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Assessing optimal treatments for intracellular infection: host immunity, heterogeneity, and the antibiotic resistance challenge

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Resumo alargado

Os modelos matemáticos são ferramentas usadas no estudo das dinâmicas das doenças infecciosas. Muitas vezes servem para estimar parâmetros biológicos que são dispendiosos ou difíceis de obter com a realização de experiências. No ramo das doenças infecciosas, o crescente aumento de resistência antimicrobiana dos patogénios coloca grandes desafios à comunidade científica e médica. A comunidade científica está a ficar consciente dos riscos que as prescrições longas de antibióticos trazem não só para a infecção mas também para a criação de resistência no microbioma residente no hospedeiro. Dado que o número de agentes bacterianos resistantes a um ou mais medicamentos antimicrobianos continua a crescer anualmente e que a indústria farmaceutica não consegue acompanhar com a criação de novos antibióticos, pede-se urgentemente uma descoberta sobre como lidar e remover agentes patogénicos resistentes de forma eficaz, evitando também o seu surgimento em novas infecções. O uso de modelos biomatemáticos nesta temática tem permitido significativos avanços nos últimos anos. Actualmente, abordagens agressivas e moderadas estão a ser discutidas como estratégias terapêuticas para o tratamento de infecções bacterianas na presença de resistência a antibióticos.

Neste trabalho, estudamos as dinâmicas da infecção intracelular combinando os efeitos do tratamento com antibióticos e respostas imunes adaptativas. Os modelos ODE são baseados em processos das bactérias que causam infecções agudas como a listeriose, provocado pela bacteria *Listeria monocytogenes* e crónicas, como a tuberculose provocado pela bacteria *Mycobacterium tuberculosis*. Estas bactérias desenvolvem-se intracelularmente, invandindo células do hospedeiro para a proliferação da população. Primeiro, procedemos à análise do modelo recorrendo a duas linguagens de programação, Mathematica e MatLAB. Encontramos a combinação de parâmetros entre macrófagos-bactérias-imunidade que possibilitam a manutenção da infecção no hospedeiro ou a sua remoção. Para haver sobrevivência da espécie bacteriana no hospedeiro, a infecção não pode ser suficiente forte para matar o hospedeiro, mas também não pode ser fraca de modo a haver proliferação da bacteria. Para tal, há uma combinação crítica nos seus parâmetros de contágio e morte que permite a manutenção no hospedeiro.

Estudamos, também, as consequências da administracção de tratamento antimicrobiano relativo a várias medidas da infecção, incluindo duração, carga bacteriana, patologia e resistência. Notamos que diferentes combinações de duração e dose do tratamento podem levar a infecções bastante semelhantes e de que o mesmo tratamento tem efeitos diferentes dependente de se a administração é realizada no ínicio da infecção ou no estágio final do seu desenvolvimento. Além disso, o tratamento nem sempre é benéfico para o paciente. Por exemplo, tratamentos

mais longos tendem a seleccionar mais bacterias resistentes devido à maior pressão sobre as populações susceptiveis ao medicamento, permitindo muitas vezes a recaída da infecção com a fixação total de populações resistentes aos medicamentos antimicrobianos.

Comparamos durações de tratamento com curta (3 dias) versus longa (7 dias) duração, utilizando medidas mais próximas dos testes clínicos. Consideramos um horizonte apenas de 7 dias após a finalização do tratamento e observamos as medidas de infecção nesse ponto. Os resultados apontam que os tratamentos mais longos são mais eficientes, com uma maior taxa de resolução de infecção. No entanto, há regimes em que tratamentos mais curtos apresentam melhores resultados, nomeadamente quando a infecção está completamente desenvolvida ou quando se usam doses baixas. Além disso, procuramos comparar os tratamentos segundo outra hipótese: conservando o uso total de antibiótico no paciente, isto é, quando se escolhe tratamentos mais longos, reduz-se a dose de antibiótico e vice-versa. Os resultados foram surpreendemente diferentes, apesar de verificarmos uma continuação da eficiência dos tratamentos com longa duração. Notou-se nos tratamentos de curta duração, que funcionariam melhor quando usados em infecções mais desenvolvidas, uma redução de eficiência. Em contrapartida, houve um aumento de resolução da infecção quando é administrado em estágios menos desenvolvidos. Este facto pode explicar o resultado que se observa em testes clínicos.

Por fim, procuramos optimizar tratamentos antimicrobianos para diferentes pacientes. Neste caso, deparamo-nos com a impossibilidade de diminuir simultaneamente todas as medidas da infecção. Além disso, observamos que para o mesmo paciente, com estágios de infecção diferentes, e para diferentes pacientes, com o mesmo estágio de infecção, o melhor tratamento não será a mesma combinação de dose de medicamento e duração do tratamento.

No geral, os resultados destacam novos tratamentos direccionados às infecções intracelulares, com o uso de menor doses de antibiótico e duracção, se combinados com acção imunológica capaz. A partir disso, extraímos princípios de optimização para vários cenários de infecções e discutimos as direcções futuras para melhoria desta área, nomeadamente a importância de biomarcadores da infecção e imunidade no início do tratamento. A quantificação destes parâmetros de forma experimental também poderá permitir um estudo mais pormenorizado das infecções bacterianas e desenvolvimentos na medicina personalizada.

Palavras-chave: Infecção, imunidade , modelos matemáticos, tratamento antibiótico, interacção bactéria-macrófagos.

Abstract

Mathematical models have been used as tools to study the dynamics of infectious diseases for a long time and to design successful control interventions, both at within-host and at the epidemiological level. Models can provide estimates of biological parameters which are difficult or expensive to obtain through experiments. Currently, in infection diseases, the growing antimicrobial resistance of pathogens poses great challenges. Recently, aggressive and moderate approaches are being debated as therapeutic strategies to deal with antibiotic resistance. The discussion is still open, as the field is becoming aware of the risks, due to higher and longer antibiotic prescriptions, not only considering the infection pathogen but also non-target resistance in resident microbiota. In this work, we study intracellular infection dynamics combining effects of antibiotic treatment and adaptive immune responses. The ODE models are based on infection processes for acute and chronic bacterial infections. We find the critical parameter combination in macrophage-bacteria-immunity interaction, dividing regimes of clearance and persistence of infection. Moreover, we study the consequences of antimicrobial treatment on many infection measures, including duration, bacterial burden, pathology and resistance. We notice that different combination of treatment duration and antibiotic doses can lead to the same infection outcomes and that the same treatment can have different effects if applied early or later during the infection course. Moreover, treatment is not always beneficial, as longer durations often select more resistant bacteria. We compare short (3 days) versus long (7 days) treatment duration in-depth. Long treatment duration is overall more efficient, with higher infection resolution. However, there are regimes where short treatment is non-inferior or even superior. Our results highlight the potential of new targeted treatments of intracellular infection, with lower antibiotic doses and duration, combined with sufficient immune action. From this, we extract optimization principles for infection over a range of scenarios and we discuss future directions for the improvement of this area, namely the importance of infection and immunity biomarkers at treatment onset.

Keywords: Infection, immunity, mathematical model, antibiotic treatment, bacteria-macrophages interaction

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Chapter 1

Introduction

Infectious diseases are a hazard to the human and animal populations. They are caused by pathogens either by disrupting the bodies normal processes and/or stimulating the defensive response, resulting in inflammation and other symptoms. The most common pathogens are different types of viruses and bacteria. Fungi and Protozoa are also known as pathogens and are responsible for various diseases. Most important, these diseases are spread, directly or indirectly, from one host to another and can lead to severe epidemics. Each human individual is susceptible to be affected by a disease. Nowadays, infectious diseases are one of the leading causes of death in developing countries. They are troublesome to contain especially due to resistance to antimicrobial therapies, which is becoming more frequent. To prevent transmission and control the diseases, there needs to be an identical pace of development of drugs/vaccines and the evolution of the pathogens. This will be the threat of this century, and it poses a challenge to the scientific and medical community. While drugs act on infected people, to treat the infection and reduce symptoms, virulence or transmission, vaccines act on the susceptible population to prevent infection by immune protection.

This thesis addresses the resistance challenge, produced by antibiotic treatments of bacteria pathogens, and contributes for the understanding of the mechanisms of treatment success and host infection dynamics characteristics, focusing on intracellular infections. The current chapter provides the state of the art data about the infection mechanisms of bacteria, immunity and the role of mathematical models in the understanding of the epidemiology of these diseases.

1.1 Bacterial pathogens

Bacteria are single-celled organisms, and most of them are not harmful. Actually, they are beneficial to the well-functioning of the human body present in the human intestine. This community of bacteria is known as microbiota. However, bacteria pathogens grow, divide and spread in the human body, causing multiple infectious diseases. Examples of these diseases are pneumonia, tuberculosis, meningitis, listeria, among others (Shlaes and Spellberg, 2012). These can be transmitted by aerosols (through coughing and sneezing), in the cases of Streptococcus and tuberculosis infection. Transmission also includes skin contact for *Staphylococcus*, through body fluids for meningitis and contaminated food or water for *Listeria*. Physicians use antibiotics to fight these infections.

1.1.1 Host-defense and immune system

In the human species, there is a powerful defense system to protect from pathogenic infections. It functions to protect the host from every attack. The first defense mechanisms are the natural barriers. The skin bars invading microorganisms unless it is physically disrupted. Mucous membranes produce secretions that have antimicrobial properties. Usually local secretions contain immunoglobins, mainly IgC and IgA, which prevent pathogens from docking to host cells (Corthésy, 2010). The respiratory tract also filters the air. In GI and GU tract, harsh environments affect pathogens survival. However, once these barriers are penetrated, the secondary defenses come forward as immune responses. Our whole immune system is divided in two components: innate immune system and adaptive immune system. The first is a nonspecific immune response. It is composed by various immune cells, such as neutrophils, killer cells, monocytes and mast cells. It is characterized by a response to the pathogen without any prior knowledge about the intruder. This natural response takes action immediately as the pathogen enters into the body. Cytokines are produced by macrophages and activated lymphocytes and they are responsible for an acute-phase protection that is developed regardless of the specific microorganism. This response involves increased production of neutrophils by the bone marrow. This inflammatory response directs immune cells to the infection locations to fight the pathogens. Immune cells also phagocyte microorganisms to prevent microbial spread. Phagocytes are drown to microbes via chemotaxis that ingest their targets. If the neutrophils action is deficient, there is a prolonged infection with a slower response to antibiotic drugs. The infections that are cleared by the innate immune system or by antimicrobial drugs can return to the host, which means the same pathogen can infect again the same host (Mantovani et al., 2011).

