Evolution of within-host Antibiotic Resistance in Gonorrhea

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Abstract

Gonorrhea is a sexually transmitted bacterial infection caused by *Neisseria gonorrhoeae* that has become resistant to a wider range of antibiotics in recent decades. We study the competition dynamics of multiple *N. gonorrhoeae* bacterial strains within a host in an effort to better understand the development of antibiotic resistance and examine individual-patient treatment regimes to determine conditions for within-host antibiotic-resistance emergence. To that aim, we propose a phenomenological model that takes into account essential ideas such as the effects of different treatment levels, the mutation rates of bacteria, and the response of the immune system. We find steady state solutions and use analytical and numerical techniques to analyze their biological significance and stability behavior. Numerical simulations also provide a more integral view of how model parameters affect the emergence of within-host resistance.

Contents

1 Introduction

Gonorrhea infects approximately sixty million people a year, making it the second largest sexually transmitted infection worldwide [5]. This infection is caused by *N. gonorrhoeae*, also known as gonococcus. Gonococcus exclusively inhabits humans and is contracted via sexual contact. *N. gonorrohoeae* normally colonizes mucosal surfaces such as the cervix and urethra [33].

Untreated gonorrhea can cause infections in newborns, ectopic pregnancy, and pelvic inflammatory disease. Gonorrhea can spread throughout an individual's body and form lesions in locations such as joints and the heart. In some rare cases, gonococcus can be found in blood clots and can attack bones [19]. Gonorrhea causes pelvic inflammatory disease in a woman and epididymitis in a man, making it one of the major causes of infertility [8] (See Appendix).

Due to the aforementioned complications associated with untreated gonorrhea, it is critical to be able to maintain treatment. The primary way to stop the spread of *N. gonorrhoeae* is through the use of antibiotics. The six main antibiotics to which some *N. gonorrhoeae* strains have acquired resistance are Sulfonamides, Penicillin, Tetracycline, Spectinomycin, Fluoroquinolones (e.g. ciprofloxacin), and Cephalosporins (e.g. ceftriaxone and cefitime). (See Appendix). Recently, strains of gonorrhea resistant to Cephalosporins, which are considered the last line of defense, have emerged and have the potential to become a threat [21].

2 Biological Overview

2.1 Antibiotic Resistance Mechanisms in Bacteria

Changes in the genetic material of *N. gonorrhoeae* can occur horizontally (gene transfer from an external source) and vertically (mutations during cell division). Both these methods serve for the developing of antibiotic resistance phenotypes in bacteria. There are three independent means of horizontal gene transfer: conjugation, transformation, and transduction. Vertical gene transfer discussed herein refers to chromosomal mutations.

Conjugation is the transmission of genetic material via bacteria cell-to-cell contact (see Figure 1). During this process, a conjugative plasmid[1] is acquired by one bacterium attaching its pili to another bacterium, resulting in the transference of a plasmid. The plasmid replicates itself, transfers the genetic material to the other bacterium, and integrates the newly replicated DNA into the other bacterial chromosome. The F^+ factor in the plasmids is the reason why bacteria can conjugate. R plasmids carry genetic code for resistance to a single or multiple drugs, meaning *N. gonorrhoeae* can obtain various means of resistance in a single step by conjugation [37]. Penicillin and Tetracycline are two good separate examples of the way *N. gonorrhoeae* obtain antibiotic resistance by plasmid conjugation.

The first stage of the development of bacterial resistance to antibiotics is generally either plasmid conjugation (whole or partial gene attainment) or chromosomal mutation, which are selected due to antibiotic pressure. After a substantial number of bacteria cells within the host become

[1] A plasmid is a circular DNA molecule that is generally independent of the chromosome and can self-replicate and repair its own DNA. The process of conjugation is centered around the plasmid.

resistant, the resistant genotype is widely spread through the population of bacteria, mainly via natural transformation [35].

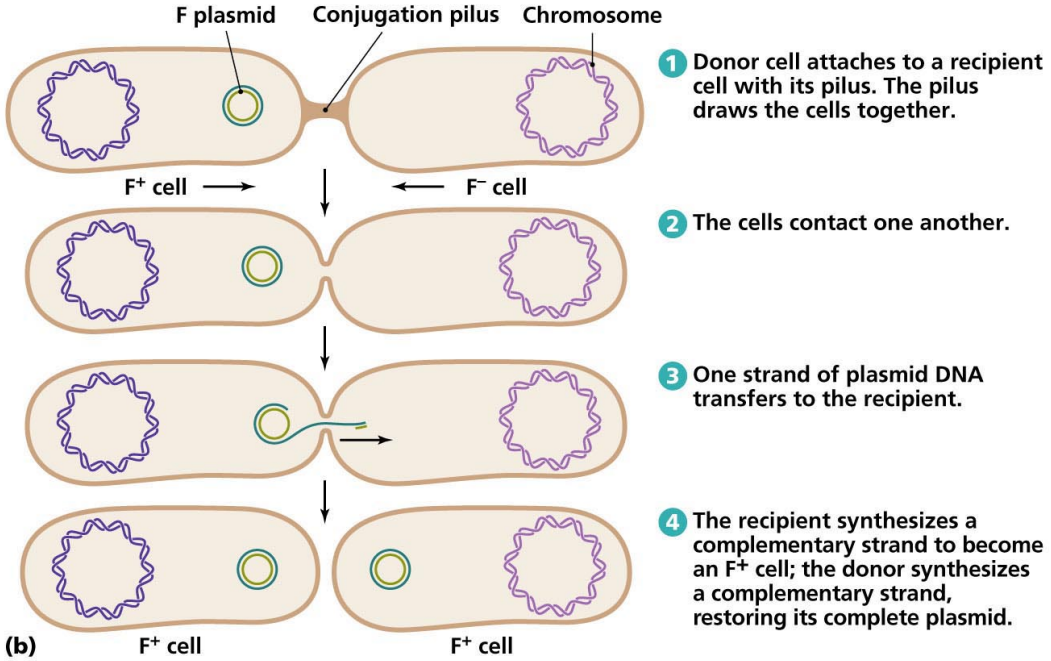

Figure 1: Conjugation: transfer of a plasmid with genetic material via bacteria cell-to-cell contact. Conjugative plasmid is acquired by one bacterium attaching its pili to another bacterium. The plasmid replicates itself, transfers the genetic material to the other bacterium, and integrates the newly replicated DNA into the other bacterial chromosome [25].

Natural transformation (see Figure 2), is the ability for bacteria to uptake DNA from the extracellular environment and efficiently incorporate the DNA into their chromosomes. *N. gonorrhoeae* is naturally competent at all phases of growth, meaning that the organism is able to transform at every phase, constituting one of only 44 species of naturally competent bacteria (e.g., *Bacillus subtilis* and *Haemophilus influenzae* [27]). Transformation has four steps: DNA donation, uptake, processing and integration into the chromosome. The transforming DNA is given by the surrounding *N. gonorrhoeae* cells by two mechanisms, secretion of chromosomal DNA into the extracellular environment or autolysis [2]. *N. gonorrhoeae* only takes up DNA that has the genus specific DNA uptake sequence (DUS), so they can readily exchange DNA with each other [23]. For instance, *Streptomycin* is an antibiotic that *N. gonorrhoeae* gained resistance to through transformation [29].

The third means of horizontal gene transfer is transduction, a type of DNA exchange that occurs when a bacteriophage (bacteria-infecting virus) takes DNA from one bacterium and transplants

[2] Autolysis is a self-destruct or self-digest mechanism that releases DNA into the surroundings [9].

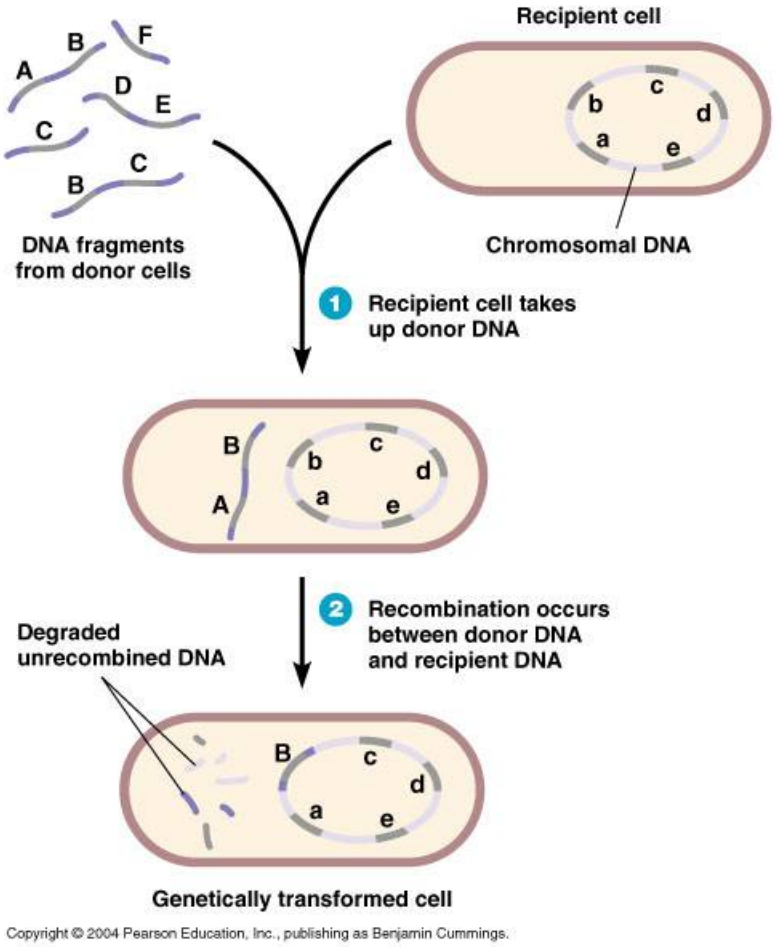

Figure 2: Transformation: direct uptake of genetic material from its external surroundings. Transformation has four steps: DNA donation, uptake, processing and integration into the chromosome. *N. gonorrhoeae* only takes up DNA that has the genus specific DNA uptake sequence (DUS) [10].

it to another bacterium. There are no phages known to infect *N. gonorrhoeae* [9]. Therefore, for purposes of this manuscript, transduction is rendered irrelevant.

Besides horizontal gene transfer, the other method for developing resistance is chromosomal mutation, or spontaneous mutation, which is a random event that alters the genes of a bacterium. This event, however, is rare due to multiple mechanisms that repair mutated genes. Penicillin and Fluoroquinolones are two examples to which *N. gonorrhoeae* gained resistance through chromosomal mutations.

If a resistant genotype is acquired through chromosomal mutations, it does so over many generations, whereas plasmid-gained resistance is a quick, one-step process [21]. Hence, chromosomal

mutations occur substantially less often than plasmid conjugation. Further, plasmid conjugation is considerably less efficient than transformation [35]. Only transformation, not plasmid conjugation, can mobilize chromosomal genes [9].

Another way *N. Gonorrhoea* can obtain resistance is by efflux pumps in the cell membrane that can eject the antibacterial out of the cell, making the bacteria resistant to the antibiotic. These pumps are antibiotic and species dependent [22].

2.2 Immune System Response to Gonorrhea

Everyday we are bombarded by a slew of extraneous infectious agents. Our defense mechanism to these disease-causing agents is the immune system. The immune system can be divided into two complementary segments, innate and adaptive. Innate immunity is characterized as being non-specific, targets any foreign substance, and fast. It is our first line of defense against foreign invaders or substances, which are also known as pathogens.

