An Analysis of Intestinal Microbiotal Homeostasis with Cellular Automata

Alexander Munoz

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Contents

1 Abstract

Recently, observational research has demonstrated discrepancies in gut-related microbiotal pathologies (such as pseudomembranous colitis, an inflammatory condition caused by the pathogenic bacteria C. difficile) in broad-spectrum antibiotic-treated patients, hypersensitive immune response patients, and Cesarean-section birthed infants. In this project, I chose to investigate whether these phenomena can be explained by a mechanism of defense from commensal intestinal flora called competitive exclusion, where commensal bacteria consume resources to block pathogenic bacteria from doing so. A cellular automaton stochastic simulation showed that while the hypersensitivity could not be explained by commensal competitive exclusion, the antibiotic-treated and Cesarean-section-birthed conditions in fact did exhibit a dependence on the presence of competitive exclusion.

2 Introduction

When the human intestinal microbiome was initially discovered, it was believed to be a solely harmful thing. For example, early scientists believed that increased populations of intestinal flora could explain gut-related pathologies. However, it was subsequently discovered that there was no correlation between quantity of intestinal microbiotal colonies (measured via colony forming units / gram of stool) and gut-related pathologies. Researchers thus concluded that intestinal bacterial species are commensal [1] (i.e. they do not appear to harm the host). Later, it was furthemore discovered that human hosts are actually quite dependent on their intestinal microbiomes and are more mutualistic than commensal [2]. For example, microbiotal species in the intestines produce Vitamin K (a clotting factor) and further breakdown nondigestible food, providing the host with nutrient-rich short-chain fatty acids (which interestingly also have trophic effects on the intestinal enterocytes) [3].

I chose to investigate the first of these two situations: the commensality of intestinal bacteria. Although bacteria do have trophic and metabolic effects on the host, for the purpose of this project, I will ignore those effects and focus on the pathogenecity of the bacteria. In fact, the most significant effect of bacteria on the host is not their metabolic effects nor their trophic effects - but just their existence, through witch commensal bacteria serve a purpose called competitive exclusion [4].

There is a finite amount of surface area in the intestines, and to survive, a bacterium must attach to the mucosal wall of the intestines. Thus, there are a finite amount of bacteria that can attach to the surface area of the colon. Just by existing, commensal bacteria take up the space on the intestinal wall that would be required for the growth of pathogenic bacteria [5] [6].

Thus, when a normal, healthy micriobiome is presented with a pathogen, the pathogen will rarely colonize, because the space has already been taken by the host's commensal bacteria (thus the name "competitive exclusion"). Recently, observational research has shown that broad-spectrum antibiotic treated patients [7], hypersensitive immune system patients (i.e. inflammatory bowel diseases) [8] [9], and Cesarean section-birthed children [10] all have increased risk of developing gut-bacteria related pathologies. In this project, I will investigate if these idiopathic conditions can be explained by discrepancies in commensal bacteria competitive exclusion.

3 Model

The system of microbiotal homeostasis is inherently stochastic and very spatially dependent, so to investigate the system I chose to implement a cellular automaton. I found this to be the best approach because it would be difficult to analytically model the plethora of 2-dimensional shapes that colony formation could occur in (because the colony distribution in 2D space is extremely stochastic and the 2D distribution greatly affects the dynamics of the population growth - i.e. having commensal bacteria spread out in 2D space is a better barrier compared to having commensal bacteria clustered in one area of the 2D space because the clustered commensal bacteria leave an opening for the colonization of a potential invading pathogenic bacterium).