On the other hand, the adaptive immune system depends on the antigen. The host keeps memory of previous pathogens, and they prevent future infections by producing a variety of antibodies, which are complex glycoproteins known as immunoglobins. These antibodies bind specific microbial antigenic targets. They are responsible for the clearance of infecting organisms, which have caused previous infections in the host. Therefore, the same pathogen cannot infect a host for a second time, unless it evolves to evade the host's adaptive immune defense antibodies. The adaptive immune system is mainly composed by B cells and T cells. This complex system is yet to be fully understood. The combination of these two systems protects our body from pathogens. In case of failure of the immune response, the result is most likely death of the host. In case there is a reduced response, it can origin pathogenic infections that last longer (e.g. chronic infections, tuberculosis). For bacterial infections, the innate immune system tends to be the main responsible for clearance, although the adaptive immune system is very important when reencountering the same pathogen (Mercado et al., 2000a) (Kikuchi et al., 2004), which is the basis for vaccine protection against bacteria. For viral infections, the adaptive system is more important (Hoebe et al., 2004). Nonetheless, there is always an interaction between the innate and adaptive arm of the host immune defense system.

1.2 Disease control

There are several strategies to control a disease. Effective ways can be reducing contacts between people and improving food quality evaluations. Nevertheless, these contacts are inevitable, as in third world countries food control is very limited and because, in a world of communication and commuting, contact is inevitable. Prevention is not always possible. In this case, vaccines are preventive tools against transmission, since they reduce the pathogen load present on infected hosts. However, when this fails, there needs to exist efficiency in treating these diseases. In that matter, antimicrobial drugs are one example, administered in diseases caused by bacteria.

1.2.1 Drugs and resistance

Focusing on antimicrobial drugs, management of this type of infections in ancient Egypt, Greece and China is thoroughly documented. In the modern era, antibiotics thrived to success, after the discovery of penicillin by Sir Alexander Fleming in 1928. They are currently used to cure millions of people worldwide. An antibiotic, also called an antibacterial, is a type of antimicrobial drug used in the treatment and prevention of bacterial infections. They can affect bacteria in

two ways: they inhibit the growth of bacteria (bacteriostatic), or they kill them (bactericidal). However, their effectiveness has lead to their overuse and misuse, instigating bacteria to develop resistance to these drugs. Nowadays, this resistance to antibiotics is considered a major problem, even leading the World Health Organization to classify antimicrobial resistance as a "serious threat that is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country". This is a worldwide problem that urges for new policies in order to reduce resistance emergence.

Only a few years after Sir Alexander Fleming discovered the first antimicrobial drug, penicillin resistance arose. This became such a significant problem that there was a need for new drug discoveries, in order to continue treating infections. However, we always observe a pattern of resistance emergence. It follows shortly after the use of a new antimicrobial drug. This means that we need to rethink the use of these drugs when treating infections, and reduce the possibility of microbes to evolve resisting these so crucial drugs (Ventola, 2015). It is estimated that by 2050, ten million people around the world will lose their lives to drug-resistant infections, even more than to cancer. In summary, resistance has been observed in almost all antibiotics developed. Increasing levels of these drugs administered in medicine and farming around the world causes pressure for bacteria to mutate and evolve, becoming resistant to antimicrobial treatments.

1.3 Mathematical models in infectious diseases

Epidemiology studies and analyses the distribution of health and disease conditions in defined populations. Infectious disease epidemiology is one of the branches of science that deals with infectious diseases. It has an impact in public health, since it shapes policy decisions by identifying risk factors for disease and targets for preventive healthcare. Epidemiologists are more focused on transmission, outbreak analysis, disease surveillance and comparison of treatment effects such as in clinical trials. To investigate these areas, scientists are increasingly relying on mathematical models and statistic tools.

Mathematical models have been used as tools to study the dynamics of infectious diseases for a long time. Recently, they increased their popularity and as a result mathematical epidemiology has risen. With the development of rapid diagnostic tests, the availability of clinical data and electronic surveillance, the application of these models is facilitated. This leads to more practical strategies to help prevention and spread of infectious diseases. Models

provide estimates of parameters of real world problems which are difficult or expensive to obtain through experiments. Also, they may be used to predict which conditions lead to resistance emergence and fixation in a population.

The first mathematical model was presented in the 18th century by Daniel Bernoulli to evaluate the benefit of inoculation of non-infected people by smallpox disease. Since then, multiple models have been created, covering numerous aspects, from spatial spread of infections to innate and adaptive immunity (Anderson and May, 1991).

1.3.1 Within-host models

One type of mathematical model is the within-host type. These are focused on pathogen population dynamics and have improved our understanding of the interactions between the host and the invading species. Most importantly, they are used to estimate the efficiency of different drug therapies as well as the strength of the immune response, both innate and adaptive. Combining the knowledge of the different aspects of the infection, we can predict outcomes, and intervene in a way to reduce the damage of infection to the host and population.

Depending on the pathogen studied, the basic model of pathogen dynamics includes essential characteristics to the disease dynamics in-host. For example, cells of the pathogen that infect host cells; life-cycle of the pathogen; time-scale of the disease; defense mechanisms of the host, among others. In the case of most bacterial infections, pathogens contain everything they need to reproduce themselves. Bacteria pathogens replicate through division/proliferation and through target-cell infection. For example, the persistence of *Mycobacterium tuberculosis*(MTB) inside a host depends on the infection of target host cells, essential in the production of bacterial machinery and proliferation of the disease. In the case of lung infection, once it is populated by the pathogen, macrophages fight the bacteria, leading to a complex and dynamic process, followed by the granuloma formation. Granulomas are collections of cells in a spherical distribution, composed by cells, bacteria and necrotic tissue. It is a physical barrier to contain Mtb inside. On the other hand, this barrier also contributes to the maintenance of the bacteria in the lung, sometimes during the entire lifetime of the host (Kirschner et al., 2017).

In this work, we explore the within-host dynamics of bacteria, immune response cells and applied antibiotic treatment in the human host. This will provide new insights on treatment application in acute and chronic infections. We look into resistance to antibiotics and how therapies drive emergence and selection. We also compare different infection measures under

different types of treatment. Our goal is to understand the infection processes in the context of intracellular and extracellular bacterial growth balance and host immune mechanisms. In addition, we want to search optimization principles for a single host and for a heterogeneous population to shed light on the topic of personalized medicine in the current era.

Chapter 2

Intracellular infection dynamics

There are multiple infectious diseases that threat human life. In this thesis we consider acute and chronic infections caused by intracellular bacteria. In the acute infection, an example can be listeriosis caused by *Listeria monocytogenes*. The mouse model of *Listeria* infection has been long used to study response mechanisms of the immune system, mostly for intracellular bacteria (Edelson et al., 1999). In this type of bacterial infections, after ingestion, the bacterium is capable of penetrating the first defence of the body and acquire a physiological state that improves bacterial survival and replication in host cells. Infection progress depends on an effective host innate immune response. In case of failure, the bacteria enter the bloodstream affecting host organs. Consequently, bacteria invade host cells and replicate in its cytosol, with the further possibility of spreading from cell to cell. This mechanism makes the bacteria avoid the immune system. Within the host cell cytosol, the bacteria replicate using nutrients present in the cell. They can be released upon burst and infect the neighbour cells, spreading in the human host body, possibly leading to his death. The boost of immune response is typically responsible for clearance of the infection. However, together with the rise of bacterial population there is a risk of killing the host due to overgrowth of immunity and bacteria, as well as increasing the pathology resulting from these processes.

On the contrary, in chronic infection the immune response is insufficient to clear the pathogen. An example could be tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB). These bacteria replicate in similar way of *Listeria*, although they are restricted to the alveolar air sacs of the lungs, in the case of lung infection. Moreover, they have a particularity that is related with the extension of infection. Most of the host population infected by these bacteria is asymptomatic, which means the bacteria are in a latent state. In case of active

tuberculosis diseases, death rates are very high without treatment (World Health Organization, 2018). This potential of change in activity between active and latent protects the bacteria from the action of the immune system, leading to longer infections, characterized by ups and downs of bacteria and immune population cells. This will cause a spectrum of infection scenarios, that shift from latency to acute infection or from acute infection to latency or chronic persistence. TB models may include systems of ordinary and partial differential equations and agent-based models as well as hybrid and multi-scale models that are combinations of these. Host dynamics in the lung, granuloma formation, roles of cytokine and chemokine dynamics of these bacteria already have been explored (Kirschner et al., 2017). However, we still do not comprehend the development of antibiotic resistance. More efforts on solving these problems are necessary in order to take action against this global threat.

Many intracellular bacteria such as *Salmonella*, *E.Coli*, *Listeria* are being struck by a rise of resistance strains. This is caused by genetic mutations conferring antibiotic resistance that arise spontaneously during replication and may be selected during treatment. Sometimes the resistant bacteria are pre-existent in the infection having acquired resistance through horizontal gene transfer, other times they arise through mutation during antibiotic treatment. The longer the antibiotic exposure these opportunist bacteria are subjected to, the greater the possibility for resistance emergence and selection. Also, they are mostly transmitted between asymptomatic carriers. Furthermore, many of these resistance genes are passed between different bacterial strains or species. Thus, antibiotic selection may lead to resistant infections of various species and antibiotics are becoming less effective, intensifying the damage caused to the human host, leading to more deaths. This is raising questions in the research community whether we should still prescribe long-course treatments (Llewelyn et al., 2017).

My study in this thesis intends to shed light on the infection processes in the context of intracellular and extracellular bacterial growth balance, affected by host immune mechanisms. Although the model is general and can encompass chronic scenarios, we will focus more on acute infections. Moreover, we study how antibiotic treatments drive the emergence and selection of bacterial resistance to these drugs. These are pivotal questions, since reducing the antibiotic resistance saves millions of people. Furthermore, money spent on developing new antibiotic compounds can be redirected for other important medical issues.

2.1 Mathematical model

In our model of intracellular infection (see diagram 2.1), we track 5 populations: 2 extracellular bacterial population and 3 macrophage compartments: susceptible (M) and infected by bacteria (I_s and I_r) plus an immune response. Thus, there are two distinct intracellular and extracellular populations of bacteria(I and B respectively), susceptible to the antibiotic treatment (I_s, B_s), and fully resistant to the antibiotic treatment(I_r, B_r). Extracellular bacteria infect susceptible macrophages, reproducing intra-cellularly in macrophages, burst and generate new extracellular infection after some time. We model the baseline dynamics of uninfected macrophages via a logistic growth with parameters r, growth rate of uninfected macrophages, and K, carrying capacity of macrophages. Modelling burst sizes through N_s and N_s (1 -γ) allows for a fitness cost of resistance to be explicit. High level resistance populations will only be controlled via resource limitation and host immunity. Sensitive bacteria B_s have the capacity to mutate with mutation probability m per cell. The process happens in the intracellular compartment and is manifested at burst of infected macrophages.

With regards to host immune defenses, we assume innate immune action (e.g. neutrophils) can be factored in the net clearance rate of extracellular bacteria, while for adaptive immune responses (e.g. T-cell, antibody responses) are attributed to effector cell populations E that are stimulated by extracellular bacteria and act by killing infected macrophages, at per capita rate v (Kaufmann and Ladel, 1994) (Harty and Bevan, 1999). We assume no decay of adaptive immunity in the time-scale of the model. E(0) = E_0 fixed (initial immune level). Immune competence expressed as: higher E_0 or higher activation rate σ, or lower antigen threshold required for half-maximal growth k, or higher killing rate v. Our assumptions are that the killing rate by the action of the adaptive immune system cells (Stromberg and Antia, 2011) is the same for B_s and B_r. Model parameters are summarized in Table 2.1.