Once a microorganism, such as *N. gonorrhoeae*, has invaded, it encounters the cellular and humoral aspects of our immune system. The cells of the innate system include phagocytes, which engulf and destroy pathogens. After these cells have engulfed the antigen (a foreign substance that induces an immune response) they will travel to localized areas to present and inform the cells of the adaptive immune system of infection.

Adaptive immunity in most cases takes much longer than the innate immune system to respond to antigen; however, it is highly specific and forms immunological memory for future invasions. This memory allows it to react faster in successive exposure. Another advantage of adaptive immunity is that it can recognize if a host cell is infected whereas the innate system cannot.

The cellular component of adaptive immunity which recognizes if host cells are infected, including innate cells, are T cells. The other components of the adaptive immune response are B cells which cannot tell if host cells are infected. Like the innate immune system, B cells recognize pathogens outside of cells. In most cases, a B cell will react to a particular antigen, engulf it, and present it to T cells for activation. In other cases, B cells do not need T cells for activation. This can occur when B cells recognize completely foreign substances to the host, such as LPS, lipo-poly-sacchride, a component of the outer membrane of gram negative bacteria.

Once activated, the B cells will undergo a process and begin secreting antibodies, which recognize the same antigen as the B cell because the antibody was once the B cell receptor. Antibodies in essence tag a pathogen for either destruction by a phagocyte or to stimulate complement, a part of the innate immune system. Complement is composed of proteins that bind to antibody or carbohydrates on a pathogen. When complement is stimulated by antibodies, it will rupture the cell. When stimulated by carbohydrates on the pathogen, it will mark the pathogen for destruction by phagocytes.

While the immune system has an array of responses to prevent and rid the body of infection, *N. gonorrhoeae* has adapted a repertoire of mechanisms to avoid the immune system. In a recent study done by Liu, Feinen, and Russel, it was shown that *N. gonorrhoeae* suppresses T cell activa-

tion by activating T regulatory cells (Treg) and specific antibody responses. Treg cells suppress T cell activation by secreting TGF-β, a chemical mediator, which is also found naturally in genital tracts and is important for reproduction. Therefore, *N. gonorrhoeae* amplifies part of the body's natural environment to survive [14].

Also, there is evidence that *N. gonorrhoeae* can survive within neutrophils, a type of phagocyte [4]. This is because *N. gonorrhoeae* has the ability to suppress intracellular oxidative burst, a main mechanism used by neutrophils to destroy pathogens [7]. *N. gonorrhoeae* has the ability to invade both phagocytes and non-immune system cells by using Opa proteins which furthers its capability to evade the immune system [18].

N. gonorrhoeae maintains a diverse population which allows it to avoid complete destruction by protein specific antibody. A diverse population is achieved through genetic polymorphism of surface proteins, recombination of genes, uptaking DNA from the environment, as well as secreting it, and phenotype switching of bacterium [14]. Another mechanism used by *N. gonorrhoeae* to increase its survival is to secrete a protease with two functions. First, the protease is able to destroy one of the two common antibodies found in the mucosal lining of vaginal tracts in infected individuals [3]. Second, the protease is believed to cleave an endosomal membrane protein which allows *N. gonorrhoeae* to survive intracellularly in host cells [13].

An additional mechanism used by *N. gonorrhoeae* to prevent antibodies binding is to create decoys. Firstly, *N. gonorrhoeae* sheds its outer membrane and creates blobs of genetic material to which antibody bind. Likewise, present on *N. gonorrhoeae* exterior is lipooligosaccharide(LOS), a type of LPS, which triggers an immediate B cell reaction. However, LOS mimics human gangliosides, used for cellular recognition and cell to cell communication [6]. The host will then naturally modify it and this allows it to prevent antibodies from binding [30]. A second advantage to this modification is that it prevents complement binding and increases the bacteria's resistance to phagocytes [18].

The complexity of the ability of *N. gonorrhoeae* to evade the immune system poses a hindrance to our ability to accurately model the immune system effect on the bacteria. However, we know that the immune system can completely clear gonorrhea [19]. We will assume the rate of adapting to *N. gonorrhoeae* by the immune system is such that the body will reach a peak saturation point where the clearing rate of *N. gonorrhoeae* by the immune system is constant. The first three models will present a scenario in which the immune system has reached a saturation point, therefore, we will use a constant clearing rate, i, for gonorrhea.

2.3 Modeling the Evolution of Within Host Antibiotic Resistance

Our modeling framework is as follows. At the infection event, the patient receives a load of drug-sensitive *N. gonorrhoeae*. These bacteria colonize and reproduce in a certain area of the host body (e.g. the cervix area). Mutations that lead to different phenotypic variants occur spontaneously. Thus, in a short amount of time after infection, there are multiple drug-sensitive bacteria strains and some drug-resistant bacteria (see Figure 3). In the absence of treatment, bacteria reproduce at a rate determined, mainly, by their relative reproductive fitness (inter-bacterial competition for space and resources) and the immune system response to the infection. Usually, the acquisition

of a drug-resistant genotype is accompanied by a reduction in the mutant bacteria's fitness (e.g. lower reproduction success). Therefore, in this non-treatment scenario, it is expected that the drug-sensitive bacteria[3] outcompetes the drug-resistant strains.

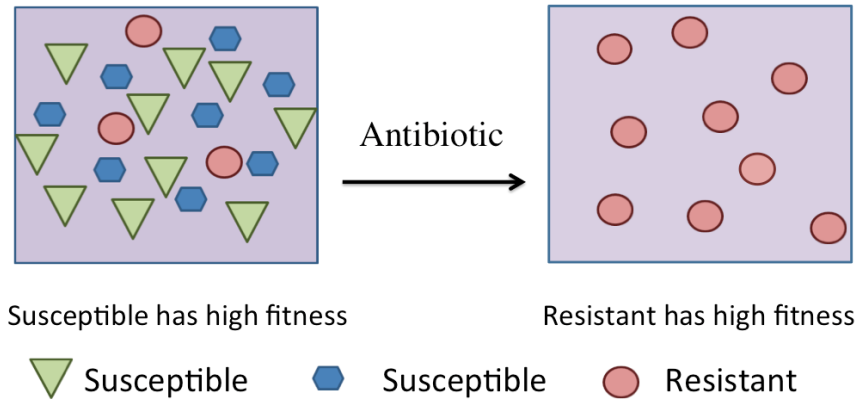

Figure 3: Schematic of Antibiotic Resistance Emergence. Soon after the initial infection, and before the onset of treatment, there are multiple drug-sensitive strains and some drug-resistant strains. In this environment, the drug-sensitive strains have a higher fitness. Treatment adds a new selection pressure into this environment, affecting mainly the drug-sensitive strains, thus giving a selective advantage to the resistant strains.

The introduction of treatment into this bacterial competition scenario changes the fitness function[4] of the environment. That is, treatment is a new selection pressure that mainly affects the drug-sensitive strains and, consequently, could confer a reproductive advantage to the drug-sensitive strains. If treatment indeed provides sufficient selection advantage to the drug-resistant strains, they would proliferate and become the prevailing strain. In this case, the patient cannot be cured with this antibiotic and serious complications could follow, including severe side effects from the disease and the spread of resistance at the population level.

Given this simplified modeling framework, our goal is to better understand the evolutionary dynamics of bacteria resistance in gonorrhea within a host and propose treatment strategies to mitigate the emergence of resistance in treated patients and, consequently, its spread at the population level. To that aim, next we present a phenomenological model that intends to capture the dynamics described above. Section 3.1 presents a simpler version of the original model that makes the simplifying assumption that bacteria grow exponentially with no inter-bacterial competition. Section 3.2, on the one hand, reincorporates the logistic growth within the bacteria population. On the other hand, we reduce its complexity by excluding nonlinear interaction terms (conjugation). The dynamics of these two simplified models are studied analytically. In Section 3.3 we present the full model with both logistic growth and conjugation integrated. This last version is analyzed numerically, providing a more complete understanding of the factors that determine how antibiotic resistance emerges within an infected host. In addition, Section 3.4 presents a sensitivity analysis,

[3]Also known as wild-type because of its high prevalence at the host-population level.
[4]Since treatment changes the environment, it also changes what the optimal phenotype is.

quantifying the relative effects of changes in controllable parameters such as treatment and the immune system.

3 The Gonorrhea Strain Competition Model

In this manuscript, we focus on the within-host dynamics of antibiotic resistance, applied to the case of gonorrhea. A phenomenological (non-mechanistic) mathematical model is built aiming to mimic the coevolution of drug-sensitive and drug-resistant bacteria (see Figure 4). As discussed previously, bacteria experience spontaneous mutations, horizontal gene transfer, and drug-resistance-based selection among the bacteria. These factors play an important role in the relative strain abundance distribution (proportion of each bacteria strain type in the population).

Based on susceptibility to antibiotics, we assume that the *N. gonorrhoeae* bacteria in the host can be classified as one of three strains: drug-sensitive (S), resistant with low fitness (R_l), and resistant with high fitness (R_h). The model we consider is a system of non-linear differential equations given by:

$$\frac{dS}{dt} = S\left[b_S\left(1-\frac{N}{K}\right) - i - T - m_1\right] - pS(R_l + R_h) \quad (1)$$

$$\frac{dR_l}{dt} = R_l\left[b_l\left(1-\frac{N}{K}\right) - i - \alpha T - m_2\right] + m_1 S + pR_l(S - R_h) \quad (2)$$

$$\frac{dR_h}{dt} = R_h\left[b_h\left(1-\frac{N}{K}\right) - i - \alpha T\right] + m_2 R_l + pR_h(S + R_l) \quad (3)$$

Each of these bacteria strains are produced at a per-capita rate b_s, b_l and b_h, respectively. An important aspect of this evolutionary scenario is the inter-bacteria competition for the limited space and resources inside the host. Moreover, in general, bacteria do not die from old age, but rather from food scarceness. A simple way to capture this is to assume that bacterial growth (i.e., cell division rate) follows a logistic growth pattern. To integrate this assumption into the model, the per-capita growth rate of the x bacterial strain, b_x, becomes

$$b_x\left(1-\frac{N}{K}\right)$$

where $N = S + R_l + R_h$ is the total population of strains (not necessarily constant), and K is the carrying capacity or maximum number of bacteria that can survive in the infected region (e.g., a section of the cervix). Notice that the carrying capacity term depends on the entire bacterial population, not only the x strain, better mimicking the inter-bacteria competition. Due to transformation from the genus *Neisseria* and mutations during cell division, drug-sensitive bacteria become drug-resistant at a per-capita rate m_1. Normally, horizontal gene transfer from the already resistant bacterial flora (other than gonorrhea) does not contribute to m_1 due to the DUS [5] requirements for gene transfer.

Mutation from the drug-sensitive strain to the low-fitness antibiotic-resistant strain by acquisition of a resistant phenotype is typically accompanied by a fitness cost, and generally reflected in

[5] DUS stands for a DNA uptake sequence. Gonorrhea bacteria only absorb DNA with a genus specific DUS, and so it cannot uptake genetic material from other bacterial flora within the host.

a decreased birth rate for the low fitness resistant strain. Thus our parameter values must satisfy $b_l < b_s$, which implies that, in the absence of treatment, the drug-sensitive bacteria typically has higher fitness than the low-fitness drug-resistant strain.

Compensatory mutations occur at a per-capita rate m_2, which includes all vertical and horizontal gene transfers that lead to a high-fitness resistant strain. In this model, compensatory mutations increase the birth rate of the high-fitness drug-resistant strain without compromising this resistant phenotype. In other words, $b_h > b_l$.

When two bacteria interact, resistance encoding genetic material can be transferred through bacterial conjugation at a rate p. Moreover, it is assumed that p is the rate of conjugation between all three strains of bacteria because the exchange mechanisms are comparable for each strain.