I chose to run the simulation over a small patch of intestine tissue of 1 square mm. Because bacteria cling on to the wall, there is negligible chemotaxis and I will assume all bacteria stay fixed in one position over the course of the simulation. I divided the 1 square mm tissue patch into 10,000 square units (each 10 um in side length). Bacteria are on that scale in length plus a little extra room since they don't pack in completely tight; thus, each unit square represents a spot where one bacteria can live. I modeled five bacterial subpopulations which I numbered 0-4 as follows:

- 0: empty (no bacterium is currently at this location)
- 1: faecalibacterium prausnitzii (one of the most common commensal bacteria in the human intestinal microbiome)
- 2: bacteroides fragilis (another one of the most common commensal bacteria in the human intestinal microbiome)
- 3: other commensal bacteria (commensal species not contained in the above 2 species)
- 4: clostridium difficile (pathogenic bacteria)

I ran the cellular automaton under five different overarching homeostatic conditions as follows:

- normal, healthy homeostasis (control condition)
- normal, healthy homeostasis presented with the introduction of a potential pathogenic infection (i.e. Clostridium difficile)
- normal, healthy homeostasis plus wide-range antibiotic treatment, followed by infection
- overactive immune system (hypersensitivivity) followed by infection
- normal, healthy homeostasis presented with infection; followed up with fecal bacteriotherapy transplant
- Cesarean section baby presented with infection

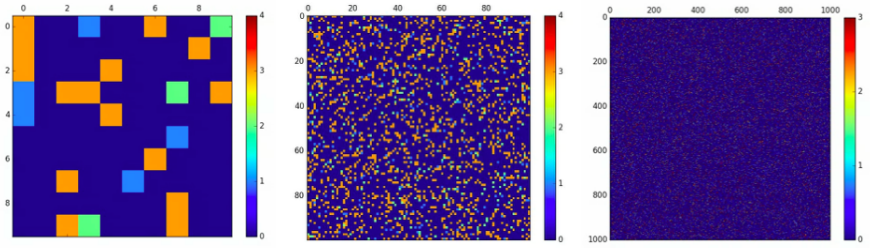
4 Paramaterization

Parameters were chosen from peer-reviewed literature to best model the human biological system.

4.1 Tissue patch size

The 1 square mm size may seem arbitrary at first. I simply chose this size such that the model was small enough to be visualizable via a heatmap but also large enough to minimize edge conflicts (ie bacteria are blocked from growing outwards at the edge). This balance can be visualized in Figure 1.

Figure 1: Leftmost heatmap has a length of 10 bacterial units. A high percent of growths are edge conflicts occur (a bacteria would divide but it cannot divide across the edge). The rightmost heatmap squares are too small (width of 1000 bacteria), there is too much information to visualize. I thus chose the middle heatmap, which shows a balance.



4.2 Initial Percent Full

There are about 10^{12} bacteria in the intestines. With each bacterium having length 10um, we can assume an area of about 100 square um per bacteria. Thus the total bacterial area is about 100 square meters. The surface area of the human adult intestines is 250 square meters. Thus, the average percent of intestinal area filled by bacteria is 40%. When we initialize the intestines, we will ergo assume that an average of 40% of unit squares are filled with bacteria.

4.3 Subpopulation Distribution

The percent of the microbiome that is contained in the subpopulations that we listed in section 3 vary from person to person. Surprisingly, only about 13% of bacterial species are conserved between individuals' microbiomes, illustrating the incredible breadth of diversity. As fascinitating as this diversity of subpopulation possibilities is, we will focus on just the most abundant and conserved commensal populations: faecalibacterium prausnitzii and bacteroides fragilis (which both represent 10% each of normal flora populations) [11] [12] [13]. We

will lump the remaining 80% of commensal flora into the "Other commensals" category (because they're diversity is too variant to represent accurately across all humans).