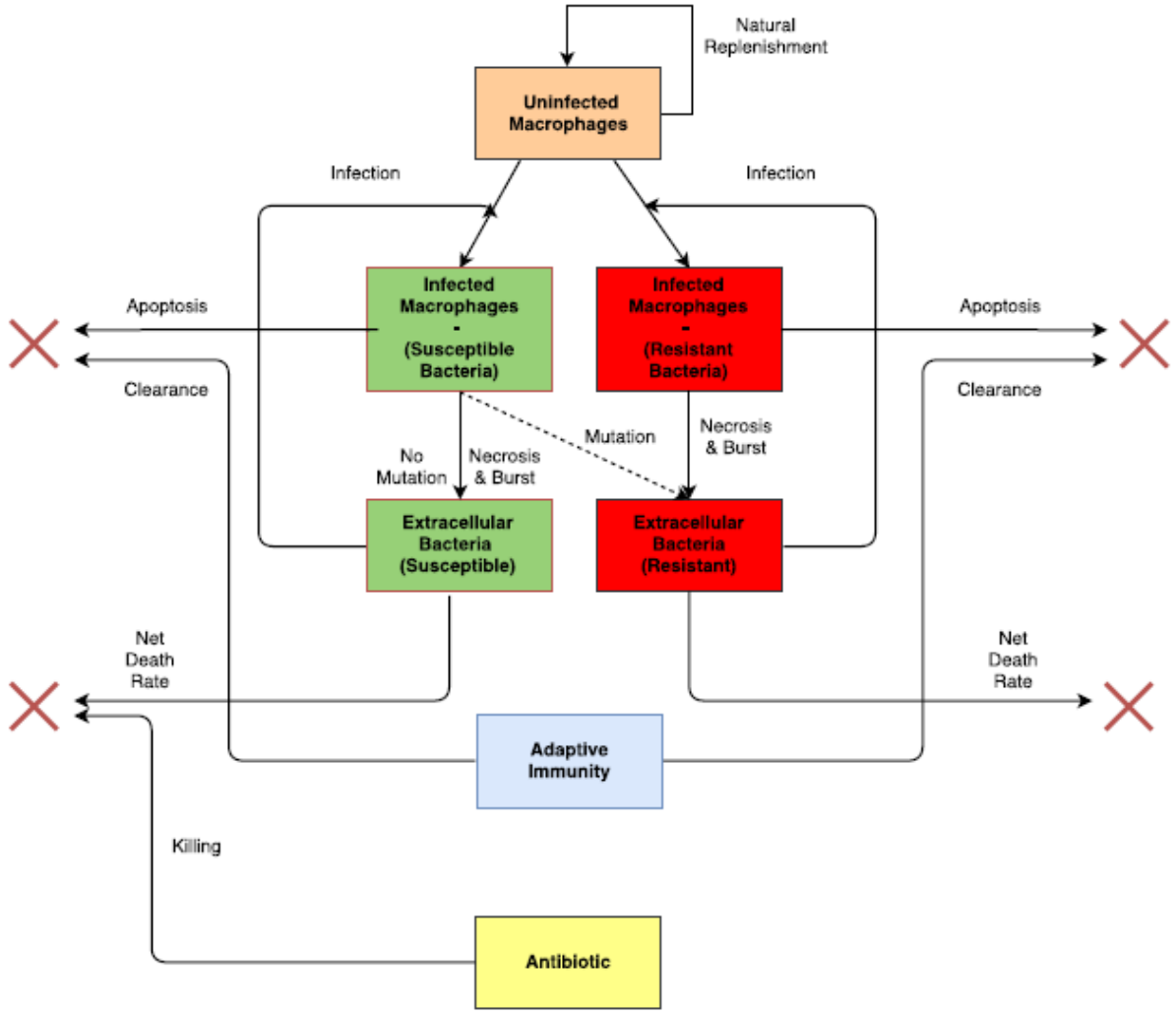


Figure 2.1: Diagram of the model. The infection process of uninfected macrophages by extracellular bacteria is modelled via mass-action kinetics. Once infected, macrophages become the niche for bacterial growth. Infected macrophages can burst through necrosis and they can die via apoptosis. In the extracellular environment, free bacteria die or are cleared. We consider two bacterial sub-populations: drug-sensitive B_s and drug-resistant B_r. Resistant bacteria are generated through mutation in the burst of infected macrophages. Adaptive immunity acts on infected macrophages, antibiotic affects extracellular sensitive bacteria population.

$$\frac{dM}{dt} = rM(1-\frac{M}{K}) - \beta M(B_s+B_r) \tag{2.1}$$

$$\frac{dI_s}{dt} = \beta M B_s - I_s[\delta + a + vE] \tag{2.2}$$

$$\frac{dI_r}{dt} = \beta M B_r - I_r[\delta + a + vE] \tag{2.3}$$

$$\frac{dB_s}{dt} = N_s(1-m)I_s\delta - \beta M B_s - (c+A_m)B_s \tag{2.4}$$

$$\frac{dB_r}{dt} = N_s(1-\gamma)I_r\delta + mN_sI_s\delta - \beta M B_r - cB_r \tag{2.5}$$

$$\frac{dE}{dt} = \sigma E\frac{B_s+B_r}{B_s+B_r+k} \tag{2.6}$$

Most simulations are based on these parameter values, likely to apply to a range of different infections types. The initial conditions of the model are $M(0) = K$ $I_s(0) = 0$ $I_r(0) = 0$ $B_s(0) = 100$ $B_r(0) = 0$ $E(0) = 200$.

Extinction of the bacteria populations (I_s, I_r, B_s and B_r) were verified by an extinction threshold event in the simulations (B_{ext}). This extinction level of bacteria and macrophages population is set as 0.1, which means if the value of bacteria/macrophages get below this value, it will be considered extinct and set to value 0. In addition, we added a generation event which impose growth only when the extracellular or intracellular populations growth rate cause reproduction from zero to the B_{ext} value. Until it happens, there is no resistant strain dynamics.

First, we will consider a specific case of no antibiotic, thus $A_m = 0$, but in the next chapter we deal with treated infections. This chapter provides insights on the baseline dynamics of bacterial infection that are not undergoing any treatment.

2.1.1 Typical acute infection dynamics

To illustrate the acute infection dynamics (figure 2.2), we use the parameter values described in Table 2.1, to echo the typical dynamic infection produced by intracellular bacterial pathogens, e.g. *Listeria monocytogenes*. This infection is characterized by an initial decrease of macrophages and a replenishment of these cells when the infection is being cleared. Also, susceptible bacteria (intracellular and extracellular) rise until immunity begins to be stimulated. In this case, this growth of immunity causes the decrease of both populations of susceptible bacteria after a few days. Intracellular bacteria in infected macrophages get extinct first.

Concerning resistant bacteria, they are generated from sensitive bacteria and their population grows until a peak that is lower when compared to the peak of susceptible bacteria due to the fitness cost. Clearance of the infection is ultimately caused by immunity, which brings the intracellular compartment to extinction, stopping the infection proliferation.

Initially, the growth of intracellular and extracellular bacteria populations stays the same. As the infection progresses and reaches its peak, the intracellular population, which is affected by immunity, starts to get depleted from the host, although the extracellular population maintains growth. Soon after the decrease of intracellular population, the extracellular presents the same behaviour (starts to decline), but always with higher load at that time. This will cause the intracellular population to get extinct first.

Table 2.1: Model parameters for acute dynamics Unit of time is days.

Parameter	Interpretation	Acute Infection (default)
r	Growth rate of uninfected macrophages	0.09 (day^{-1})
K	Carrying capacity of macrophages	10^8
β	Infection rate of macrophages by bacteria	1.2×10^{-7} (day^{-1})
δ	Necrosis rate of infected macrophages	0.2 (day^{-1})
a	Apoptosis rate of infected macrophages	12 (day^{-1})
N_s	Burst size of infected macrophages with drug-sensitive bacteria	100
γ	Fitness cost of high-level resistance, HLR (reduction factor for burst size in B_r)	0.1
c	Natural death/removal rate of bacteria (e.g. neutrophils)	2 (day^{-1})
σ	Maximal growth rate of the adaptive immune response	2 (day^{-1})
k	Half-saturation constant for antigen-dependent immunity	10^4 (CFU)
m	Mutation probability leading to HLR per bacterial cell	10^{-7}
A_m	Net action rate by antibiotic on drug-sensitive bacteria (bacteriocidal/bacteriostatic)	$1 - 30$ (day^{-1})
v	Killing rate effected by adaptive immunity on infected macrophages	1×10^{-5} (day^{-1})
B_{ext}	Pathogen extinction threshold	0.1 (Colony-forming cells, CFU)
B_0	Initial inoculum	100 (Colony-forming cells, CFU)
E_0	Initial immunity	200 (number of cells)

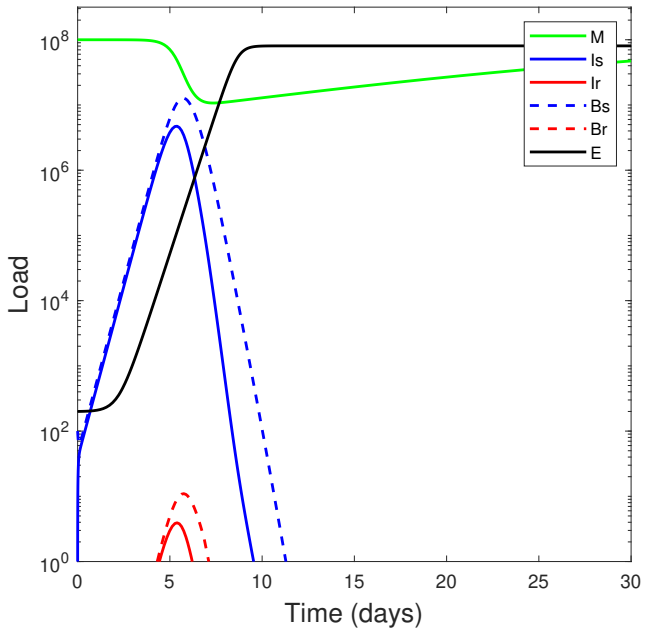


Figure 2.2: Model dynamics of an acute infection over 30 days. Blue lines refer to susceptible bacteria populations. Red lines refer to resistant bacteria populations. Dashed lines representing the extracellular populations, while solid lines represent the intracellular populations (infected macrophages). Green line reflects the population of uninfected macrophages. Black line shows the growth of immunity. Simulation run with the default parameters from Table 2.1.

2.2 Methodology

2.2.1 Materials

Wolfram Mathematica

For conducting analysis of the mathematical model, we used Wolfram Mathematica language. It contains multiple functions and libraries for ODE solvers as well as tools for asymptotic evaluation of the model. Wolfram Mathematica is a modern technical computing system spanning most areas of technical computing such as mathematics. It was conceived by Stephen Wolfram and is developed by Wolfram Research of Champaign, Illinois.