A holistic view of the effect of the immune system on the bacterial infection is adopted. That is, the components of the immune system (phagocytes, T-Cells, B-Cells) and its infection-clearing mechanisms (antigen-binding affinity, etc.), are not explicitly modeled. Rather, an emphasis is placed on the combined effect of all these components on the bacterial population. Thus, it is assumed that the immune system clears the infection at a per-capita rate i. Moreover, the immune system will affect all three bacterial strains with the same rate.

Antibiotic treatment comes in different forms (e.g. penicillin, cephalosporins, fluoroquinolones, and tetracyclines), and consequently affects the bacteria's life cycle differently. In this model it is considered that treatment either disrupts the bacteria cell division process or directly eliminates them. In either case, these phenomena can be modeled by assuming that treatment clears the drug-sensitive bacteria at a per-capita rate T_s. Additionally, both the drug-resistant bacteria variants are affected by treatment at a per-capita rate T_r. A simple way to model the fact that drug-resistant bacteria are less affected by treatment than drug-sensitive, is through a constitutive relation between T_r and T_s given by

$$T_r = \alpha T_s, \text{ where } 0 \leq \alpha \leq 1.$$

For values of α between 0 and 1, this parameter is representative of increasing levels of partial resistance to the antibiotic. For $\alpha = 0$, the drug-resistant bacteria has complete resistance to the antibiotic, which therefore cannot decrease the population of drug-resistant bacteria. For $\alpha = 1$, the drug-resistant bacteria are cleared by the antibiotic in the same manner as the drug-sensitive bacteria. Thus α adds richness to our model by allowing us to explore the impact of different levels of resistance on the overall coevolutionary dynamics. For notational simplicity, we will symbolize the treatment clearing rate by T.

3.1 Model I: Absence of Inter-Bacteria Competition

In this version of model (1)-(3), all bacteria strains are, as before, intrinsically produced at a per-capita rate b_s, b_l and b_h, respectively. However, the competition for space and resources is not considered (i.e. $K \to \infty$). Instead, bacteria naturally die at a rate μ. The system of non-linear

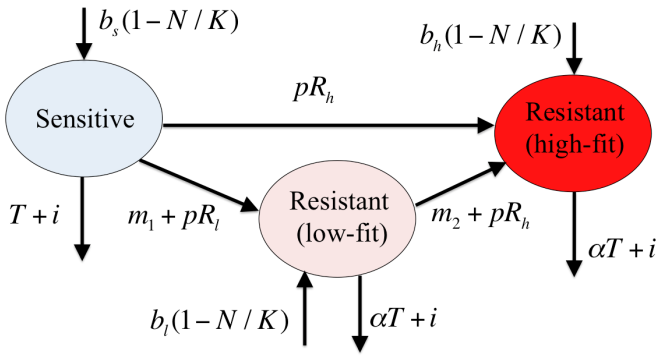

Figure 4: Flow Diagram of the Compartmental Model of the Dynamics between Drug-Sensitive and & Drug-Resistant Bacteria in system (1)-(3). Three strain types are featured: a drug-sensitive (S), a low-fitness (R_l) and a high fitness (R_h) resistant strains. All strains grow logistically and different mutation rates (m_1, m_2, p) convert drug-sensitive strains into drug-resistant strains. Additionally, strains are cleared by the immune system (i) and treatment (T).

ordinary differential equations describing this scenario is as follows:

$$\frac{dS}{dt} = S(b_s - \mu - i - T - m_1) - pS(R_l + R_h) \tag{4}$$

$$\frac{dR_l}{dt} = R_l(b_l - \mu - i - \alpha T - m_2) + m_1 S + pR_l(S - R_h) \tag{5}$$

$$\frac{dR_h}{dt} = R_h(b_h - \mu - i - \alpha T) + m_2 R_l + pR_h(S + R_l) \tag{6}$$

3.1.1 Non-dimensionalization of Model I

The system (4)-(6) can be non-dimensionalized by introducing the following new dimensionless state and time variables:

$$s = S\frac{p}{m_1} \qquad l = R_l\frac{p}{m_1} \qquad h = R_h\frac{p}{m_1} \qquad \tau = tm_1$$

In these new variables, system (4)-(6) becomes

$$\frac{ds}{d\tau} = sA - s(l + h) \tag{7}$$

$$\frac{dl}{d\tau} = l\,(B - M) + s + l(s - h) \tag{8}$$

$$\frac{dh}{d\tau} = hC + lM + h(s + l) \tag{9}$$

where

$$A = \frac{a}{m_1} - 1 \qquad B = \frac{b}{m_1} \qquad C = \frac{c}{m_1} \qquad M = \frac{m_2}{m_1}$$

$$a = b_s - \mu - i - T \qquad b = b_l - \mu - i - \alpha T \qquad c = b_h - \mu - i - \alpha T$$

This new system (7)-(9) has only four parameters, compared to ten in the original one.

Let G_s, G_l, and G_h be the reproductive numbers for the sensitive, low-fitness and high-fitness resistant bacterial strains, respectively. We define these quantities as follows

$$G_s := \frac{b_s}{\mu + i + T + m_1} \qquad G_l := \frac{b_l}{\mu + i + \alpha T + m_2} \qquad G_h := \frac{b_h}{\mu + i + \alpha T}$$

Biologically, these quantities can be interpreted as follows: G_x is the average number of viable bacteria offspring that a typical strain x bacteria produces in its lifetime. Notice that $G_s \leq 1 \iff A \leq 0$ and $G_s \geq 1 \iff A \geq 0$. Also $G_l \leq 1 \iff B - M \leq 0$ and $G_l \geq 1 \iff B - M \geq 0$. Finally, $G_h \leq 1 \iff C \leq 0$ and $G_h \geq 1 \iff C \geq 0$. Also $G_h \geq G_l$ since G_h has a larger numerator ($b_h \geq b_l$), and a smaller denominator than G_l.

3.1.2 Analysis of Equilibria

The system (7)-(9) has four steady states[6], P_1^1, P_1^2, P_1^3 and P_1^4. The first two points are

$$P_1^1 = (0, 0, 0) \qquad P_1^2 = \left(0, C\left(-1 + \frac{M}{B}\right), B - M\right)$$

The third and fourth points are of the form (S^*, R_l^*, R_h^*), with $S^* \neq 0,\ R_l^* \neq 0,$ and $R_h^* \neq 0$ and are presented in the Appendix. The first steady state, P_1^1, is the infection-free equilibrium. The second one, P_1^2, represents the worst case scenario in which the resistant strains completely colonize the host. The third and fourth equilibria represent possible coexistence scenarios in which both strain types are present in the bacterial infection.

We will focus on finding the conditions for P_1^2 to be biologically significant, and also derive the parameter constraints that determine the stability of P_1^1 and P_1^2. Moreover, we are interested in finding conditions for the scenario in which the host has cleared the infection, i.e., when P_1^1 is locally stable and P_1^2 unstable.

For P_1^2 to be biologically significant, all its coordinates must be in the first octant. The R_h coordinate is non-negative if $B - M \geq 0$, which also implies $G_l \geq 1$. Regarding the R_l coordinate, if $B - M \geq 0$, since $M \geq 0$, then $B \geq 0$ as well. Consequently, $B - M \geq 0 \implies \frac{M}{B} \leq 1 \implies -1 + \frac{M}{B} \leq 0$. Then, for the R_l coordinate to be non-negative, the condition $C \leq 0$ must be satisfied. Recalling that $C \leq 0 \implies G_h \leq 1$, R_l is non-negative whenever

$$G_h \leq 1 \leq G_l$$

However, this does not make biological sense in our model. Since the R_h strain is by definition more fit than the R_l strain, we have that $b_h > b_l$, so the numerator of G_h is greater than that of G_l. Also, since the denominator of G_h is smaller than that of G_l, we know that $G_l < G_h$. Hence, P_1^2, the resistant-only equilibrium, is never biologically relevant in this specific context.

[6]Subscript stands for the model, and the superscript stands for the steady state number in that model

3.1.3 Local Stability Analysis

The local stability of the steady states can be established by finding the corresponding eigenvalues of the Jacobian matrix of the system (7)-(9) evaluated at the respective steady states [32]. The sign of the real part of these eigenvalues will determine the local stability. For any given steady state, if all three eigenvalues have a negative real part, then that steady state is locally stable.

Eigenvalues for the infection-free equilibrium P_1^1

$$\lambda_1^1 = A \qquad \lambda_2^1 = B - M \qquad \lambda_3^1 = C$$

We can readily see that the infection-free steady state is locally stable if

$$G_s < 1, \quad G_l < 1, \quad \text{and} \quad G_h < 1.$$

The biological interpretation of this result is straightforward: if none of the strains can produce more than one viable offspring in their lifetime, then the infection dies out.

The main finding derived from Model I is that if all the reproductive numbers G_s, G_l and G_h are less than one, the infection-free equilibrium is locally asymptotically stable. Also of interest is that the equilibrium in which only both resistant strains coexists is not biologically relevant to the specific problem addressed in this work.

3.2 Model II: Absence of Conjugational Gene Transfer

For the purpose of analytic tractability, this simplified version of the model in (1)-(3) disregards the acquisition of the resistant phenotype by means of conjugation, that is, $p = 0$. With these modification, Model II becomes:

$$\frac{dS}{dt} = S\left[b_S\left(1 - \frac{N}{K}\right) - i - T - m_1\right] \tag{10}$$

$$\frac{dR_l}{dt} = R_l\left[b_l\left(1 - \frac{N}{K}\right) - i - \alpha T - m_2\right] + m_1 S \tag{11}$$

$$\frac{dR_h}{dt} = R_h\left[b_h\left(1 - \frac{N}{K}\right) - i - \alpha T\right] + m_2 R_l \tag{12}$$

3.2.1 Non-Dimensionalization of Model II

The system (10)-(12) will be non-dimensionalized by rescaling the bacterial populations with the carrying capacity K, and rescaling time with the mean sensitive-bacteria-doubling time $\frac{1}{b_s}$. The new dimensionless variables s, l and h are defined as follows:

$$s = \frac{S}{K} \quad l = \frac{R_l}{K} \quad h = \frac{R_h}{K} \quad \tau = b_s t.$$

With this change of variable the new system is now

$$\frac{ds}{d\tau} = s(1-s-l-h) - \frac{s}{G_s} \tag{13}$$

$$\frac{dl}{d\tau} = F_l\left[l(1-s-l-h) - \frac{l}{G_l} + sU\right] \tag{14}$$

$$\frac{dh}{d\tau} = F_h\left[h(1-s-l-h) - \frac{h}{G_h} + lW\right] \tag{15}$$

with

$$F_l = \frac{b_l}{b_s}, \quad F_h = \frac{b_h}{b_s}, \quad U = \frac{m_1}{b_l} \quad \text{and} \quad W = \frac{m_2}{b_h}.$$

Notice that F_l and F_h are the relative fitness of the low-fitness and high-fitness bacteria with respect to the sensitive strain, respectively (thus $F_h > F_l$). G_s, G_l and G_h are defined as in 3.1.1, with the modification of $\mu = 0$, where μ was the natural death rate of the bacteria in Model I:

$$G_s = \frac{b_s}{i+T+m_1} \qquad G_l = \frac{b_l}{i+\alpha T+m_2} \qquad G_h = \frac{b_h}{i+\alpha T}$$

3.2.2 Analysis of Equilibria

In our non-dimensionalized model 13-15, we found four equilibria. They are listed as follows,

Infection-Free Equilibrium: $P_2^1 = (0,0,0)$

High-Fitness Resistant-Only Equilibrium: $P_2^2 = \left(0, 0, \frac{G_h-1}{G_h}\right)$

Resistant-Only Equilibrium: $P_2^3 = \left(0, \frac{F_h(-1+G_l)(-G_h+G_l)}{G_l(F_h(-G_h+G_l)+G_hG_lW)}, \frac{G_hW(-1+G_l)}{F_h(-G_h+G_l)+G_hG_lW}\right)$

Coexistence Equilibrium:

$$P_2^4 = \left(\frac{F_hF_l(G_s-G_h)(G_s-G_l)(-1+G_s)}{G_s\psi}, \frac{UF_hG_l(G_s-G_h)(-1+G_s)}{\psi}, \frac{UWG_sG_lG_h(-1+G_s)}{\psi}\right)$$

where $\psi = F_h(-G_h+G_s)(F_l(-G_l+G_s)+G_lG_sU)+UWG_s^2G_lG_h$.