4.4 Growth Rate

Faecalibacterium prausnitzii has a doubling rate of about 30 minutes [14]. Bacteroides fragilis has a doubling time of about 2 hours [15]. Other commensal bacteria in the intestines have a doubling time of 1 hour. Clostridium difficile also has a doubling time of 1 hour [16]. To convert these doubling times to probabilities (such that we can use them in our stochastic model), we will assume an exponential distribution of waiting times. While we do not expect the doubling times to be fully memoryless like the exponential distribution requires, the fact that a bacteria didn't divide in the first of it's doubling time doesn't drastically increase its probability of dividing in the second doubling time because a biological error could have occurred halting the cell cycle (i.e. DNA repair is occurring before division continues). We will thus apply the cumulative distribution function of the exponential distribution:

$$F(x) = 1 - e^{-\lambda x}$$

where λ is the rate parameter (divisions per hour). Thus probability of division in an hour can be calculated as $F(1)$ using the above equation (assuming a maximum of 1 division per timestep).

4.5 Death Rate

When we initialize the gut tissue patch, we will assume healthy homeostasis. In healthy homeostasis, the microbiotal populations will not be drastically changing, and thus, the individual subpopulations should be at roughly equilibrium. Because I could not find consistent results for microbiotal species-specific death rates in the literature (they seem to vary quite significantly), I chose to rely on the assumption of equilibrium - I ergo selected the death rate for each subpopulation that "matches" the growth rate. The growth rate matches the death rate such that the subpopulation is neither growing nor dying significantly over time and remains at a dynamic equilibrium.

4.6 Antibiotic Efficiency

This parameter is difficult to precisely parametrize because of the huge diversity of flora in the 80% of micriobiotal species that we labeled as "other commensals." Many of those species will react quite differently to broad-spectrum antibiotics like ampicillin or tetracycline. Although the antibiotic intestinal efficacy has surprisingly not been well studied on human intestinal flora, I found an article describing the effect of tetracycline on chicken stool flora. The study determined that approximately 10% of chicken intestinal flora survived the broad-range antibiotic treatment [17]. Thus, the tetracycline antibiotic is about 90% effective.

4.7 Overactive Immune System

T-helper cells are a class of immune cells developed in the thymus. They are trained in the thymus to have receptors against non-self while refraining from possessing self receptors, all under the transcriptional control of the Aire gene [18]. However, in hypersensitivity pathologies such as inflammatory bowel diseases (i.e. Crohn's Disease or Ulcerative Colitis), the training of the T cells can fail in the thymus, potentially due to altercation in correct Aire functionality. Included in this hypersensitivity, is the chronic inflammation characteristic of mounting an immune response toward commensal intestinal flora.

At first look, I thought of modeling the immune response as introducing another population to the model: T-helper cells that could kill commensals and pathogenics in their vicinity. However, upon further research, I noted that this is not in fact how the immune system operates in the intestines. Interestingly, the immune response is mediated transcellularly across the gut mucosal membrane of the intestinal enterocytes via pockets of dense lymphoid tissue called Peyers' Patches. I highly recommend a Nature Immunology educational video on Peyers' Patches for a visualization of this phenomenon (youtu.be/gnZEge78_78).

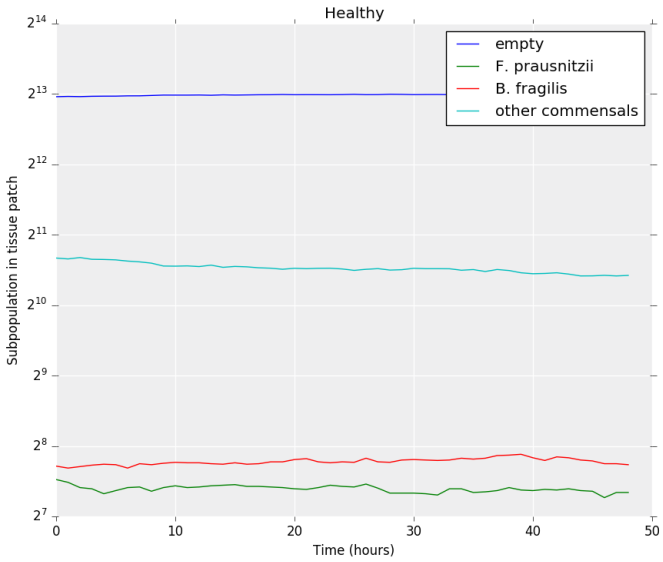
Nevertheless, the immune response is not spatially dependent like the bacterial growth, as it is trancellularly mounted. Thus the rate-limiting step of the T-helper cell killing is not the collisions with bacteria but the actual downstream effectors. The kinetics of T-helper cell mediated killings show a 17.4% efficacy in 1 hour [19].