MATLAB

The choice of the software and programming language was based on previous work on this matter. Also, it has present scientific software packages and library for numerical computation and data visualization and plotting. In this matter, we created tools to generate and manipulate data for global use. MATLAB (matrix laboratory) is a multi-paradigm numerical computing environ-

ment and proprietary programming language developed by MathWorks. Moreover, MATLAB is intended primarily for numerical computing, which is essential for simulating data using models. Cleve Moler, the chairman of the computer science department at the University of New Mexico, was responsible for the development of the first MATLAB version in the late 1970s. We use this programming language for computing simulations and also to develop the numerical integration of ODE's and plotting.

2.3 Equilibria for the case of untreated infections

2.3.1 Sub-model without immunity and a single bacterial population

Fixed points

For more understanding of the model, we conduct an asymptotic analysis using Mathematica. First, we evaluate a sub-model not taking into account the treatment ($A_m = 0$), immunity and resistant and susceptible strains. This means only 3 populations: an extracellular bacterial population (B) and 2 macrophage compartments: susceptible (M) and infected by bacteria (I). In this case, extracellular bacteria infect susceptible macrophages, reproducing intra-cellularly in macrophages, burst and generate new extracellular infection after some time.

$$\frac{dM}{dt} = rM(1 - \frac{M}{K}) - \beta MB \tag{2.7}$$

$$\frac{dI}{dt} = \beta MB - I[\delta + a] \tag{2.8}$$

$$\frac{dB}{dt} = N_s I\delta - \beta MB - cB \tag{2.9}$$

After, we evaluate the equilibria points from a model with 2 strains and without immunity. Then, we move into the full model to better understand the processes governing the infection. We find the equilibria by solving a system of equations where all the derivatives of the models are set to 0. Each of the equilibrium points may represent real scenarios that can be labelled as persistence of infection or clearance. In persistence of infection, the level of bacterial population and macrophages remains above zero, reflecting a chronic scenario. Clearance represents any case that leads to the absence of bacterial populations, either through immunity or resource limitation and it can be achieved after some initial growth, or directly as a decline

dynamics from specific initial conditions.

We find three equilibria: S_{sm1} the trivial equilibrium where all populations are equal to zero. S_{sm2} the healthy (clearance) equilibrium where uninfected macrophages are at their carrying capacity and no infection is present. S_{sm3}, the persistence of infection. The analytical expressions are given below:

$$\mathrm{S}_{sm1} = \begin{bmatrix} M^* = & 0 \\ I^* = & 0 \\ B^* = & 0 \end{bmatrix} \quad \mathrm{S}_{sm2} = \begin{bmatrix} M^* = & K \\ I^* = & 0 \\ B^* = & 0 \end{bmatrix} \tag{2.10}$$

$$\mathrm{S}_{sm3} = \begin{bmatrix} M^* = & \frac{-((c(a+\delta))}{(\beta(a+\delta-\delta N_s)))} \\ I^* = & \frac{-((c((a+\delta)(c+\beta K)-\beta\delta K N_s)r)}{(\beta^2 K(a+\delta-\delta N_s)^2))} \\ B^* = & \frac{(((a+\delta)(c+\beta K)-\beta\delta K N_s)r)}{(\beta^2 K(a+\delta-\delta N_s))} \end{bmatrix} \tag{2.11}$$

We find this sub-model admits 3 possible infection outcomes: i) containment, when bacteria do not grow in the host but are gradually cleared (S_{sm2}) ii) growth, followed by clearance yielding acute infection (S_{sm2}) iii) growth, followed by persistent infection (S_{sm3}). The three scenarios are illustrated in Figure 2.3.

The first scenario results when death processes dominate over infection of host cells and replication inside macrophages is too weak. The second scenario results when growth processes are very strong and rapid, leading to fast resource consumption and a drastic decline of the bacterial population, such that it hits the extinction threshold before the target cells have recovered to optimal levels. The third scenario results when growth and death within host balance to maintain infection at intermediate levels. The macrophage-bacteria system undergo a finely-tuned prey-predator type dynamics.

Naturally, for persistence scenario, a balance between many parameters is required, and this is reflected in the following analytical condition for the existence of the persistence equilibrium:

$$\frac{K\beta}{c}\left(\frac{\delta N_s}{\delta + a} - 1\right) > 1,$$

where an obvious sub-condition is $N_s > \frac{a+\delta}{\delta}$. For example, in Figure 2.4, we show how bacterial level at the persistence equilibrium changes with the apoptosis rate of infected macrophages and burst size of infected macrophages. There is increased severity at the peak level when decreasing

the death rate or by increasing burst size. Moreover, we illustrate how the persistence equilibrium depends on critical model parameters. Figure 2.4A shows the bacteria can persist only if burst size N_s is sufficiently big, relative to infected macrophage apoptosis rate, and that the equilibrium level of extracellular bacteria decreases with burst size. Figure 2.4B shows how the bacterial peak depends on burst size, for three values of apoptosis rate of infected macrophages. So far, we looked at existence criteria for the persistence equilibrium. It is important to check also the stability properties.

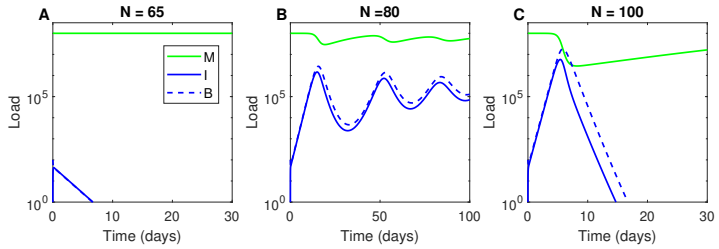


Figure 2.3: Three scenarios of infection dynamics. A) Containment of bacteria, clearance of infection. B) Growth and persistent infection. C. Growth and acute infection followed by clearance (B hits the stochastic extinction threshold). In this illustration, the burst size from infected macrophages, N_s, was varied to describe how bacterial replication capacity in host cells can produce big qualitative changes in the dynamics. Other parameters as in Table 2.1.

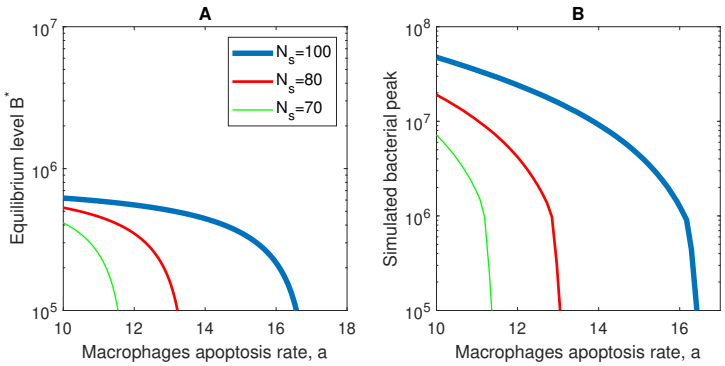


Figure 2.4: Bacterial persistence level and peak infection severity decrease with the apoptosis rate of infected macrophages. A) Analytical expression of the equilibrium level of extracellular bacteria B^* is plotted as a function of a, for three different burst size values N_s. B) Peak bacterial load from simulations is plotted as a function of a: this corresponds to the maximum of $B(t)$ reached in the first growth peak.

Stability

Equilibria can be stable or unstable. To study the stability of this equilibria, we evaluated the Jacobian matrix 2.13 at each equilibria S_{sm1}, S_{sm2}, S_{sm3} and analyzed the eigenvalues.

$$\mathrm{J}_1 = \begin{bmatrix} -\beta B - \frac{Mr}{K} + (1 - (\frac{M}{K})r & 0 & -\beta M \\ \beta B & -a - \delta & \beta M \\ \beta B & \delta N_s & -c - \beta M \end{bmatrix} \quad (2.12)$$

We consider a equilibrium stable if every eigenvalue of the Jacobian matrix of that equilibrium has a negative real part, which is equivalent to require that only the dominant eigenvalue has negative real part:

$$max(Re(\lambda_i)) < 0 \quad (2.13)$$

On the contrary, we consider a equilibrium unstable if any eigenvalue of the Jacobian matrix of that equilibrium is positive:

$$\exists_i s.t. Re(\lambda_i) > 0 \quad (2.14)$$

The trivial equilibrium S_{sm1} is unstable because it has positive eigenvalues. This can be explain by the adding of macrophages will shift the system into a new equilibrium at the carrying capacity K.

For the carrying capacity equilibrium S_{sm2}, it is stable when the burst size is not high enough to cause an infection, therefore:

$$N_s < \frac{(a + \delta)(c + \beta K)}{\beta \delta K} \quad (2.15)$$

Since the eigenvalues for the S_{sm3} were very extensive, a numerical analysis was done to understand the stability of this equilibrium. The clearance equilibrium or carrying capacity equilibrium S_{sm2} always exists. When the clearance equilibrium is stable, the persistence equilibrium does not exist, but, when the clearance equilibrium is unstable, the persistence equilibrium exists. Nevertheless, S_{sm3} is not always stable when it exists. In Figure 2.5 we show how the persistence equilibrium varies with burst size N_s and when it is stable it is manifested

through oscillations. We can clearly see three different possibilities for the equilibrium S_{m3}. In blue, it is unstable, green means it is stable manifested through oscillations and in red it is also stable but without oscillations.

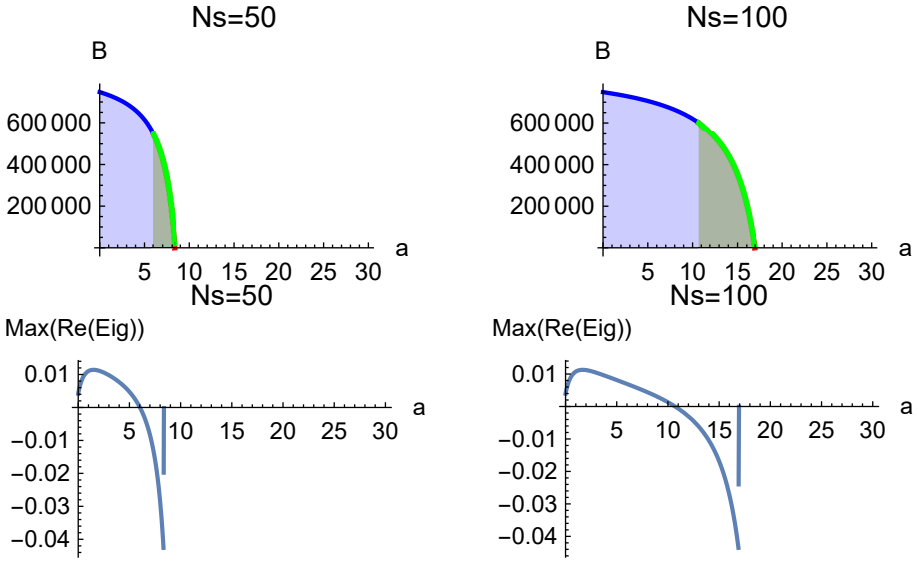


Figure 2.5: Numerical evaluation for the dominant eigenvalue of the Jacobian matrix associated to S_{m3} as a function of infected macrophage death rate. Representation of the stability of equilibrium in the first line. Blue means the existence of equilibrium. Green means stable through oscillations. Red defines the regions where it is stable without oscillations. Second line represents the maximum of the real eigenvalues for the default parameters. We may have stable and unstable equilibrium.