We are able to determine analytical conditions for the biological relevance and local stability of the first three equilibria. The fourth one was analyzed numerically.

High-Fitness Resistant-Only Equilibrium: P_2^2

The high-fitness resistant-only equilibrium is biologically relevant if $\frac{G_h-1}{G_h} \geq 0$ holds. This is only true if $G_h \geq 1$. Hence, we need the reproductive number of the high-fitness resistant bacteria to be greater than one for this equilibrium to be admissible.

Resistant-Only Equilibrium: P_2^3

If this equilibrium is to be biologically relevant, both the l and h components must be nonnegative. In the case of h we have

$$h = \frac{G_h W(-1+G_l)}{F_h(-G_h+G_l)+G_h G_l W} \geq 0$$

Hence, $(-1+G_l)$ and $(-F_hG_h+F_hG_l+G_hG_lW)$ must be either both positive or both negative, so we have either

$$G_l \geq 1 \text{ and } -F_hG_h+F_hG_l+G_hG_lW \geq 0 \text{ or}$$
$$G_l \leq 1 \text{ and } -F_hG_h+F_hG_l+G_hG_lW \leq 0.$$

We also need

$$l = \frac{F_h(-1+G_l)(-G_h+G_l)}{G_l(F_h(-G_h+G_l)+G_hG_lW)}$$

to be positive. From the h coordinate we have that $\frac{(-1+G_l)}{F_h(-G_h+G_l)+G_hG_lW} \geq 0$, so in order for this point to be biologically relevant, the term $(G_l - G_h)$ must be positive, implying that $G_l \geq G_h$. However, this is not true in this specific biological context, as previously explained in Section 3.1. Therefore, for the purposes of this model, the Resistant-Only Equilibrium is never biologically relevant. Interestingly, we arrived at the same conclusion using Model I, suggesting that the two resistant strains cannot coexist alone. This result is intuitively sound since, in the absence of a drug-sensitive strain (which "feeds" the low-fitness resistant strain at a rate m_1) the high-fitness resistant strain is more fit (higher reproduction rate) than the low-fitness one, thus the former one will always outcompete the latter.

Coexistence Equilibrium: P_2^4

For the coexistence equilibrium to be biologically relevant, we must have its $h, l,$ and s components to be non-negative. Let us first consider when the h component is non-negative. This happens when the denominator and the numerator are either both non-negative, or both negative. Hence, we have two cases:

$$G_s \geq 1 \text{ and } F_h(-G_h+G_s)(F_l(-G_l+G_s)+G_lG_sU)+UWG_s^2G_lG_h \geq 0,$$

and

$$G_s \leq 1 \text{ and } F_h(-G_h+G_s)(F_l(-G_l+G_s)+G_lG_sU)+UWG_s^2G_lG_h \leq 0.$$

Let us next consider when l is non-negative. If either of the above cases hold, this implies that $(G_s - G_h) \geq 0$ in order for l to be non-negative.

Finally, consider when the s component is non-negative. If either of the above cases hold together with the condition that makes the l component non-negative, then the s component is non-negative when $(G_s - G_l) \geq 0$. To summarize, the conditions determining the biological relevance of the coexistence equilibrium P_2^4 all rely on the value of G_s in the following forms:

Case I: $0 \leq F_h(-G_h+G_s)(F_l(-G_l+G_s)+G_lG_sU)+UWG_s^2G_lG_h$; $\quad 1 \leq G_s$; $\quad G_h \leq G_s$ and

$G_l \leq G_s$

Case II: $0 \geq F_h(-G_h + G_s)(F_l(-G_l + G_s) + G_l G_s U) + UWG_s^2 G_l G_h$; $\quad 1 \geq G_s$; $\quad G_h \leq G_s$ and $G_l \leq G_s$

Biologically, these two cases can be interpreted as follows. The last three conditions in cases I and II imply that for the coexistence equilibrium to be admissible, the drug-sensitive strain must have a higher reproductive number than its resistant counterparts, and that it *does not* matter how many viable offspring a typical drug-sensitive bacterium produces in its lifetime.

To sum up, P_2^2 is biologically significant if and only if $G_h \geq 1$, P_3^2 is never biologically relevant in the context of this model, and for P_4^2 to be biologically relevant, it is necessary (but not sufficient) that $G_l \leq G_h \leq G_s$.

3.2.3 Local Stability Analysis

Infection-Free Equilibrium

For the infection-free equilibrium to be locally stable, each of the eigenvalues of the Jacobian matrix evaluated at P_2^1 have a negative real part. The eigenvalues are

$$\lambda_1 = F_h\left(1 - \frac{1}{G_h}\right), \lambda_2 = F_l\left(1 - \frac{1}{G_l}\right), \text{ and } \lambda_3 = 1 - \frac{1}{G_s}.$$

Thus, this point is locally stable if and only if

$$G_h < 1, \quad G_l < 1, \text{ and } \quad G_s < 1.$$

Therefore, in order for the infection-free equilibrium to be locally stable, the reproductive number of each strain of *N. gonorrhoeae* must be less than one. The biological interpretation of these conditions are equivalent to those given in Model I, that is, if none of the strains can produce more than one viable offspring in their lifetime, the infection dies out.

High-fitness Resistant-Only Equilibrium: P_2^2

The eigenvalues for this equilibrium are

$$\lambda_1 = F_h\left(-1 + \frac{1}{G_h}\right), \lambda_2 = F_l\left(\frac{1}{G_h} - \frac{1}{G_l}\right), \text{ and } \lambda_3 = \frac{1}{G_h} - \frac{1}{G_s}.$$

Hence, the conditions for local stability of this point are

$$1 < G_h, \quad G_l < G_h, \text{ and } \quad G_s < G_h.$$

This means that for the high-fitness resistant-only equilibrium to be locally stable, its reproductive number must be greater than one, and greater than the reproductive numbers of both the low-fitness resistant and drug-sensitive strains.

Since the Resistant-Only Equilibrium is not biologically relevant, its stability is not considered. The stability of the coexistence equilibrium was not analyzed analytically. However, a necessary condition about its stability can be deduced. Since the infection-free equilibrium is stable if $G_s < 1$,

then $G_s > 1$ must necessarily hold for the coexistence equilibrium to be stable. In the next section we provide, in addition to this analysis, a numerical characterization of the stability behavior of the system.

A summary of the analytical results regarding the biological significance and stability of Model II is presented on Table 1:

Table 1: Conditions for biological significance and local stability for the equilibria in Model II

	Biological Significance	**Local Stability**
P_2^1	Always	$G_s < 1, G_l < 1, G_h < 1$
P_2^2	$G_h \geq 1$	$G_h > 1, G_h > G_l, G_h > G_s$
P_2^3	Never	
P_2^4	$G_l \leq G_h \leq G_s$ (necessary)	$G_s > 1$ (necessary)

Additionally, Table 1 provides the following remarks:

- If P_2^1 is stable, then P_2^2 is not biologically relevant or stable.
- If P_2^2 is stable, then P_2^1 is not stable and P_2^4 is not biologically relevant. Thus, when G_h crosses 1 (from below), if $G_h > G_s$, P_2^2 becomes stable and P_2^1 loses its stability.
- If P_2^4 is biologically admissible, P_2^2 is not stable. Moreover, if $G_s < 1$, and P_2^4 is admissible, then P_2^1 is stable. In this case, the phase space has two equilibria, with P_2^1 as the stable one. It also seems that, since P_2^1 can lose its stability when G_s crosses 1 (from below), if P_2^4 is admissible, then the latter point becomes stable, through a transcritical bifurcation.

3.2.4 A Numerical Perspective of the Stability Analysis

In this section, we numerically analyzed the equilibria of Model II. For parameter values used in the numerical computation, see Table 2 in Appendix 5.3.

To have a clearer idea of when the high-fitness resistant only equilibrium (P_2^2) and the coexistence equilibrium (P_2^4) are biologically significant as a function of the immune system clearing rate i and the treatment level T, Figure 5 shows for what values of i and T these two equilibria are biologically significant (white). In the small shaded triangular area P_2^2 and P_2^4 are both biologically relevant, thus, for these parameter values the phase plane has three admissible equilibria (including the infection-free equilibrium). It may appear that i plays no role in the P_2^2 case because of the vertical line separating the black and white regions. However, in order for P_2^2 to be biologically relevant, $G_h = \frac{b_h}{i+\alpha T} \geq 1$, so i does play some role. P_2^4 is admissible only for lower values of i. Interestingly, high treatment makes P_2^2 inadmissible, probably because high treatment would clear out the sensitive bacteria before it can mutate into resistant strains, rendering P_2^1 as the only admissible equilibrium. P_2^4 is only admissible when treatment is medium-to-low. It is intuitive that high treatment levels would not allow the coexistence point to exist since all the sensitive bacteria would be cleared from the host.

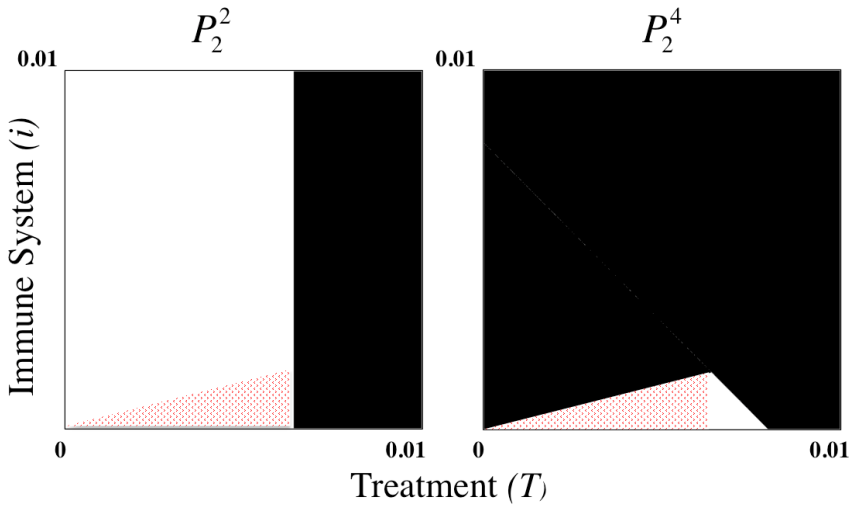

Figure 5: Biological Significance of P_2^2 and P_2^4 as a function of immune system (i) and treatment (T) in system 1-3. Black stands for *no* biological significance, white stands for biological significance. The parameter values used for these numerical simulations are the same as those in Table 2 in Appendix 5.3, with the exception that i and T are allowed to vary.

