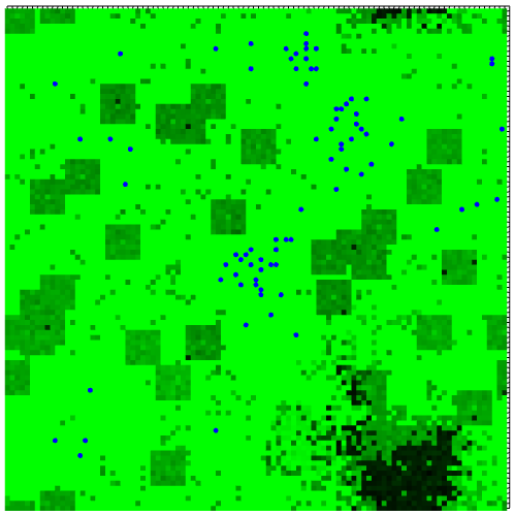

Figure 15.Immune system cells in the Repast Simphony simulation environment at the 753rd time step

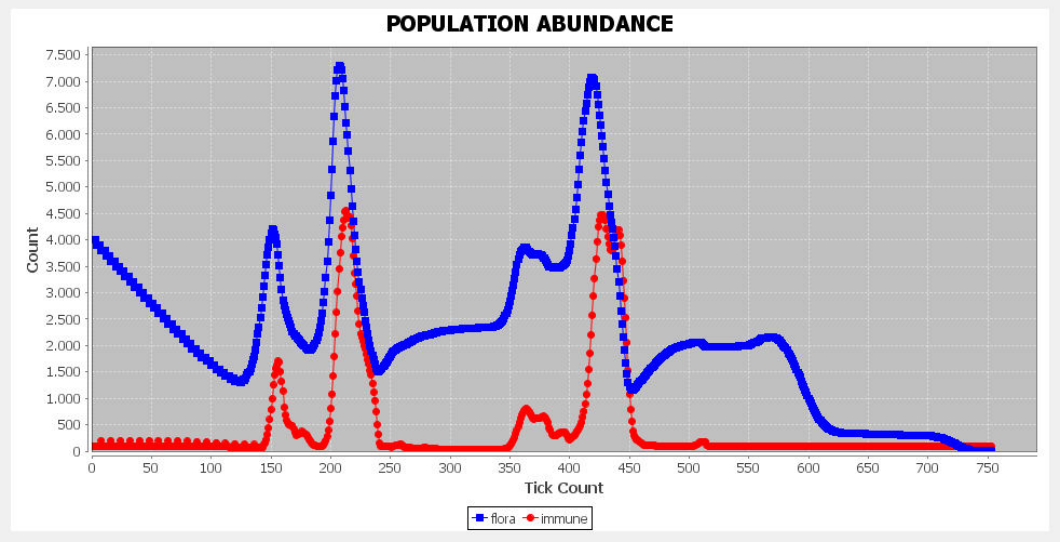

Figure 16. Populations graph of immune system cells and bacterial cells

6. Conclusions

In this study, we usedABMStechnique to observe the competition between bacteria traits and the interaction between the bacteria and immune system cells. We observed the effects of variability of antibiotic resistance on the infection process. In our experiments, we show that resistance to antibiotics increaseswith the survival of bacterial cells that are immune to the effects of the antibiotic. Their daughter cells inherit this resistance, thereby creating a population of resistant bacteria.Besides, we show that the effect of the suppressed immune

system on the infection process and the necessity of the use of second antibiotic for the treatment of the infection caused by the antibiotic-resistant bacteria.

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