Even though we do not provide a full formal stability analysis of the persistence equilibrium, numerical simulations show that it is typically reached through oscillations, indicating complex eigenvalues of the Jacobian matrix evaluated at this equilibrium.

The amplitude of these oscillations around the equilibrium value, for some parameter values, can be very big (e.g. when bacteria grow too fast and deplete their resource too much). In such cases, even though theoretically we expect persistence, due to the large amplitude oscillation, B can hit the extinction threshold, and thus numerically correspond to a clearance scenario. These are the "acute" infection scenarios (see Figure 2.3 C) that sometimes can drive the system to (K,0,0) and sometimes to (0,0,0) depending on how fast the healthy macrophages are depleted.

We have explored the criteria needed to be satisfied in this simple system for stability of clearance and stability of persistence. Now, taking this information into account, we add more complexity to the model, and add two bacteria strains to simulate competition in the infection. We expect similar equilibria and principles are going to be applied. The question is now how competition governs the infection and if there is any impact in this equilibria.

2.3.2 Model with 2 bacterial sub-populations

Introducing the two strains (for example drug-resistance and drug-sensitive) to the sub-model 2.9, we now grow in complexity, and we can have competition between the two strains on the course of infection. We have now 5 different populations of cells. We assume that the difference between the two sub-populations is in the fitness cost of B_r, manifested in a smaller burst size (where $\gamma < 1$) and there is a mutation process generating B_r from B_s growing intracellularly. Again, we assume no treatment ($A_m = 0$) for simplicity of analysis and illustration purposes.

$$\begin{aligned}
\frac{dM}{dt} &= rM(1-\frac{M}{K}) - \beta M(B_s + B_r) && (2.16)\\
\frac{dI_s}{dt} &= \beta M B_s - I_s[\delta + a] && (2.17)\\
\frac{dI_r}{dt} &= \beta M B_r - I_r[\delta + a] && (2.18)\\
\frac{dB_s}{dt} &= N_s(1-m)I_s\delta - \beta M B_s - cB_s && (2.19)\\
\frac{dB_r}{dt} &= N_s(1-\gamma)I_r\delta + mN_sI_s\delta - \beta M B_r - cB_r && (2.20)
\end{aligned}$$

Fixed points

We now find four equilibria similar to the sub-model 2.9 with the addition of one equilibrium with persistence of only resistance strain, denoting possibility of out-competition of the susceptible strain. By setting all the equations (derivatives) to zero, and solving for all variables, we find the equilibria of the system. In this case we have 4 equilibria described as:

$$\mathrm{S}_1 = \begin{bmatrix} M^* = & 0 \\ I_s^* = & 0 \\ I_r^* = & 0 \\ B_s^* = & 0 \\ B_r^* = & 0 \end{bmatrix} \quad \mathrm{S}_2 = \begin{bmatrix} M^* = & K \\ I_s^* = & 0 \\ I_r^* = & 0 \\ B_s^* = & 0 \\ B_r^* = & 0 \end{bmatrix} \quad (2.21)$$

$$\mathrm{S}_3 = \begin{bmatrix} M^* = & \frac{-((c(a+\delta))}{(\beta(a+\delta+\delta(-1+\gamma)N_s)))} \\ I_s^* = & 0 \\ I_r^* = & \frac{-((c((a+\delta)(c+\beta K)+\beta\delta(-1+\gamma)KNs)r)}{(\beta^2 K(a+\delta+\delta(-1+\gamma)N_s)^2))} \\ B_s^* = & 0 \\ B_r^* = & \frac{(((a+\delta)(c+\beta K)+\beta\delta(-1+\gamma)KN_s)r)}{(\beta^2 K(a+\delta+\delta(-1+\gamma)N_s))} \end{bmatrix} \quad (2.22)$$

$$\mathrm{S}_4 = \begin{bmatrix} M^* = & \frac{-((c(a+\delta))}{(\beta(a+\delta+\delta(-1+m)N_s)))} \\ I_s^* = & \frac{-((c(\gamma-m)((a+\delta)(c+\beta K)+\beta\delta K(-1+m)N_s)r)}{(\beta^2\gamma K(a+\delta+\delta(-1+m)N_s)^2))} \\ I_r^* = & \frac{-((cm((a+\delta)(c+\beta K)+\beta\delta K(-1+m)N_s)r)}{(\beta^2\gamma K(a+\delta+\delta(-1+m)N_s)^2))} \\ B_s^* = & \frac{((\gamma-m)((a+\delta)(c+\beta K)+\beta\delta K(-1+m)N_s)r)}{(\beta^2\gamma K(a+\delta+\delta(-1+m)N_s))} \\ B_r^* = & \frac{m((a+\delta)(c+\beta K)+\beta\delta K(-1+m)N_s)r)}{(\beta^2\gamma K(a+\delta+\delta(-1+m)N_s))} \end{bmatrix} \quad (2.23)$$

The first case (S_1) refers to the trivial equilibrium situation when there are no populations present in the system, which is not realistic at any point during infection, since macrophages are always present in the healthy human host. The second case (S_2) refers to a healthy or a pre-infection situation, where macrophages are at carrying capacity or after an infection (clearance). The third, and worst case scenario (S_3), is when there is only resistant population persisting. Finally, the last case (S_4) refers to a chronic situation with both susceptible and resistant populations coexist in the system.

Features of the persistence equilibria

The calculations for the following analysis are present in Appendix A. For both S_3 (fixation of resistance) and S_4 (coexistence) equilibria to exist, they must fulfil a condition such as, for S_3 equilibria to exist:

$$K > \frac{c(a+\delta)}{\beta\big[\delta(N_s-\gamma N_s-1)-a\big]} \quad (2.24)$$

and for S_4 to exist:

$$K > \frac{c(a+\delta)}{\beta\big[\delta(N_s-mN_s-1)-a\big]} \quad (2.25)$$

Starting from these inequalities, we can derive a further sub-condition (denominator to be positive) by requiring that, in S_3 and S_4, burst size satisfies:

$$N_s > \frac{a+\delta}{\delta(1-\gamma)} \quad and \quad N_s > \frac{a+\delta}{\delta(1-m)} \tag{2.26}$$

Moreover, considering positive values of all variables, in order to exist equilibrium S_4, the fitness cost γ must be greater than the mutation rate m. Therefore, if the fitness cost of the drug-resistant strain is greater than the mutation rate, we will have coexistence with both susceptible and resistant bacteria populations. In the case of the mutation rate higher than the fitness cost, it favours the selection of resistance and consequently, it will only be possible the resistant equilibrium S_3.

Regarding the bacterial population, susceptible and resistance strains compete each other for the resources and proliferation. We find that in the coexistence equilibrium, susceptible and resistant populations present the same ratio intracellularly and extracellularly:

$$\frac{I_s^*}{I_r^*} = \frac{B_s^*}{B_r^*} = \frac{\gamma - m}{m} = \frac{\gamma}{m} - 1 \tag{2.27}$$

Taking into account our default parameter values, the mutation rate is considerably lower than the fitness cost. In this case, in the absence of immunity, only the coexistence equilibrium is possible to achieve (Figure 2.7), since coexistence requires of both populations higher fitness cost when compared to the mutation rate.

Furthermore, at the coexistence equilibrium, we can have dominance of the resistant bacteria if:

$$\frac{Is^*}{Ir^*} < 1 \Leftrightarrow \frac{\gamma}{m} - 1 < 1 \Leftrightarrow \frac{\gamma}{m} < 2 \tag{2.28}$$

When the ratio $\frac{\gamma}{m}$ is higher than the value 2, there will be more advantage conditions for susceptible population to grow. The fitness cost is high enough that does not allow resistant bacteria to win over the susceptible population. On the other hand, when the ratio $\frac{\gamma}{m}$ is lower than $2m$, there will be a higher number of resistance bacteria and sensitive bacteria at persistence equilibrium.

In summary, there will be different scenarios depending on the fitness cost γ and mutation rate m:

- When $\gamma < m$, only the resistant equilibrium is possible. Resistant strains goes to fixation

within host, assuming deterministic mutation constantly maintaining a flux from sensitive to resistant bacteria.

- When $\gamma > m$, only the coexistence equilibrium is possible.
- When $m < \gamma < 2m$ resistant bacteria population dominates over susceptible bacteria population in coexistence.
- When $\gamma > 2m$ susceptible bacteria population dominates over resistant bacteria population in coexistence.

Moreover, in the persistence equilibria we can have different behaviours (Figure 2.6) with bigger oscillations from decreasing the value of intracellular apoptosis rate, eventually leading to extinction of the bacteria populations, as we can see in the case A of the Figure 2.6. However, by the information retrieved from the stability analysis in Figure 2.8 C, we expect that will be a persistent equilibrium when the intracellular death rate is 12 and we do not see that on the simulations. We explain it by the use of the extinction threshold, which impose the death of population when they reach that threshold value. Therefore, if we reduce the extinction threshold we see bigger oscillations again, as expected (see Figure A.1), reaching to a persistence equilibrium.

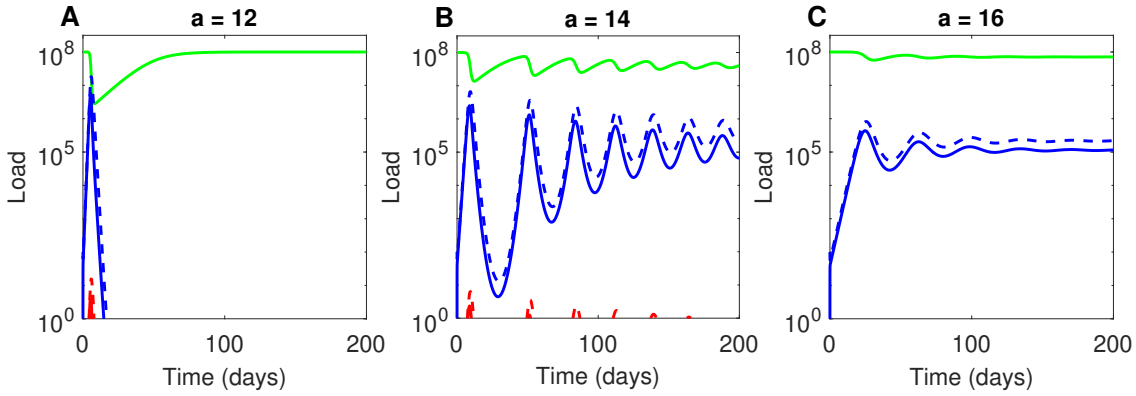


Figure 2.6: Model dynamics of an infection over 200 days without immunity for three different infected macrophage apoptosis rate values. Blue lines refer to susceptible bacteria populations. Red lines refer to resistant bacteria populations. Dashed lines representing the extracellular populations, while solid lines represent the intracellular populations (infected macrophages). Green line reflects the population of uninfected macrophages. Simulations run with the default parameters from Table 2.1. Oscillatory behaviour decreases with the increasing of infected macrophages death rate. In A we do not observe due to a larger oscillatory behaviour which reach the B_{ext}, imposing extinction of bacterial populations.