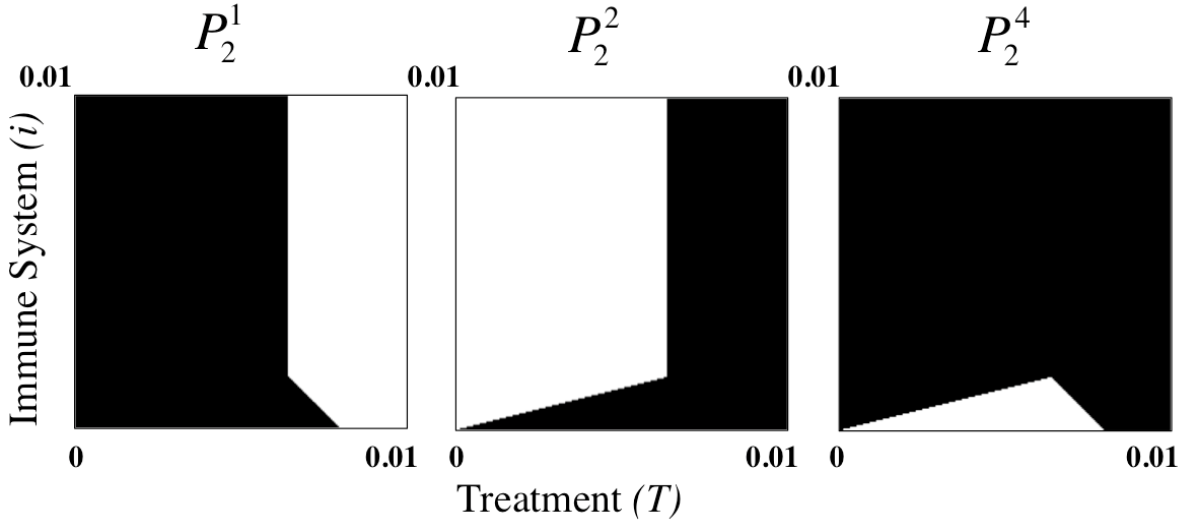

Figure 6: Stability of P_2^1 , P_2^2 and P_2^4 as a function of immune system (i) and treatment (T) in system 1-3. Black stands for *not* stable, white stands for stable. The parameter values used for these numerical simulations are the same as those in Table 2 in Appendix 5.3, with the exception that i and T are allowed to vary.

Figure 6 depicts, through the same numerical approach, the values of i and T for which P_2^1, P_2^2 and P_2^4 are stable [7] (white). Interestingly, this figure suggests that there cannot be two equilibria stable at the same time (notice that the white zones are exclusive and add up to the entire square). That is, local stability suggests global stability (at least numerically). In the shaded triangular area, where the three equilibria are present, P_2^4 is globally stable. Moreover, comparing Figure 5 and 6 we readily see that whenever P_2^4 is admissible, it is also globally stable.

3.3 The Gonorrhea Strain Competition Model: Numerical Simulations

In this section we numerically explore the model given in System (1)-(3) (see in Table 2 for parameter values). More specifically, we examine the role played by some of the key parameters in our model, especially those that are variable from within one patient to within another patient (e.g., immune system and treatment levels), and those whose values are uncertain and/or difficult to measure (e.g., mutation rates and relative fitness of resistant bacteria). When parameters are not varied in the plots, they are fixed at the values in Table 2, including setting $\alpha = 0$ to model full resistance. We will focus on the prevalence of each bacterial variant after one month of the infection event in Figures 7-14. The reasoning behind this particular time frame is that symptoms take 1-2 weeks to show, then treatment takes up to a week to clear the infection, adding up to about 20 days in which the patient was either treated and his/her symptoms disappeared if treatment was effective, or the patient did not seek for medical help and the disease took its "natural" course. Mathematically, we observe that for the parameter values of Table 2, the system after one month is extremely close to its equilibria levels, (see Figure 7). In addition, we assume in the ensuing figures that the initial bacterial population starts as entirely drug-sensitive, with no resistant strains present initially.

To ensure that our parameter values yield expected results, we simulate our system with and without treatment. In the case of no treatment we expect the drug-sensitive bacteria to prevail in the population, while the resistant variants are kept at lower levels. Conversely, in the presence of treatment, a severe selection pressure is placed on the sensitive strain, and as a result the high-fitness resistant strain can rise, eventually becoming more prevalent than the sensitive strain. Figure 7 shows these scenarios, adding to the validity of our model.

Role of Treatment and Different Levels of Drug-Susceptibility

Figure 8 (left) depicts the effect of treatment on the bacterial prevalence after one month. As expected, treatment severely affects the drug-sensitive population, while it gives a significant selective advantage to high-fitness resistant strains. Figure 8 (right) shows that, in the presence of treatment, increasing the drug-susceptibility (α) of the drug-resistant strain will decrease the after-one-month prevalence of the drug-resistant strains and also favor the dominance of the drug-sensitive strain.

Role of Carrying Capacity

The value of the carrying capacity as a proxy for the level of inter-bacteria competition plays a significant role in the frequency of bacteria in the population after one month. Moreover, Figure

[7] To decide which points were stable (white) in the (T, i) phase space, we picked those whose three eigenvalues had negative real part.

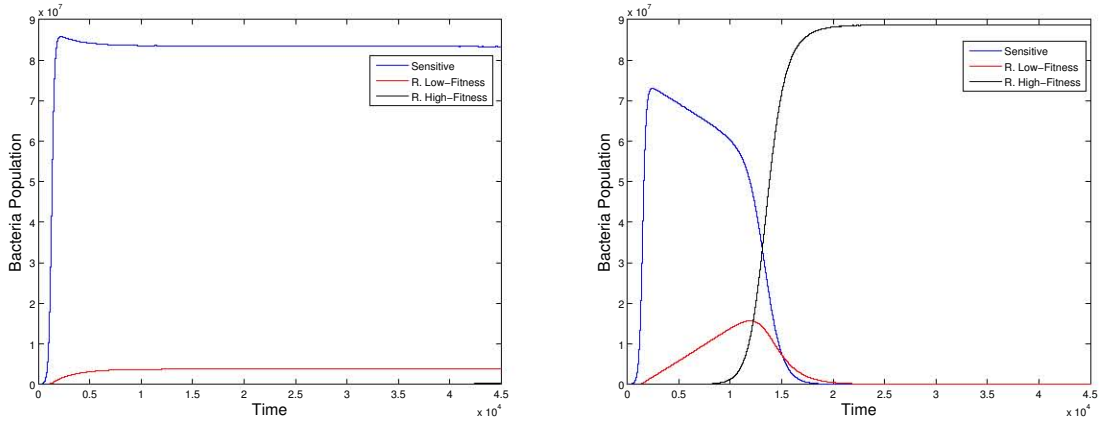

Figure 7: Bacterial Population with no Treatment (left) and with Treatment (right, $T = 1 \times 10^{-3}$), where the time is measured in minutes. With no treatment, the drug-sensitive prevails. In the presence of treatment the high-fitness resistant dominates the strain competition.

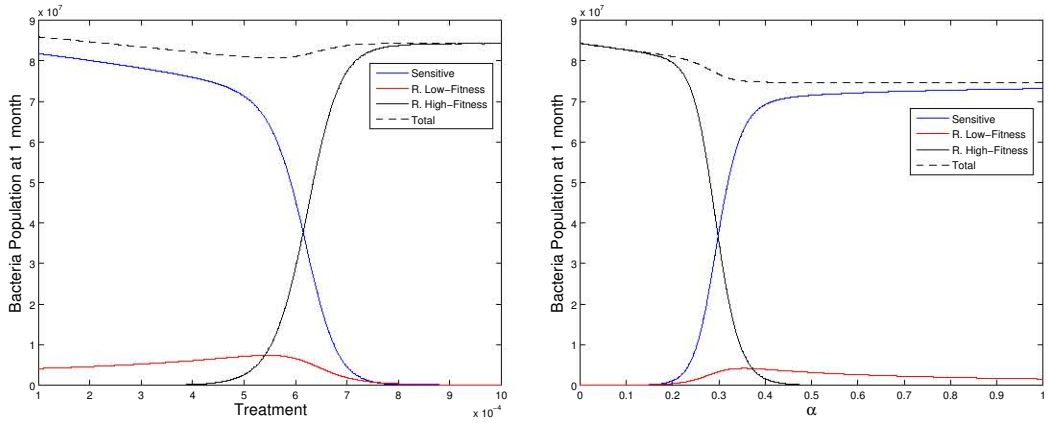

Figure 8: Bacterial Population after one month of initial infection with different levels of treatment (left) ($\alpha = 0$) and drug-susceptibility of the resistant strain (right) ($T = 1 \times 10^{-3}$). For low levels of treatment, the drug-sensitive strain is widespread within the host. As treatment increases, the high-fitness resistant strain begins to increase its prevalence until it completely colonizes the host. As α increases, the high-fitness resistant strain loses its fitness advantage and the drug-sensitive strain outcompetes it.

9 shows that increasing the carrying capacity will linearly favor the uprise of the drug-resistant population, while the drug-sensitive and low-fitness drug-resistant strain populations are at zero, meaning they are cleared from the host. That is, when treatment is present, a decrease in the inter-bacteria competition *mainly* benefits the drug-resistant strain. This unexpected result suggests that if the resource availability increases (larger K), the strain with the highest fitness takes most advantage of this rich envoronment.

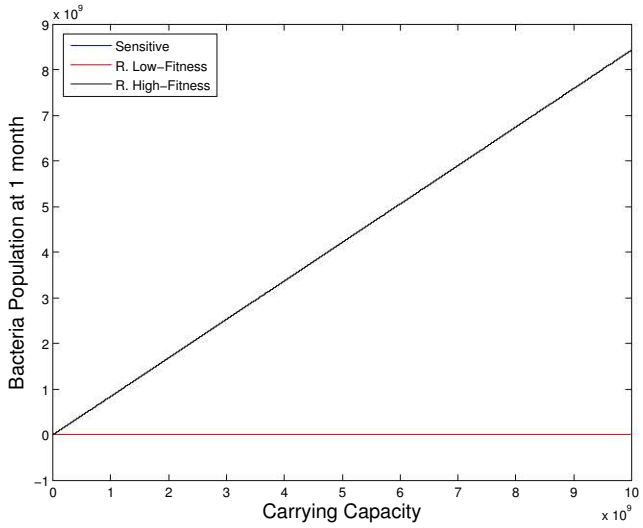

Figure 9: Bacterial Population after one month with Treatment and varying the carrying capacity K. As the resource availability increases within the host (larger K), the high-fitness resistant strain increases linearly with K, while the drug-sensitive and low-fitness drug-resistant strain populations are at zero, meaning they are cleared from the host. The other two strains go extinct regardless of the value of K.

Role of immune system

A patient that gets infected with gonorrhea could be immunocompromised and unable to fight the disease, increasing the risk of suffering from side effects or even death. Conversely, the patient could possess a healthy immune system that allows him/her to keep the infection at low levels, remaining infectious (not latent) but decreasing the risk of incurring further gonorrhea-related complications. It is thus our interest in this section to explore how different levels of immune activity, along with different treatment levels, can impact the prevalence of the different bacteria strains.

Figure 10 shows the populations of drug-sensitive strain (left) and high-fitness resistant strain (right) after one month. In this plot the treatment and immune system are varied. It is clear that for low-medium immune system levels and low treatment regimes, the drug-sensitive strain outcompetes the high-fitness resistant strain. However, if treatment is high and the immune system is low, the resistant strain takes over the host. Note also that higher values of the immune system clears both infections. This last observation hints about the importance of a healthy immune system in fighting the disease.