5 Results & Discussion

5.1 Healthy homeostasis

In the normal, healthy homeostasis conditions, the patients microbiome is at a dynamic equilibrium, where there are no large changes in population dynamics over short times. Thus in figure 2, where we plot the bacteria in a given group over time, we notice that there is indeed a dynamic equilibrium with the parameterization. The death rate is about equal to the division rate for the bacterial subpopulations and as a result, the population lines appear horizontal over time.

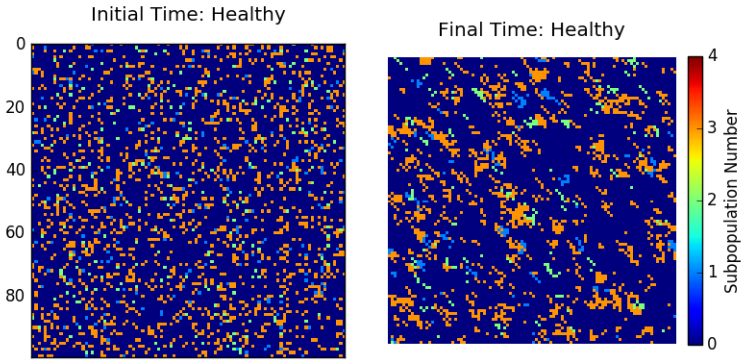
Figure 2: Subpopulation count over time. The horizontal lines verify the parameterization, as healthy homeostasis indeed presents with a dynamic equilibrium where birth rate equates with death rate



Next, I examined the microbiotal dynamics via a heatmap (Figure 3). During the initialization of the gut tissue patch, bacteria were spread via a 2D uniform distribution over the patch (Figure 3, left plot). However, 2 days of simulation caused discrepancies in the 2D uniform distribution. This is because all bacteria have an equal chance of dying, but only bacteria will divide to create a new bacterium adjacent to itself. As a result, bacteria will cluster into their subpopulations over time. In fact, this colony-formation indeed occurs in reality as well and is not just an artifact of the simulation.

This healthy control serves as a control for the remained of the project. We now

Figure 3: In healthy homeostasis, bacteria cluster into their subpopulations over time because all bacteria have equal chances of dying but divide to create a new bacterium adjacent to themselves (same subpopulation).



know that we expect constant subpopulation bacterial counts but clustering of the subpopulations over time. Deviations from these expectations will be due to the altered homeostatic conditions.

5.2 Healthy homeostasis plus infection

In this homeostatic condition, the patch is initialized to the typical 2D uniform and is then given 5 hours to begin clustering. After the 5 hour interim, the patch is presented with a pathogenic infection at the middle square. Figure 4 shows the subpopulation count plot over time. At the infection point (time = 6 hours), a C. difficile bacterium appears and begins to divide. However, the growing colony quickly dies off after running into a commensal colony and losing room in which to grow into.

Furthermore, I produced a heatmap visualization of the automaton immediately after the introduction of the pathogenic infection and after the 48 hours of the simulation (Figure 5).

Figure 4: After presentation with pathogenic infection, a colony begins to grow but quickly dies out after encountering a cluster of commensal bacteria.