Stability

Now, we investigate the stability again for each equilibria. 2.29 is the Jacobian matrix for the model without immunity. We look again at the eigenvalues for each equilibrium and determine at which conditions there may be stability.

$$\mathrm{J}_2 = \begin{bmatrix} \frac{(-\beta(B_r+B_s)-(Mr)}{K+(1-M/K)r} & 0 & 0 & -\beta M & -\beta M \\ \beta B_s & -a-\delta & 0 & \beta M & 0 \\ \beta B_r & 0 & -a-\delta & 0 & \beta M \\ -\beta B_s & \delta(1-m)N_s & 0 & -c-\beta M & 0 \\ -\beta B_r & \delta m N_s & \delta(1-\gamma)N_s & 0 & -c-\beta M \end{bmatrix} \tag{2.29}$$

First equilibrium S_1 is unstable. It has positive eigenvalues. The addition of macrophages will cause the system to move into another state, namely to the S_2 equilibrium with macrophages at the carrying capacity.

Considering S_2, the equilibrium where the macrophages are present at the carrying capacity, we may have stability. This equilibrium always exists, but it is not always stable. In this case, the condition for stability depends also on burst size as in previous sub-model, as follows:

$$N_s < \frac{(a+\delta)(c+\beta K)}{\beta\delta(1-\gamma)K} \tag{2.30}$$

We just have the introduction of fitness cost to the condition of stability described previously. The burst size must be lower than a threshold making the infection weak enough to cause an infection. Therefore, the introduction of bacteria will lead again to the previous state of clearance since there is no proliferation.

For the other cases, since the eigenvalues expression for the equilibrium is too extensive, a numerical evaluation was carried taking into account the default parameters: S_3 equilibrium is stable when the infection is strong enough to proliferate in the host, but not too strong to kill. This means that when S_3 exists it is not always stable. There are regimes when it is unstable, namely for very slow or very fast infections. On the other hand, S_4 seems to always unstable when exists. Resistance persistence equilibrium, which is the worst scenario, is the only case whether we can have a stable persistence equilibrium in the host. However, it is dependent on the fitness cost being lower than the mutation rate.

Nevertheless, our analysis points that the critical condition for a persistence equilibrium

to exist is, in fact, a relationship between multiple parameters, and not just dependent on the carrying capacity of the system. It is also dependent on the fitness cost and mutation rate as we saw previously at section 2.3.2. Therefore, using default parameters for the intracellular and extracellular death rate and the burst rate, we calculated the threshold in which is possible to observe each equilibrium, as described below. Both burst size and burst rate are related to the proliferation of bacteria in the host's body; fitness cost and mutation rate with the emergence and persistence of the resistant bacteria population; intracellular and extracellular death rate with the growth and maintenance of the populations.

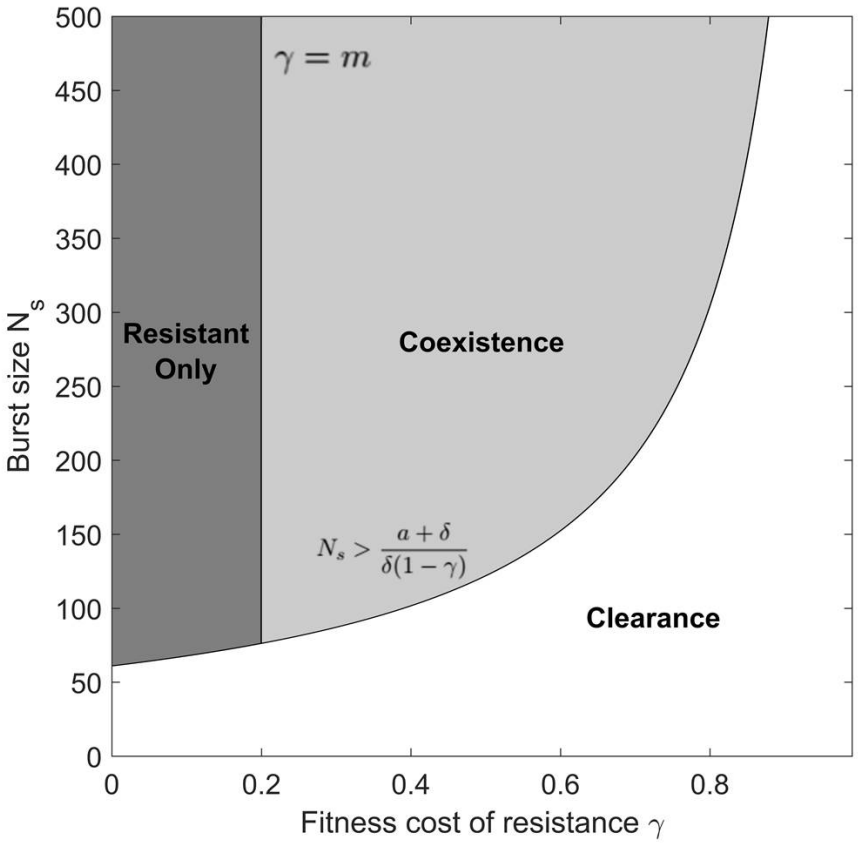


Figure 2.7: How the intracellular and extracellular balance leads to different outcomes in the absence of immunity. Asymptotic analysis shows the possibility of three equilibria: Persistence with resistant bacteria; Persistence with sensitive and resistant bacteria and Clearance. Burst size must counterbalance the net lifespan of infected macrophages for the infection to persist. When the fitness cost is low enough compared to the mutation rate, there is room for resistant bacteria to dominate. There is another condition for existence of equilibria depending on K: $K > \frac{c(a+\delta)}{\beta[\delta(N_s - \gamma N_s - 1) - a]}$. Also, mutation rate is set to 0.2 to better understand visually the possibility of equilibria according to their conditions.

When the burst size N_s is too low, the infection do not progress fast enough and infection cannot be sustained. In this case, death dominates over growth, and we achieve clearance. When N_s is big enough to sustain an infection, there can be a persistence opportunity

for the infection. In this case, the comparison of both the fitness cost γ and the mutation rate m defines whether the resistant bacteria will coexist with sensitive ones, or will persist alone. Moreover, when we alter the value of the intracellular apoptosis rate (a), we may shift the system from a persistence equilibrium to a clearance equilibrium (see Figure 2.8 A and B). Notice, however, that like in the previous sub-model, if the oscillations around the persistence equilibrium are too big, bacteria may hit the extinction threshold and thus numerically we may see effective clearance even inside the gray region above the critical line in Figure 2.7.

2.3.3 Analysis for the full model with 2 sub-populations of bacteria and immune dynamics

We understood previously how the infection progresses in the absence of immunity dynamics (no E-equation). Adding immunity to the model, we now investigate how immunity changes the dynamics of the infection. We look into the equilibria points and its stability as previously, although now we have the input information of what governs the infection.

We go back to the model described in section 2.1 by 2.1 to 2.6 equations. The most important difference is that we have a non-decreasing immune response $dE/dt > 0$ as long as $B > 0$ and $E > 0$. This will act as a definite negative feedback on the bacterial population growing during infection. When we have some immunity in the system and some bacteria, the only outcome possible will be eventual clearance.

Fixed Points

$$\mathrm{S}_{e1} = \begin{bmatrix} M^* = & 0 \\ I_s^* = & 0 \\ I_r^* = & 0 \\ B_s^* = & 0 \\ B_r^* = & 0 \\ E^* = & 0 \end{bmatrix} \quad \mathrm{S}_{e2} = \begin{bmatrix} M^* = & K \\ I_s^* = & 0 \\ I_r^* = & 0 \\ B_s^* = & 0 \\ B_r^* = & 0 \end{bmatrix} \tag{2.31}$$

$$\mathrm{S}_{e3} = \begin{bmatrix} M^* = & \frac{-((c(a+\delta))}{(\beta(a+\delta+\delta(-1+\gamma)Ns)))} \\ I_s^* = & 0 \\ I_r^* = & \frac{-((c((a+\delta)(c+\beta K)+\beta\delta(-1+\gamma)KNs)r)}{(\beta^2 K(a+\delta+\delta(-1+\gamma)Ns)^2))} \\ B_s^* = & 0 \\ B_r^* = & \frac{(((a+\delta)(c+\beta K)+\beta\delta(-1+\gamma)KNs)r)}{(\beta^2 K(a+\delta+\delta(-1+\gamma)Ns))} \\ E^* = & 0 \end{bmatrix} \quad (2.32)$$

$$\mathrm{S}_{e4} = \begin{bmatrix} M^* = & \frac{-((c(a+\delta))}{(\beta(a+\delta+\delta(-1+m)Ns)))} \\ I_s^* = & \frac{-((c(\gamma-m)((a+\delta)(c+\beta K)+\beta\delta K(-1+m)Ns)r)}{(\beta^2\gamma K(a+\delta+\delta(-1+m)Ns)^2))} \\ I_r^* = & \frac{-((cm((a+\delta)(c+\beta K)+\beta\delta K(-1+m)Ns)r)}{(\beta^2\gamma K(a+\delta+\delta(-1+m)Ns)^2))} \\ B_s^* = & \frac{((\gamma-m)((a+\delta)(c+\beta K)+\beta\delta K(-1+m)Ns)r)}{(\beta^2\gamma K(a+\delta+\delta(-1+m)Ns))} \\ B_r^* = & \frac{m((a+\delta)(c+\beta K)+\beta\delta K(-1+m)Ns)r)}{(\beta^2\gamma K(a+\delta+\delta(-1+m)Ns))} \\ E^* = & 0 \end{bmatrix} \quad (2.33)$$

At first look, we observe the same equilibria points from the model without immunity. However, we have one small difference: The carrying-capacity equilibrium now sustains any value of immunity. The other 3 equilibria values are exactly the same as previously, with the addition of zero immunity.

We revisit the equilibria explanation. The first case (S_{e1}) refers to the trivial equilibrium situation when there are no populations present in the system, which is not realistic at any point during infection, since macrophages are always present in the healthy human host. The second case (S_{e2}) refers to a healthy or a pre-infection situation, where macrophages are at carrying capacity. Immunity can have any value for this to be true. This scenario could also be a post-infection scenario, after immunity has grown to a very big level, E^* sufficient to keep bacterial death bigger than bacterial proliferation. The third, and worst case scenario (S_{e3}), is when there is only resistant population persisting. In this case, immunity can only assume a value equal to zero for the system to remain equilibrium. Finally, the last case (S_{e4}) refers to a chronic situation with both susceptible and resistant populations are present in the system. As in the previous case, immunity here can only assume the value zero in order for the equilibrium to be a real equilibrium. In this case, the derivatives are zero for all equations, including $\frac{dE}{dt}$.