Role of Mutation Rates

How fast bacteria can mutate to resistance is clearly a major determinant in antibiotic resistance development. In this section we focus on how each mutation rate (m_1, m_2, p) affects the disease

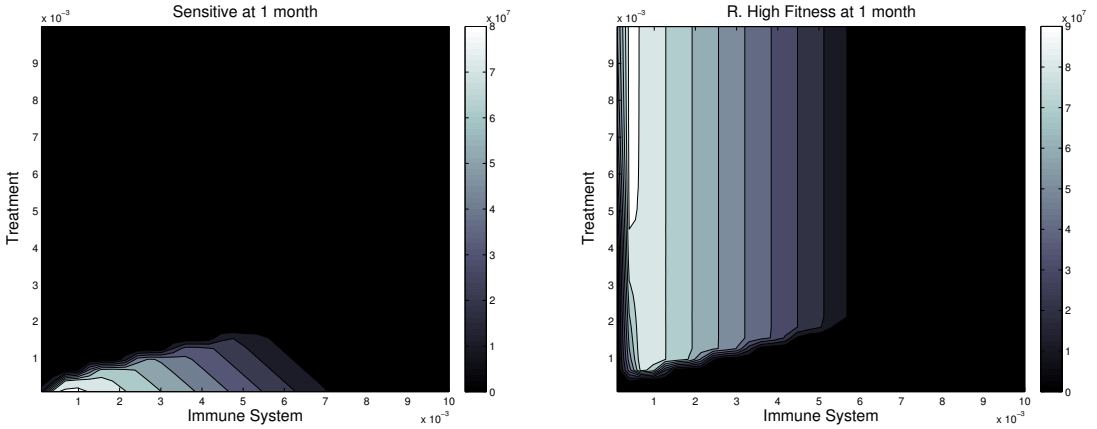

Figure 10: Drug-sensitive strain (left) and high-fitness resistant strain (right) populations after one month with varying treatment and immune system. For low-medium immune system levels and low treatment regimes, the drug-sensitive strain outcompetes the high-fitness resistant strain. If treatment is high and the immune system is low, the resistant strain takes over the host. Higher values of the immune system clears both infections.

prevalence.

Figure 11 and Figure 12 show, in essence, that varying the values of the mutation rates m_1 and m_2 does not have a large impact on the emergence of resistance within the host. However, if treatment surpasses a certain threshold, resistance will likely emerge in the patient. If instead, treatment levels are low enough, resistance will not emerge and the sensitive strain will still prevail in the host.

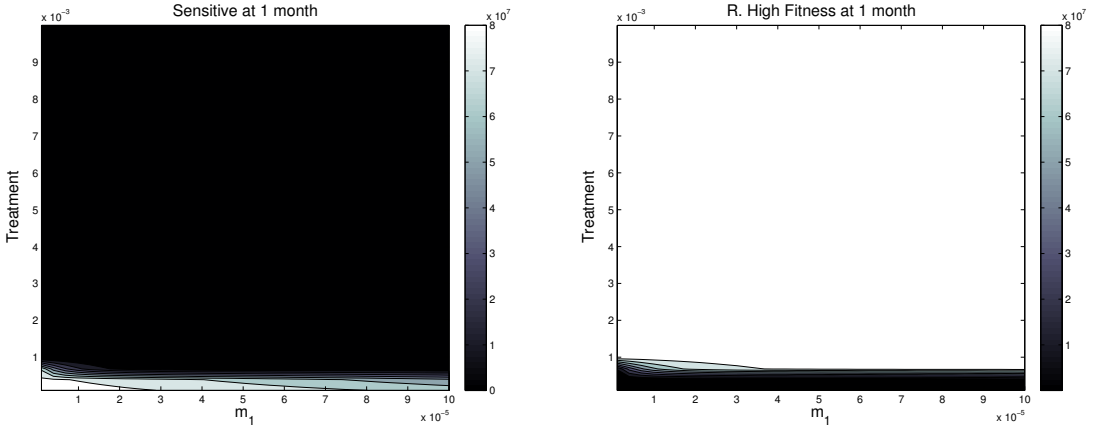

Figure 11: Drug-sensitive strain (left) and high-fitness resistant strain (right) populations after one month with varying treatment and mutation rate m_1. Low treatment doses hinders the emergence of resistance in the host. For higher values of treatment, and with almost no regard for the values of m_1, resistance emerges.

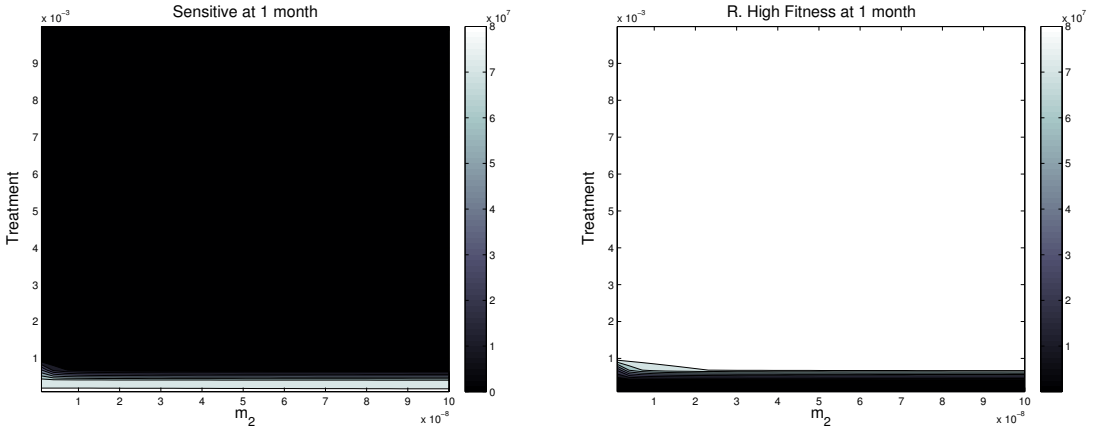

Figure 12: Drug-sensitive strain (left) and high-fitness resistant strain (right) populations after one month with varying treatment and mutation rate m_2. Low treatment regimes hinders the emergence of resistance in the host. For higher values of treatment, and with almost no regard for the values of m_2, resistance emerges.

Figure 13 displays the role of the conjugational rate p. It can be readily observed that low values of treatment, together with low values of p, will halt the rise of the resistant strain in the infected host. High values of treatment, however, will increase the likelihood of resistance emergence. Two interesting features of this plot is (i) even for low very low treatment values, if p is large enough ($p > 6 \times 10^{-12}$), resistance will emerge, and (ii) in the region given by $3 \times 10^{-12} < p < 5 \times 10^{-12}$ and $1 \times 10^{-4} < T < 0.5 \times 10^{-3}$, neither strain colonizes the host. This observation suggests that, if p could be estimated, levels of treatment can be deduced such that the infection gets cleared.

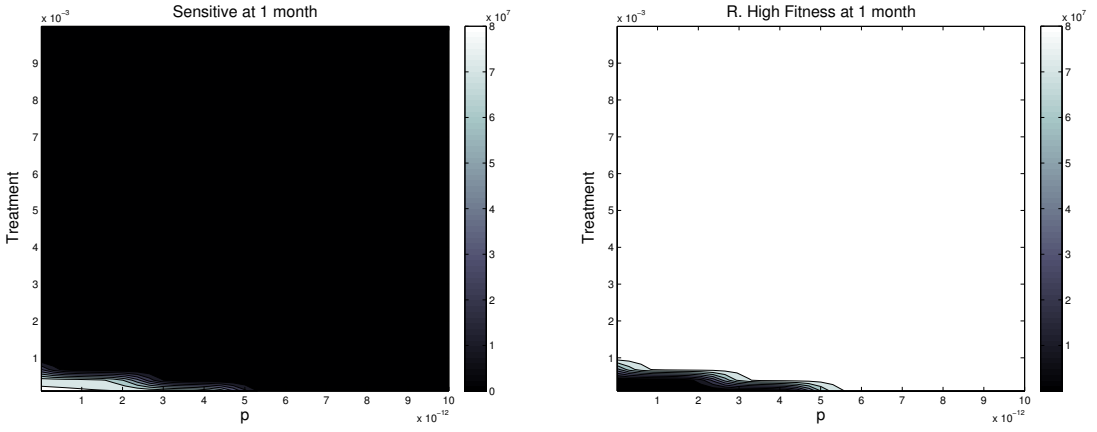

Figure 13: Drug-sensitive strain (left) and high-fitness resistant strain (right) populations after one month with varying treatment and mutation rate p. Low treatment and p levels hinders and the emergence of resistance in the host. For higher values of treatment, regardless of p, resistance emerges.

Role of Relative Fitness

Even though mutation rates affect the pace of resistance development, the fitness cost of resistance is also a crucial factor in the risk for resistance development [1]. For instance, in the case when resistant mutants form at a high rate, if the resistant phenotype severely reduces fitness, the resistant mutants might not take over the population. In this model, we assume that the reduction in fitness is expressed by a reduction in the birth rate. Let $F_h = \dfrac{b_h}{b_s}$ be the relative fitness of the high-fitness resistance bacteria with respect to the sensitive bacteria. Hence, F_h is a simple way to represent the biological cost of resistance: lower F_h implies a higher cost, whereas higher F_h implies resistance is less costly. Generally $F_h < 1$ implies that the resistant phenotype hinders the reproduction rate. However, compensatory mutations could reverse this situation, yielding $F_h > 1$.

Figure 14 shows that for high treatment levels and relative fitness F_h, the high-fitness resistant strain outcompetes the other strains. Lower values of fitness and high treatment, instead, favor the rise of the low-fitness strain. Finally, low fitness values and low treatment regimes, allow the drug-sensitive to increase its prevalence in the infected host. These observations constitute one of the most interesting results in this section. Figure 14, along with Figures 11, 12, and 13, suggest that in the presence of treatment, the relative fitness F_h, and not the mutation rates m_1, m_2 and p, plays a chief role in the inter-bacteria competition dynamics and in whether resistance will emerge within the host. This possibility was suggested in [1], and here we provide supporting evidence, at least for the specific parameters used here (see Table 2).

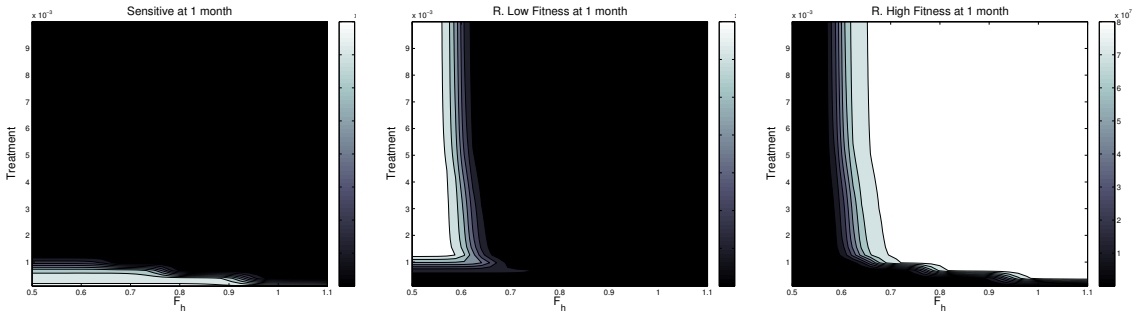

Figure 14: Drug-sensitive strain (left), low-resistance strain (middle) and high-fitness resistant strain (right) populations after one month with varying treatment and mutation rate F_h. For high treatment levels and fitness, the high-fitness resistant strain outcompetes the other strains. Lower values of fitness and high treatment favor the rise of the low-fitness strain. Low fitness values and low treatment levels, allows the drug-sensitive to prevail in the host.

Note: All of these observations were made for a certain choice of parameters, (see Table 2). Any observations herein arrived at may be biased by this fact, thus we cannot necessarily extend their applicability to another region of the parameter space.