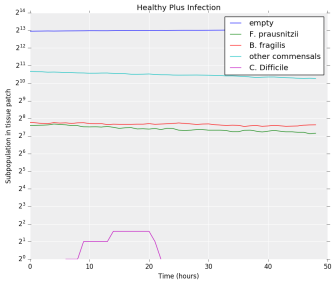
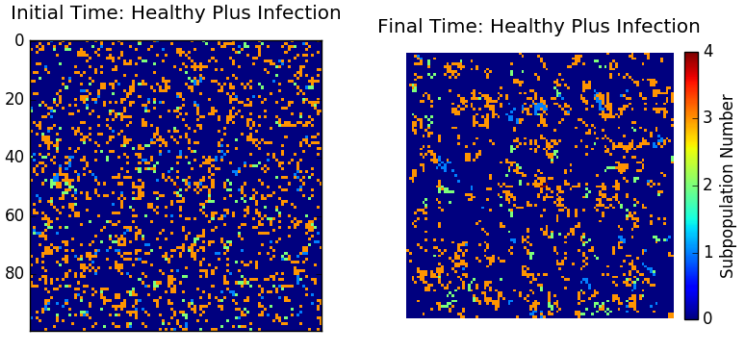


Figure 5: The pathogen is introduced in the leftmost plot and begins to divide in the rightmost plot but is encountered by a cluster of commensal bacteria (the orange in the middle of the rightmost plot). The pathogenic bacteria will die of soon after the collision.



5.3 Antibiotic treatment plus infection

In this homeostatic condition, the patch was first initialized as in the normal, healthy condition. After 5 hours, a broad-range antibiotic was introduced, which dramatically dropped the number of commensal bacteria (Figure 6). A pathogenic C. difficile bacterium was simultaneously introduced to the center of the patch as in the previous homeostatic condition. However, unlike the previous condition, this C. difficile managed to begin to colonize the patch. Figure 6 shows that the C. Difficile colony is steadily growing over time (note that this is a semilog plot so linear slope is exponential growth base 2).

This growth of the C. difficile colony occurs in the antibiotic homeostatic condition but not the healthy homeostatic condition because the antibiotic removed the competitive exclusion that prevent the colonization of the pathogen in the previous condition.

The heatmap visualization in Figure 7 demonstrates the caliber of the antibiotic treatment. The broad-spectrum antibiotic drastically change the microbiotal dynamics of the intestinal patch, giving the C. difficile colony plenty of room to grow without worrying about being blocked by any commensals.

Figure 6: The patch begins at normal homeostasis. At hour 5, the patch is treated with a broad-range antibiotic and the patch is simultaneously infected with a pathogenic C. difficile bacterium. The bacterium colonizes, as can be seen by the positive slope of the purple line.

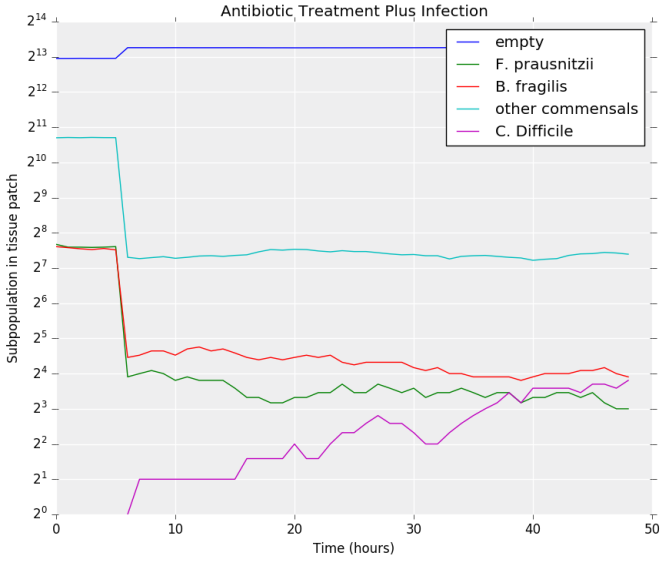
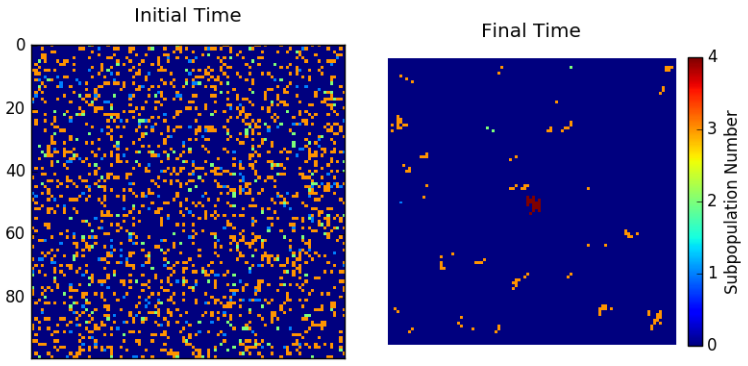