In the fixation of resistance scenario (S_3), we can compare the intracellular population with the extracellular population. Intracellular population is lower than the extracellular population if:

$$N_s > \frac{a+c+\delta}{\delta(1-\gamma)} \Leftrightarrow I_r^* < B_r^* \tag{2.34}$$

and the reverse otherwise. This means that if the burst size N_s is sufficiently big, extracellular population wins and dominates over intracellular.

Focusing now on the last case (S_4), which refers to the coexistence equilibrium, the ratio of extracellular populations and intracellular is similar to the S_3, diverging only on one parameter that conserve the same position in the equation. In the case of the S_3 equilibrium, the parameter is the fitness cost. In the case of the S_4 equilibrium, the parameter is the mutation rate as it follows:

$$\frac{I_r^* + I_s^*}{B_r^* + B_s^*} = \frac{c}{N_s(1-m)\delta - (a+\delta)} \tag{2.35}$$

Moreover, the bacterial peak observed in the simulations (see Figure 2.8 D) is much higher for the same parameter values than the equilibrium we expect them to reach, even with the presence of immunity. The oscillations around the equilibrium will cause higher peaks than the actual value expected for the bacteria to persist in the host when time goes to infinity (actual meaning of the theoretical equilibrium). Presence of immunity in this case prevent the relapses after the first peak, eliminating the entire bacteria present.

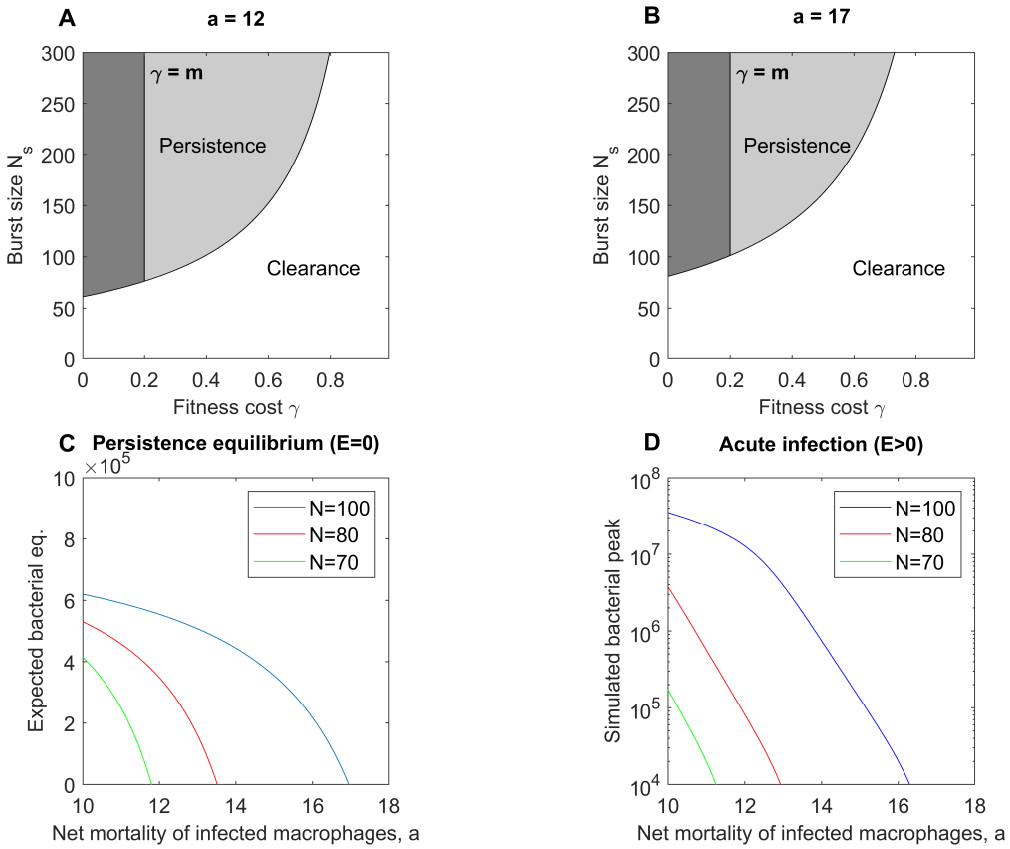


Figure 2.8: Persistent infection vs clearance: role of the apoptosis rate of infected macrophages. This follows the Figure 2.7, which describe the conditions for the existence of the equilibria S_3 and S_4. Asymptotic analysis shows the possibility of three equilibria: Persistence with resistant bacteria (dark grey); Persistence with sensitive and resistant bacteria (light grey) and Clearance (white). A)Conditions for existence of the equilibria with the intracellular death rate with the value 12. B)Conditions for existence of the equilibria with the intracellular death rate with the value 17. C) Value of the bacteria population in the persistence equilibrium for different values of the intracellular apoptosis rate and burst size. No immunity present. D) Bacterial peak for acute infections with different intracellular apoptosis rate and burst size. Immunity is activated.

Stability

To understand how immunity affects the stability of the system, we performed a last stability analysis to the equilibria that arose from the model. Once again, the mathematica files are presented in Appendix A with further explanations. Denoting equation 2.36, the Jacobian matrix in its general form reads as follows:

$$U = \frac{-(((B_r + B_s)E\sigma)}{(B_r + B_s + k)^2)} + \frac{(E\sigma)}{(Br + Bs + k)} \tag{2.36}$$

$$
\mathrm{J}_3 = \begin{bmatrix}
\frac{(-\beta(B_r+B_s)-(Mr)}{K+(1-M/K)r} & 0 & 0 & -\beta M & -\beta M & 0 \\
\beta B_s & -a-\delta-E1v & 0 & \beta M & 0 & -I_s v \\
\beta B_r & 0 & -a-\delta-E1v & 0 & \beta M & I_r v \\
-\beta B_s & \delta(1-m)N_s & 0 & -c-\beta M & 0 & 0 \\
-\beta B_r & \delta m N_s & \delta(1-\gamma)N_s & 0 & -c-\beta M & 0 \\
0 & 0 & 0 & U & U & \frac{((B_r+B_s)\sigma)}{(B_r+B_s+k)}
\end{bmatrix} \tag{2.37}
$$

First equilibrium S_{e1} is unstable. It has positive eigenvalues. The addition of macrophages will cause the system to move into another state, namely to the S_{e2} equilibrium with macrophages at the carrying capacity.

Considering S_{e2}, the equilibrium where the macrophages are present at the carrying capacity with immunity present, but no infection, we may have neutral stability, after or before clearance. We may have two situations: when the value of immunity is too low, addition of bacteria will cause a acute infection followed by clearance. Another case is when the value of immunity rises to a level high enough that any perturbation of bacteria/macrophages will not shift the system into a different state with different levels of bacteria. However, these two cases reflect changes in E^* value, since there is always growth of immunity when we introduce bacteria. This causes the appearance of zero values in eigenvalues, making it only neutrally stable. If immunity at this equilibrium is higher than

$$
E^* > \frac{1}{v}\Big[\frac{\delta N_s(1-m)}{\frac{c}{K\beta}+1} - (\delta+a)\Big]
$$

we will have direct clearance of bacteria. However, if immunity is lower than this condition we will have growth followed by decline and immunity eventually reaching a super-critical level.

We performed a numerical analysis for the other cases since the eigenvalues expression was too extensive as in previous section. We find every numerical eigenvalue bigger than zero. This means that all consist in unstable equilibriums. So, in theory, any perturbation will lead the system to a new equilibrium. This can be understood because of the action of immunity. In an only-growing immunity model (2.6), if the infection persistence equilibrium is perturbed with any quantity of immunity and there is bacteria present, $\frac{dE}{dt}$ will be made positive and E will start to grow until it will be able to clear the infection. Therefore, the system will go to clearance. In our model, the carrying capacity equilibrium remains the most plausible.

2.3.4 Extension to chronic persistence with immunity

Until now we have seen persistence of infection to arise only in the the S_3 or S_4 equilibria. Persistence of bacteria can involve coexistence of susceptible and resistant bacteria or be fully resistant. The previous equilibria are only possible with $E = 0$, thus complete absence of immunity or a immune suppressed individual. However, it is known that in real life persistence of infection can be observed also with some level of immunity. Therefore, for this model to capture a chronic case with immunity present, we can add a new feature to the model by restraining the action of the immune response to a limited time-window of activation. In the acute case, immune response will grow for an indefinite amount of time stimulated by antigen, enabling final pathogen clearance. One way to generate a chronic infection sustained by suboptimal immunity is to assure a limited window of time post-infection (short) $[0; T_{off}]$ over which immunity can grow (Mercado et al., 2000a). This limits its peak level, and thus constrain the net rate of killing on the pathogen. In this case we could obtain (as shown in Figure 2.7) a persistent infection maintained by sub-optimal immune levels. Obviously in any system where additional bacterial control is coming from the immune response, the persistence level of infection will be lower. Thus, even though immunity may not be strong enough to drive the infection to clearance, it can still contribute to maintain infection levels below the extreme values expected under no immunity at all.

The host immune system is able to eliminate most of the invading bacteria. However, in certain conditions, the bacteria can evade the immune system and persist within the host, leading to asymptomatic infections for long periods of time. During this period, they may also reactive into clinically significant diseases. There are many factors that contribute to the ability of bacteria to establish chronic infections, including both host and bacterial factors. These persistent infections have typically slow-growth mechanisms when compared to acute infections.

Therefore, to illustrate an example of persistent infection, we will assume other combination of parameter values for the chronic infection pathogen, similar to what might be expected for *Mycobacterium tuberculosis* (MTB), summarized in Table 2.2.

In the chronic case, the bacteria persist in the host. Immunity rises and fights the infection. Although it is not sufficient to clear it, it still maintains at a certain level. Consequently, there tends to be a equilibrium, similar to a predator-prey type of dynamics, with oscillatory behaviour between the bacteria as the "predator" and macrophages as the "prey" (see Figure 2.9). The analysis of the model for chronic persistence with static immunity (unable

Table 2.2: Model parameters for chronic dynamics. Unit of time is days. Bold parameters changed their values from the acute Table 2.1.