3.4 Sensitivity Analysis

This section presents a sensitivity analysis of two equilibria of system (1)-(3), to determine the relative impact on the bacterial population of changes in parameters that can be controlled to fight

off the infection, (i.e. treatment, T, and the immune system, i).

Sensitivity analysis considers the percent change of the state variable over the percent change of the parameter:

$$\frac{\%\text{change in state variable}}{\%\text{change in parameter}}$$

More formally, from sensitivity analysis theory, the sensitivity index S (also known as elasticity) of a function $\xi(t,p)$ with parameter p is

$$S_p(\xi(t)) = \frac{\frac{\delta\xi}{\xi}}{\frac{\delta p}{p}} \approx \frac{\xi(p+\epsilon p,t)-\xi(p,t)}{\epsilon p}\cdot\frac{p}{\xi(p,t)} \approx \frac{\partial\xi(p,t)}{\partial p}\frac{p}{\xi(p,t)}$$

For our sensitivity analysis, $\delta p = \epsilon p$ with $\epsilon = .0001$.

The analysis will be performed numerically (see Table 2 for parameter values) with the system starting at an equilibrium point. Our equilibrium points for equations (1)-(3) were solved numerically and we obtained the infection-free, the resistant-only, high-fitness only, and coexistence equilibria. Since the resistant-only equilibrium was uninteresting in the previous models, we will not be considering it here. We will not consider the infection-free equilibrium, $(0,0,0)$, in this analysis when it is stable since it is not sensitive to changes in either the treatment or the immune system. Consider first the effect of small changes in the immune system on the high-fitness resistant only equilibrium, (see Figure 15 (left)). For a 1% increase in the clearing rate of the immune system, we observe a roughly 2% negative impact on the high-fitness antibiotic-resistant equilibrium. This is to be expected since this strain is still affected by the immune system. However, this equilibrium is not sensitive to changes in treatment, as the high-fitness antibiotic-resistant strain has complete resistance to antibiotics (see Figure 15 (right)).

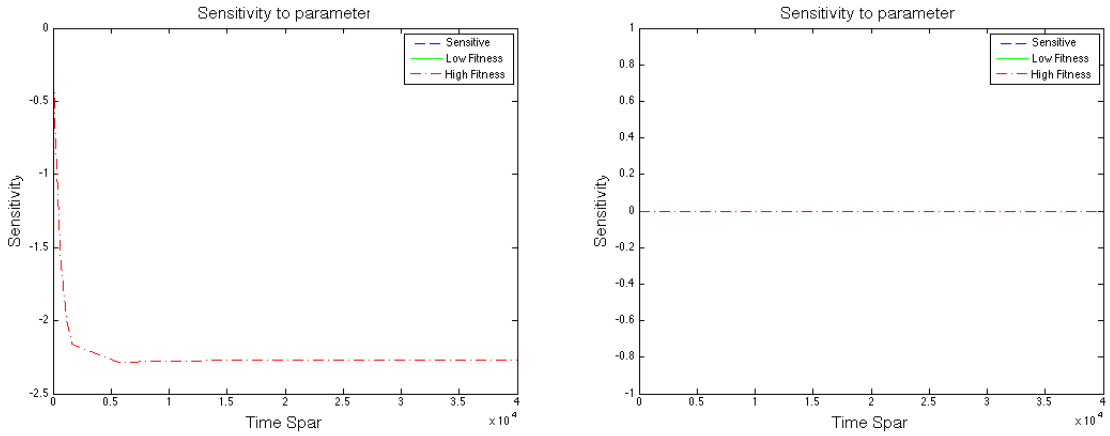

Figure 15: Sensitivity of high-fitness resistant only equilibrium on Immune System (left) and Treatment (right) on High Fitness Equilibrium. The high-fitness resistant strain is negatively affected by changes in i, while T has no effect on it.

Consider now the coexistence equilibrium, ($S \approx 10^7$, $R_l \approx 10^5$ and $R_h \approx 10^1$ at this point of

the parameter space). A 1% increase in the clearing rate of the immune system negatively affects all strains (see Figure 16 (left)). Interestingly, a 1% increase in the immune system appears to affect the high-fitness antibiotic-resistant strain the most with an approximately 4.5% decrease. A plausible explanation is that since the immune system affects all strains at the same rate, the high-fitness resistant strain is not only directly affected by the immune system but also indirectly because of the reduction in the incoming population from the sensitive and the low-fitness resistant strains (due to pSR_h, m_2, and pR_lR_h).

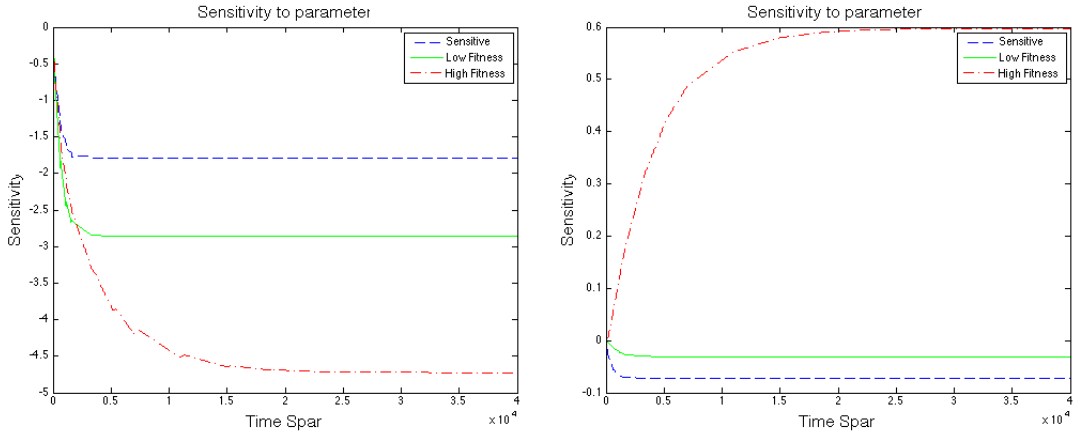

Figure 16: Sensitivity Analysis of the *coexistence* equilibrium of model (1)-(3). A small increase in i negatively affects all strains; the high-fitness resistant strain is impacted the most. As T is slightly increased, the drug-sensitive and the low-fitness resistant strain are negatively affected; the high-fitness resistant strain is positively and largely impacted.

At this equilibrium all strains are sensitive to changes in the treatment. For a small increase in treatment, both the sensitive strain and the low-fitness antibiotic-resistant strain are negatively impacted Figure 16 (right). The sensitive strain is directly negatively impacted by the increase in treatment, whereas the low-fitness resistant strain is indirectly negatively impacted by the treatment because any change in the population size of the sensitive strain changes the amount of incoming sensitive strain (due to m_1S and pSR_l). The increase in treatment, however, has a positive, and relatively larger, impact on the high-fitness antibiotic-resistant strain. While the incoming amount of sensitive and low-fitness antibiotic-resistant strains (due to pSR_h, m_2, and pR_lR_h) is decreased as treatment increases, this is negligible in comparison to the reproductive advantage conferred by treatment on the high-fitness resistant strain.

4 Discussion

Gonorrhea is a bacterial infection that has developed resistance to many of the most accessible antibiotics and it is on the rise globally. A mathematical model that qualitatively captures the dynamics of antibiotic resistance of gonorrhea within a host could help to investigate the role of key factors in the emergence of resistance within a host. Modeling the dynamics of within host antibiotic resistance in gonorrhea proves to be an exciting, yet complex and challenging task. Observing the effects of treatment alone yields unsatisfying results given the interplay between

different factors such as the immune system, bacterial mutations and inter-bacteria competition. Our model shows that, as suspected, increasing the treatment dose poses a selective pressure on the drug-sensitive strain, facilitating the spread of the high-fitness resistant strain in the patient. We also found that the high-fitness resistant strain cannot coexist with the low-fitness strain alone, as it will outcompete it thanks to its fitness advantage in a limited resource environment. Moreover, unless the treatment and immune system together have a high enough impact to clear the infection, we will see either a stable high-fitness only equilibrium or a stable coexistence equilibrium.

A very interesting finding of this project that is suggested by numerical simulations is that, in the presence of treatment, the relative fitness of the the high-fitness resistant strain, and not the mutation rates, plays a leading role in the likelihood of resistance emergence within the host. This observation provides supporting evidence to conjectures made elsewhere.

Immune response to gonorrhea is a very important factor in preventing the development of resistance in the host. In our model, the immune system does not distinguish between resistant and sensitive strains. Thus, the immune response has a greater impact on the growth dynamics of all bacteria variants. As the immune response is increased, eventually all gonorrhea strains will be cleared from the host. However, as critical as the immune system is in this model, it is inherently difficult to quantify. In future studies, the immune system response mechanisms must be studied in more detail if drug-resistance is to be more effectively controlled [11, 28].

This model takes an incredibly complex microbiological process into account and through simplifications, explores how important evolutionary processes at smaller space and time scales are in our everyday life.

Possible Improvements and Future Research

In order to obtain analytical results, we neglected the inherent complexity of factors such as the immune system and the stochasticity of phenotype switching within the population of *N. gonorhoeae*. Rather than create a mechanistic model, we assumed the immune system clears out all different bacterial strains at a constant rate. Further research must be done on the immune system response in humans, because it is a key factor affecting resistant gonorrhea strains. Such studies would improve understanding of how exactly resistance develops, which in turn would inform treatment policies to hinder the spread of gonorrhea without inducing resistance.

Phenotype variation as well as random drug-resistance conferring mutations should be studied using stochastic methods. We should consider a stochastic mutation model due to the fact that mutations occur as events in time and not as continuous happenings over time.

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5 Appendix

5.1 Complications with Untreated Gonorrhea

In the late 1800s and early 1900s, before the introduction of antibiotics, gonorrhea caused severe complications in colonized patients. In primary infection sites, such as the urogenital and perigenital, common symptoms include inflammation of tissues and glands, lesions, discharge, and cysts. According to Charles C. Norris (1913) [19], untreated *N. gonorrhoeae* can cause rupturing of the fallopian tubes, ectopic pregnancy, pregnancy in the fallopian tubes (which does not result in viable births), and gonorrhea infections in newborns. Secondary infection sites include lesions in the lungs, tissues, joints, bones, and the heart. In some rare cases, gonococcus can be found in blood clots and can attack bones, the frequent target being the heel bones [19][24].

Despite modern treatment availability, gonorrhea can still cause severe health problems in both men and women, mainly because gonorrhea has become increasingly asymptomatic. The primary infections documented by Norris still occur today, including ectopic pregnancy and pelvic inflammatory disease (PID). PID can damage the Fallopian tubes to the point where a woman is unable to have children. In the case of infected males, if gonorrhea spreads from the urethra to the testicles, it can develop into epididymitis, inflammation of a certain part of the testicles[8][20]. Hence, gonorrhea is one of the major causes of infertility in both women and men.