Figure 7: The left heatmap shows the intestinal patch before broad-spectrum antibiotic treatment. The right heatmap shows the intestinal patch after broad-spectrum antibiotic treatment. As can be clearly seen from this visualization, the broad-spectrum antibiotic removes the large majority of the commensal bacteria which typically have essential homeostatic competitive exclusion effects. As a result of the lost of these commensals, the pathogenic C. difficile colony introduced in the right plot begins to grow.



5.4 Hypersensitive immune system plus infection

In the hypersensitivity homeostatic condition, an overactive immune system was introduced. The system was first initialized to the 2D uniform and presented with a pathogenic C. difficile in the middle of the patch as before. However, in this condition, we additionally chose to investigate if increased incidence of gut-related pathologies is people with allergies (i.e. hypersensitivity disorders, inflammatory bowel disease) could be due to competitive exclusion.

The simulation was run but this time with pathologic Peyers' patch influence - a hyperactive immune system that targets commensal bacteria. As can be seen from Figure 8, the hypersensitivity caused a decrease in all commensal bacterial colonies. Furthermore, the C. difficile pathogen did not colonize. This occurs because the extra killing of the hypersensitive immune system toward the commensal bacteria tipped the homeostasis scale. Typically, homeostasis ensures that birth rate matches death rate and the subpopulation will remain stable (as seen previously in Figure 2). However, the hypersensitivity of the immune system added extra to the death process of the commensal bacteria, thus tipping the homeostasis scale and causing the decline of all commensal bacteria popula-

tions. Besides destroying commensal bacteria, this scenario also prevented the pathogenic invasion of the C. difficile colony because it was destroyed too.

As a result, we can conclude that competitive exclusion is not a valid explanation for the increased incidence of intestinal hypersensitive pathologies - for hypersensitivity affects not only the commensals but also the pathogenics (thus causing an equally difficult environment for all subpopulations).

Figure 8: The introduction of a hypersensitive immune reaction tips the homeostatic balance causing the decline of the commensal bacteria, while also preventing a pathogenic C. difficile infection.

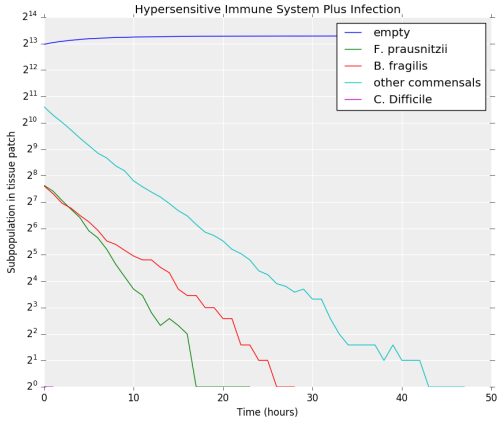
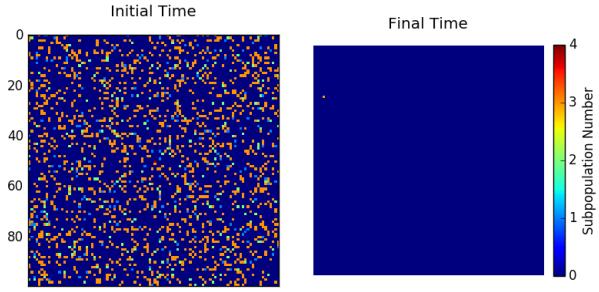


Figure 9: As expected, the rightmost heatmap shows little micriobiotal presence as a result of the hypersensitive immune system. Both commensal and pathogenic flora subpopulations are under stress from the immune system that does not differentiate between the two.



5.5 Infection plus fecal bacteriotherapy transplant

As mentioned previously, fecal bacteriotherapy transplant are a new method to treat gut related pathologies (specifically pseudomembranous colitis, an infection caused by C Difficile typically present after broad-spectrum antibiotic treatment!) [20].

This homeostatic condition begins with antibiotic treatment of a 2D uniform commensal distribution. Following the antibiotic treatment, a C difficile bacterium was introduced to the middle of the patch. After 10 hours with the infection, the patient is transplanted with healthy stool (lacking pathogenic C. difficile).

Figure 10 shows that C. difficile is increasing in numbers before the 10 hour mark. However, at the 10 hour mark, when healthy stool is transplanted, the pathogenic colony is almost immediately extinguished, as it is surrounded by commensal bacteria such that it cannot divide.

Figure 11 shows a heatmap visualization of the patch immediately after antibiotic treatment and then immediately after fecal transplant. The fecal bacteriotherapy serves to emulate the normal levels of commensal bacteria in healthy homeostasis. Via this emulation of healthy conditions, the commensal bacteria can serve their function of competitive exclusion and can block the pathogenic invasion.

Figure 10: Before 10 hours, a pathogenic C. difficile colony grows an an antibiotic-treated patch. At 10 hours, healthy stool bacteria is transplanted to the patch, emulating the competitive exclusion of healthy hemostasis. The C. difficile colony quickly dies off after the introduction of the healthy bacteria.

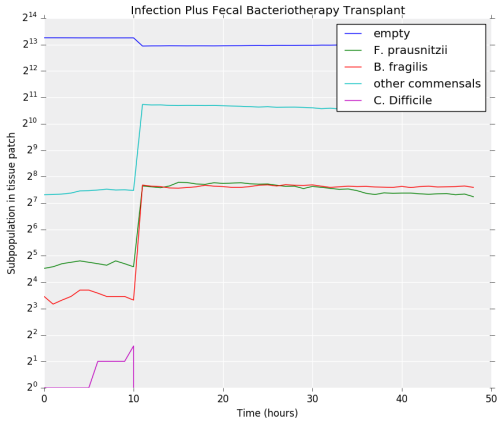
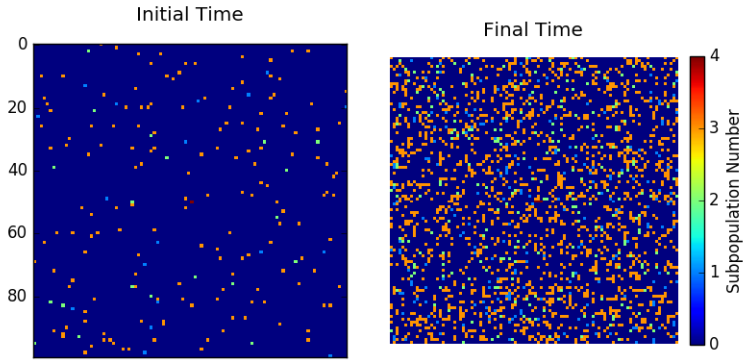


Figure 11: The leftmost heatmap represents the intestinal patch immediately following antibiotic treatment and introduction of pathogenic C. difficile. The pathogen will begin to colonize. The rightmost plot represents the patch after fecal bacteriotherapy transplant. Normal homeostatic conditions are emulated by supplementing the commensal bacteria, thus boosting the effect of competitive exclusion as the pathogen has less possible directions in which it can grow.