5.2 Brief History of Antibiotic-Resistance in Gonorrhea

The six main antibiotics which some *N. gonorrhoeae* strains have acquired resistance to are Sulfonamides, Penicillin, Tetracycline, Spectinomycin, Fluoroquinolones (e.g. ciprofloxacin), and Cephalosporins (e.g. ceftriaxone and cefixime). Sulfonamides were first listed as a treatment for gonorrhea in 1937, but *N. gonorrhoeae* developed resistance several years later [21]. Penicillin, the "new miracle drug", became the widespread treatment for gonorrhea in 1943. Through the decades the minimum dosage of Penicillin required to cure gonorrhea increased, until in the mid-1980s, when *N. gonorrhoeae* gained complete resistance [21] [35]. When penicillin was still widely used, tetracycline was used as a substitute for those individuals whom penicillin did not effectively treat. In the mid to late 1980s tetracycline was no longer a treatment option [35]. Yet another alternative to penicillin was spectinomycin. However, gonorrhea developed resistance to it as well [21]. Subsequently, Fluoroquiolones, such as ciprofloxacin, became the treatment option for gonorrhea in the mid-1980s. Resistance to fluoroquinolones were seen in the early 2000s, and they were taken off the recommended treatment list by the Center for Disease Control and Prevention (CDC) in 2007 [12]. *N. gonorrhoeae* has recently gained resistance to Cephalosoporins, which are considered the last line of defense [21]. More ominous is the fact that many *N. gonorrhoeae* strains have acquired multiple-antibiotic resistance, allowing them to resist eradication by a broad spectrum of antibiotics.

5.3 Parameter Calculations

Our population of bacteria will be measured using the units of CFU, or colony forming units. One CFU= one healthy, reproducing bacteria. Our time scale will be measured in minutes.

In S, the doubling time ranges from 45 to 115 minutes, so we decided to choose 80 minutes for our calculations [26]. Using properties of exponential growth, we have determined that if a population follows the growth pattern $\frac{dN}{dt} = rN$, then $r = \frac{\ln(2)}{t}$, where t is the doubling time of

the population. So $b_s = \frac{\ln(2)}{80}[1/\text{min}] = 8.664 \times 10^{-3}[1/\text{min}] \approx 8 \times 10^{-3}[1/\text{min}]$, which is the value used in our model. The doubling time of R_l can vary, but must be greater than that of S, so we shall choose $b_l = 4 \times 10^{-3}[1/\text{min}]$ to observe a resistant low-fitness strain with half the growth rate of the drug-sensitive strain. The doubling time of R_h can also vary, but must be less than that of R_l, and typically no smaller than that of S, so we choose to make $b_h = 0.8 \times b_s = 6.4 \times 10^{-3}[1/\text{min}]$.

We have calculated m_1 to be a combination of spontaneous mutations and transformations, which we found to be 10^{-8} to 10^{-9}[34] and 3.6×10^{-3} (with a standard error 1.1×10^{-3}[29]), respectively, where all units are mutations per cell division. However, the spontaneous mutations occur at a substantially lower rate than the transformation rate and therefore are not included in the calculation of m_1. To calculate the transformation rate of mutations we converted the units to [1/min] by calculating $m_1 = (\frac{3.6 \times 10^{-3} \text{ mutations}}{\text{cell division}})(\frac{\text{cell division}}{80\text{minutes}}) = 4.5 \times 10^{-5}[1/\text{min}]$, which is in the same value range as $2 \times 10^{-5}[1/\text{min}]$, the chosen value.

Although we have not been able to find data on the compensatory mutation rate for *N. gonorrhoeae*, the compensatory mutation rate for*Salmonella typhimurium* is greater than 10^{-7} per cell per generation [15], and we will assume that the compensatory mutation rate for *N. gonorrhoeae* is comparable to this rate. To convert this rate from terms of mutations per cell generation to mutations per minute, we will divide by the doubling time of 110 minutes versus 80 minutes, to account for a longer doubling time for the R_l strain. So we obtain $m_2 \geq (\frac{10^{-7} \text{ mutations}}{\text{cell division}})(\frac{\text{cell division}}{110 \text{ min}}) = 9.09 \times 10^{-10}[1/\text{min}]$. We will use $9 \times 10^{-9}[1/\text{min}]$ as our m_2 parameter, since it falls into this range of values.

We will assume that p is the rate of conjugation between all three strains of bacteria because the exchange mechanisms are comparable for each strain. In our model, we will consider a gonorrhea infection at the cervix, which is near the section of largest vaginal width, 32.5 mm[2]. Under the assumption that the shape of the cervix is similar to a disk with radius 32.5 mm, then its surface area is around 814.3322317 mm^2. If we assume that region which *N. gonorrhoeae* inhabits is only 1 mm thick, then the volume we will study is 814.3322317 $\text{mm}^3 = 0.814$ mL 1 mL. We know that for *Escherichia coli*, the bulk conjugation rates for conjugation are: 10^{-8} to 10^{-15} [mL/(CFU×min)] [36]. Multiplying the bulk conjugation rate and the volume of the section of the cervix under study yields a value in the range of 10^{-8} to 10^{-15} [1/(CFU×min)]. For our study, we choose the value $p = 5 * 10^{-13}$ [1/(CFU×min)].

For K, the carrying capacity, we shall choose 10^8CFU [31].

For the initial population values, we choose $S_o = 10^4$CFU, and $R_{lo} = R_{ho} = 0$.

5.4 Model I: P_1^3 and P_1^4

In section 6.2, the system $(1)-(3)$ has four steady state points where the first two are mentioned and analyzed in the section. Here we include the last two, P_1^3, P_1^4 which represent possible coexistence scenarios. If we set

$$\phi = 2(A+1)M(A(A-B+c+1)-c) + (A(A-B+c+1)+c)^2 + (A+1)^2M^2,$$

Table 2: Parameter and State Variable Initial Condition Description

Param.	Description	Value	Ref.
S_o	Initial Population of Sensitive strain	$10^4 cfu$	
R_{lo}	Initial Population of Resistant strain with *low* fitness	$0\ cfu$	
R_{ho}	Initial Population of Resistant strain with *high* fitness	$0\ cfu$	
b_s	Birth rate of the S strain	$8 \times 10^{-3}[1/min]$	[26]
b_l	Birth rate of the R_l strain	$4 \times 10^{-3}[1/min]$	[26]
b_h	Birth rate of the R_h strain	$6.4 \times 10^{-3}[1/min]$	[26]
K	Carrying capacity of bacterial population	$10^8[cfu]$	
m_1	Mutation rate to Low-fitness Resistant strain	$2 \times 10^{-5}[1/min]$	[29] [17]
m_2	Compensatory mutation rate	$9 \times 10^{-9}[1/min]$	[16]
p	Bacterial conjugation rate	$5 \times 10^{-13}[1/(cfu \cdot min)]$	[36][2]
i	Clearing rate due to immune system each strain	$10^{-3}[1/min]$	
T	Treatment-induced mortality rate	$10^{-3}[1/min]$	
α	Level of drug-resistance of the resistant bacteria	$[0, 1]$	

$$\pi = 2(A - B + c + 1),\ \gamma = A^2(-(B + c)) + A\left(B^2 - B(M + 1) + c(-c + M - 1)\right),$$

$$\nu = A^2 - AB + Ac + AM + A - c + M,\ \tau = A^2 - A(B - c + M - 1) + c - M, \text{ and}$$

$$\sigma = (A(A - B + c + M + 1) - c + M)^2 + 4Ac(A - B + c + 1) \text{ then}$$

$$P_1^3 = \left(\frac{\gamma + (c - B)\left(\sqrt{\phi} - c + M\right)}{\pi(A + 1)}, \frac{\nu + \sqrt{\sigma}}{\pi}, \frac{\tau + \sqrt{\sigma}}{\pi}\right)$$

$$P_1^4 = \left(\frac{\gamma - (c - B)\left(\sqrt{\phi} + c - M\right)}{\pi(A + 1)(A - B + c + 1)}, \frac{\nu - \sqrt{\sigma}}{\pi}, \frac{\tau + \sqrt{\sigma}}{\pi}\right)$$

5.5 The Model with a Dynamic Immune Response and without Conjugation

As an extension of (1)-(3) (with $p = 0$), we consider the immune system to depend on the presence of the bacterial agents. More specifically, we consider that, in the presence of small quantities of bacteria, the immune system per-capita clearing rate (i) scales linearly with the size of the infection. If the bacteria population keeps increasing, the immune system reaches a point after which the per-capita clearing rate diminishes.

To mimic the dynamics between the immune system and the bacteria population we propose the following function of i

$$i(N) = \frac{\theta_1 N}{\theta_2 + N^2} \tag{16}$$

where $N = S + R_l + R_h$. The parameters θ_1 and θ_2 need to be estimated and they stretch the graph horizontally and vertically (see Figure 17).

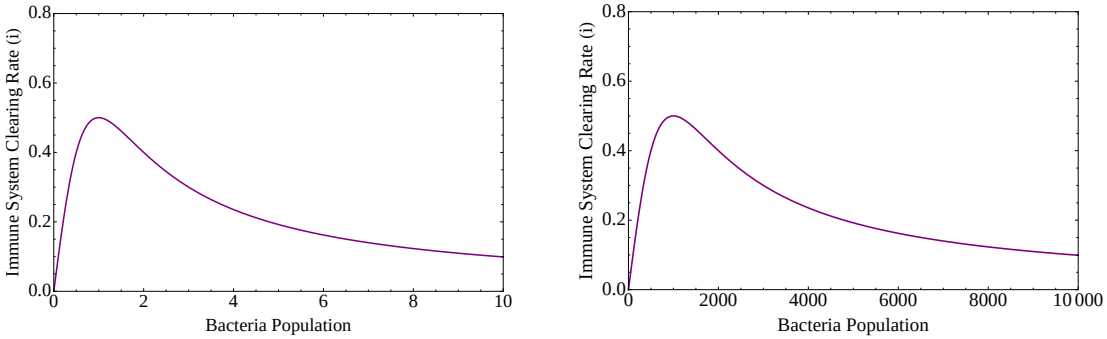

Figure 17: Immune System clearing rate, i, as a function of bacterial population, N. (Left) $\theta_1 = 10$ and $\theta_2 = 10$ in 16 and (Right) $\theta_1 = 10000$ and $\theta_2 = 1 \times 10^7$. θ_1 controls the height of the maximum, while θ_2 determines both the height and the N-coordinate of this maximum.

We used the parameters in Table 2 except for $p = 0, \theta_1 = 8.015 \times 10^4, \theta_2 = 5 \times 10^{14}$, and $T = 6.5 \times 10^{-5}$. From this we see three biologically relevant equilibrium points and they are defined as follows: the infection-free, the high resistance only and the coexistence equilibra. The high resistance only equilibrium is a saddle point and the infection-free equilibrium is unstable therefore we will only be considering the unique stable point, the coexistence equilibrium. With these parameter values, the treatment level and the immune system response are low enough for the sensitive strain to survive and the immune system response is low enough for all strains to exist.

If $\theta_1 < 8.014 \times 10^4$, the coexistence equilibrium doesn't exist and the high resistance equilibrium is stable. In terms of our model, the high resistance equilibrium emerges because treatment will clear all of the antibiotic sensitive strain leaving only the two antibiotic resistant strains. We will be left with only the high-fitness antibiotic-strain due to the high-fitness strain out competing the low fitness strain. In comparison with Model II, (10)-(12), we observe the same behavior, where the high-resistance is the only survivor. When θ_1 is extremely large, e.g $\theta_1 = 8.015 \times 10^{12}$, the coexistence stable equilibrium has all its values smaller than one, which we can interpret as the immune system being strong enough to clear all of the infection.

If we increase T keeping $\theta_1 = 8.015 \times 10^4$ constant, we never see the coexistence equilibrium and only the high-resistance stable equilibrium exists. When $T > 6.5 \times 10^{-5}$,the treatment level is high enough, no matter the immune system level, to clear out the sensitive strain and have only the high-resistance strain survive. When $T < 6.5 \times 10^{-5}$, the sensitive will survive and we see the coexistence equilibrium. This is due to the treatment level not being strong enough to clear the infection.

An important conclusion from this brief study of a dynamic immune system is that it behaves, at least qualitatively, similarly to Model II.

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