5.6 Cesarean section plus infection

Much of the infant microbiome is acquired from the vaginal canal during birthing; as a result, Cesarean-section birthed children possess decreased colony-forming units of micriobiotal species [21].

I finally chose to investigate the automaton behavior under homeostatic conditions of Cesarea-section-induced decreased microbiotal quantities in infant humans. Figure 12 demonstrates that seemingly small discrepancies in the overall bacterial quantification such as the 2-fold initialization difference shown here (between healthy initialization and C-section initialization) can actually have significant effects on the microbiotal dynamic. In the C-section infant, the C. difficile persists and begins to colonize (though more slowly than in the antibiotic-treated homeostatic condition). This corresponds with the clinical observation the Cesarean-section born children are more prone to gut-related pathologies.

Figure 13 shows that the the C-section infant after initialization on the left, and after 2 days of infection on the right. Because of the decreased raw quantity of bacteria available in the C-section infant, there is more room available

for C diffiicile growth.

Figure 12: The decreased numbers of commensal bacteria fail to prevent the growth of a pathogenic C difficile colony in a Cesarean-section born infant.

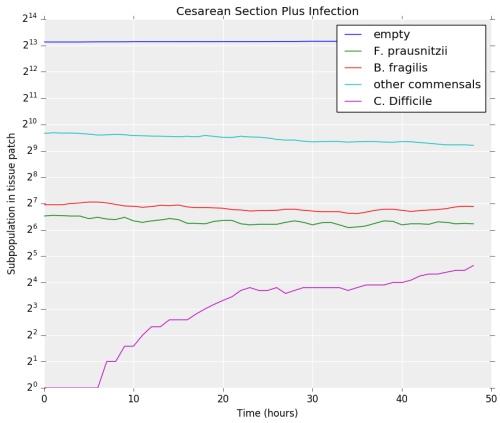
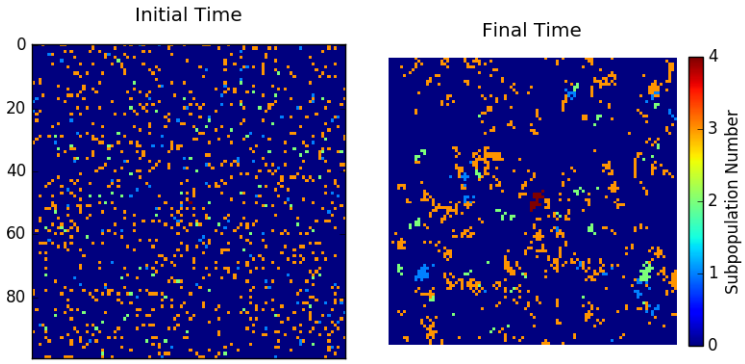


Figure 13: The leftmost heatmap shows the decreased bacterial quanitfication in the Cesarean section born-child, with the right heatmap showing the resultant openings in the plot (space where C difficile has a chance of colonizing).



6 Conclusion

The healthy homeostatic condition showed the effective parametrization of the model via the correct existence of a dynamic equilibrium between growth and death rates. Using the model, we showed that both broad-spectrum antibiotic treatment and Cesarean-section birth can increase the risk of developing a pathogenic bacterial colonization upon presentation. However, the healthy phenotype of pathogenic bacterial colonization resistance can be recovered by transplanting healthy stool via fecal bacteriotherapy. After restoring healthy bacteria levels, the commensal bacteria added serve their function of competitive exclusion and block the development of subsequent microbiotal subpopulations. Although competitive exclusion was not found to be a contributing factor to immune system hypersensitivtiy, it does indeed appear to contribute to risk of pseudomembranous colitis in Cesarean-section birthed children as well as broad-spectrum antibiotic treated patients. Because both of these situations are so common but also risky for infection, further research should be dedicated to emulating the effects of commensal bacteria (i.e. via fecal bacteriotherapy transplant or another drug that binds to intestinal mucosal membranes to simulate the commensal bacteria competitive exlclusion paradigm.

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