Parameter	Interpretation	Chronic Infection (default)
r	Growth rate of uninfected macrophages	0.09 (day^{-1})
K	Carrying capacity of macrophages	10^8
β	**Infection rate of macrophages by bacteria**	$1.2 \times 10^{-8.5}$ (day^{-1})
δ	**Necrosis rate of infected macrophages**	0.48 (day^{-1})
a	**Apoptosis rate of infected macrophages**	0.2 (day^{-1})
N_s	**Burst size of infected macrophages with drug-sensitive bacteria**	35
γ	Fitness cost of high-level resistance, HLR (reduction factor for burst size of B_r)	0.1
c	Natural death/removal rate of bacteria (e.g. neutrophils)	2 (day^{-1})
σ	**Maximal growth rate of the adaptive immune response**	1 (day^{-1})
k	Half-saturation constant for antigen-dependent immunity	10^4 (CFU)
m	Mutation probability leading to HLR per bacterial cell	10^{-7}
A_m	Net action rate by antibiotic on drug-sensitive bacteria (bacteriocidal/bacteriostatic)	$1 - 30$ (day^{-1})
v	Killing rate effected by adaptive immunity on infected macrophages	1×10^{-5} (day^{-1})
B_{ext}	Pathogen extinction threshold	0.1 (CFU)
B_0	Initial inoculum	100 (CFU)
E_0	Initial immunity	200 (number of cells)
T_{off}	**Window of time post-infection over which immunity can grow**	$7(\text{days}^(- 1))$

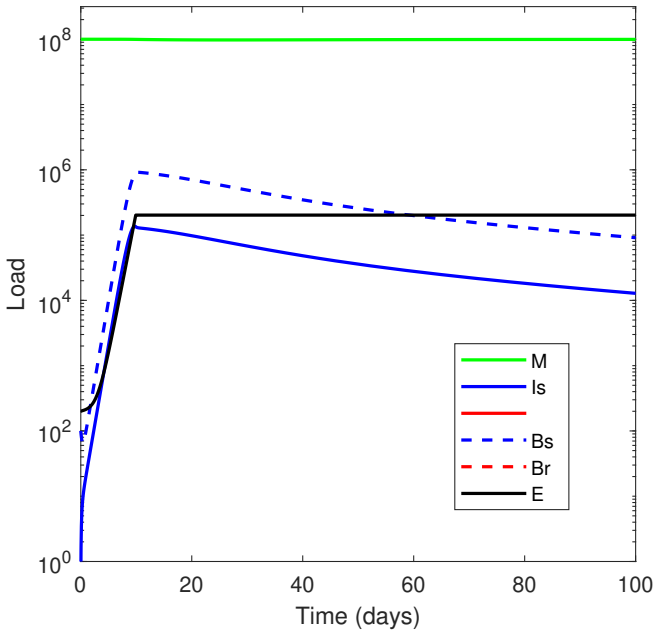


Figure 2.9: Dynamics of a chronic infection with sub-optimal immune stimulation. Blue lines refer to susceptible bacteria populations. Red lines refer to resistant bacteria populations. Dashed lines representing the extracellular populations, while solid lines represent the intracellular populations (infected macrophages). Green line reflects the population of uninfected macrophages. Black line shows the growth of immunity this time constrained only during a limited time window at the beginning of infection. Simulation run with the default parameters from Table 2.2.

to grow any further) is similar to the model without immunity, although now the intracellular death rate of infected macrophages should be higher, due to the additional marginal killing by the immune response and becomes $a + (v * E_{final})$. Taking this into account, we may have the same equilibria, and the same conditions for existence and stability. For example, in order for resistance persistence to exist:

$$K > \frac{c(a + (v * E_{final}) + \delta)}{\beta\big[\delta(N_s - \gamma N_s - 1) - a - (v * E_{final})\big]} \quad \text{and} \quad N_s > \frac{a + (v * E_{final}) + \delta}{\delta(1 - \gamma)} \tag{2.38}$$

and for S_4 to exist:

$$K > \frac{c(a + (v * E_{final}) + \delta)}{\beta\big[\delta(N_s - mN_s - 1) - a - (v * E_{final})\big]} \quad \text{and} \quad N_s > \frac{a + (v * E_{final}) + \delta}{\delta(1 - m)} \tag{2.39}$$

Regarding stability, the persistence equilibrium will not exist if:

$$E^*_{final} > \frac{1}{v}\Big[\frac{\delta N_s(1-m)}{\frac{c}{K\beta}+1} - (\delta + a)\Big] \tag{2.40}$$

and in that case the clearance equilibrium will be stable.

Thus, only if the immune response stops growing at a high-enough, super-critical level, the infection will go to clearance. If immunity saturates and gets "locked" at sub-critical levels then, we will have the persistence equilibrium.

2.4 Sensitivity analysis of the model

Now we go back to the full model, and and understand the transient dynamics. Since we do not know exactly the full biological range of the parameters of the model, to increase understanding of the infection processes and relationships between the variables in this model, we conducted a sensitivity analysis. This provides us more information to comprehend what fuels the infection and what parameters impact more the infection outcomes. From this, we can infer new therapy targets and which main process of the infection we should focus on (competition, immune stimulation or target cell limitation) and allow us to control the infection. For this, we used a matlab package named Global Sensitivity Analysis Toolbox (Cannavó, 2012). GSAT package includes routines for generic global sensitivity analysis. In particular it implements Sobol' analysis and FAST analysis to models with up 50 different input parameters. In this case, it was implemented with our model, using 9 model parameters to check how they differ in magnitude of effect and to identify the ones that produce more changes in dynamics. We use the intervals presented on Table 2.3. We note that the results may be different if we were to choose a different range. However, in the range presented, which is biologically feasible, we explore some interesting features. Some were transformed in log scale to improve computation time. It simulated infections over 100 days performing 200 iterations.

Table 2.3: Model parameters and intervals used on sensitivity analysis.

Parameter	Interpretation	Range	Scale
β	Infection rate of macrophages by bacteria	$[10^{-}9, 10^{-}6]$	Log
δ	Necrosis rate of infected macrophages	$[0.01, 1]$	Log
a	Apoptosis rate of infected macrophages	$[0.1, 100]$	Linear
N_s	Burst size of infected macrophages with drug-sensitive bacteria	$[1, 100]$	Log
γ	Fitness cost of high-level resistance, HLR (reduction factor for burst size of B_r)	$[0, 1]$	Linear
c	Natural death/removal rate of bacteria (e.g. neutrophils)	$[0.1, 20]$	Linear
σ	Maximal growth rate of the adaptive immune response	$[0.1, 4]$	Linear
k	Half-saturation constant for antigen-dependent immunity	$[10^3, 10^5]$	Log
v	Killing rate effected by adaptive immunity on infected macrophages	$[10^{-}7, 10^{-}4]$	Log

We evaluated how important were the model parameters for four different infection measures: duration of infection, peak of susceptible bacteria, peak of resistant bacteria and bacterial burden. We define these four measures as follows:

- Duration of infection - Time elapsed until the extinction of the 4 different bacterial populations.

$$T_{ext} = min_t(\ni B_s, B_r, I_s, I_r < B_{ext}) \tag{2.41}$$

- Peak of susceptible bacteria - The maximum value that susceptible bacteria acquire during an infection profile.

$$\hat{B}_s(t) = Max_t(B_s(t)) \tag{2.42}$$

- Peak of resistant bacteria - The maximum value that resistant bacteria acquire during an infection profile.

$$\hat{B}_s(t) = Max_t(B_r(t)) \tag{2.43}$$

- Pathogen/bacterial burden - Total number of bacteria present throughout the infection.

$$B_{tot} = \int_0^{T_{ext}} B_s(t) + B_r(t)dt \tag{2.44}$$

From our sensitivity analysis (see Figure 2.10), given the ranges we assumed, we find four parameters that stand out: infected macrophages death rate (a), extracellular bacteria death rate (c), burst rate (δ) and burst size (N_s). We identify, i the particular ranges studied, the death rate of infected macrophages(a) as the most disruptive parameter for all the four outcome measures. This supports the fact that the long persistence of infected macrophages is a very strong factor promoting bacterial proliferation. Thus, this may be a target for drugs to control infections. The other parameter is the extracellular bacteria death rate. If bacteria persist longer extracellularly, they can infect more macrophages, boosting infection. N_s, the burst size and, δ, burst rate, are also critical parameters for the dynamics, because they actually scale the contribution of the intracellular compartment to the proliferation of the infection. In addition, for the peak of resistance bacteria, fitness cost is also important (γ), as expected. The less cost resistant bacteria have, the easier they compete with susceptible bacteria and proliferate.

This information is helpful to understand further results, especially the relevance of intracellular and extracellular balance in the process of the infection.

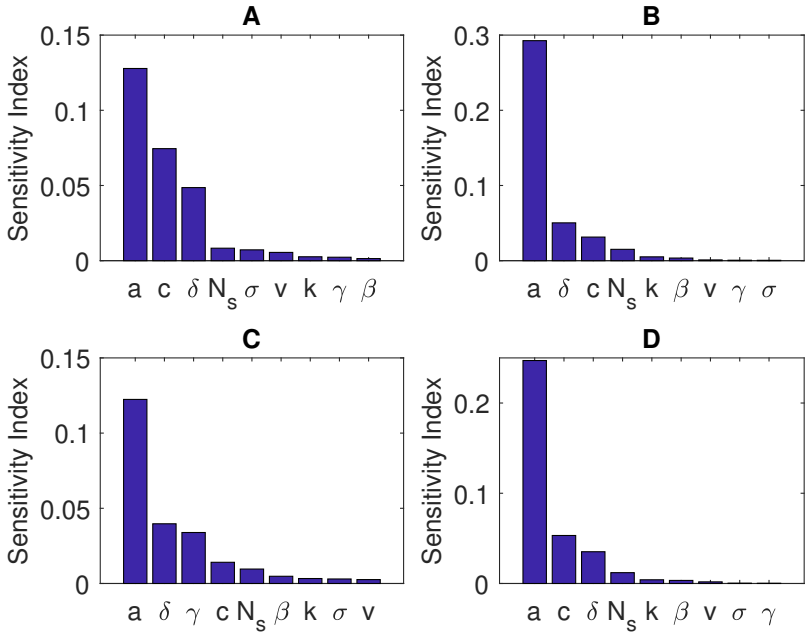


Figure 2.10: Sensitivity analysis for the model. In respect of A) Duration of infection. B) Peak of susceptible bacteria. C) Peak of resistant bacteria. D) Bacterial load. Index used was Sfast. Combination of 9 parameters(β , δ , a, γ , c, Ns, v, σ , k) with 200 iterations in an infection during 100 days. (Cannavó ,2012).

2.5 Simulations without treatment

2.5.1 Effects of bacterial traits on infection characteristics

For a clear picture of the outcomes of the infection using this model (equations 2.41-2.44) and the impact of the parameters on these values, we varied model simulations across parameters, when treatment is not applied (see Figure 2.11). This provides a general overview of the types of infections that this model can produce, and then evaluate their biological meaning. We verify that small changes in values of parameters produces the switch from an acute infection to a chronic one. This was key in defining the limits to study the acute and chronic infection more deeply.

We observe cases when there is a monotonic parameter effect on model outcome, such as extracellular bacteria death rate c, transmission rate β, mutation rate m, fitness cost γ and immune growth rate σ. However, there are parameters that do not show this monotonic effect. More specifically, both burst size and burst rate, as well as the intracellular bacteria death rate along their range of variation. They can increase infection severity and decrease it. This is a very interesting finding,pointing to the complexity of infection feedbacks mediated by these parameters.

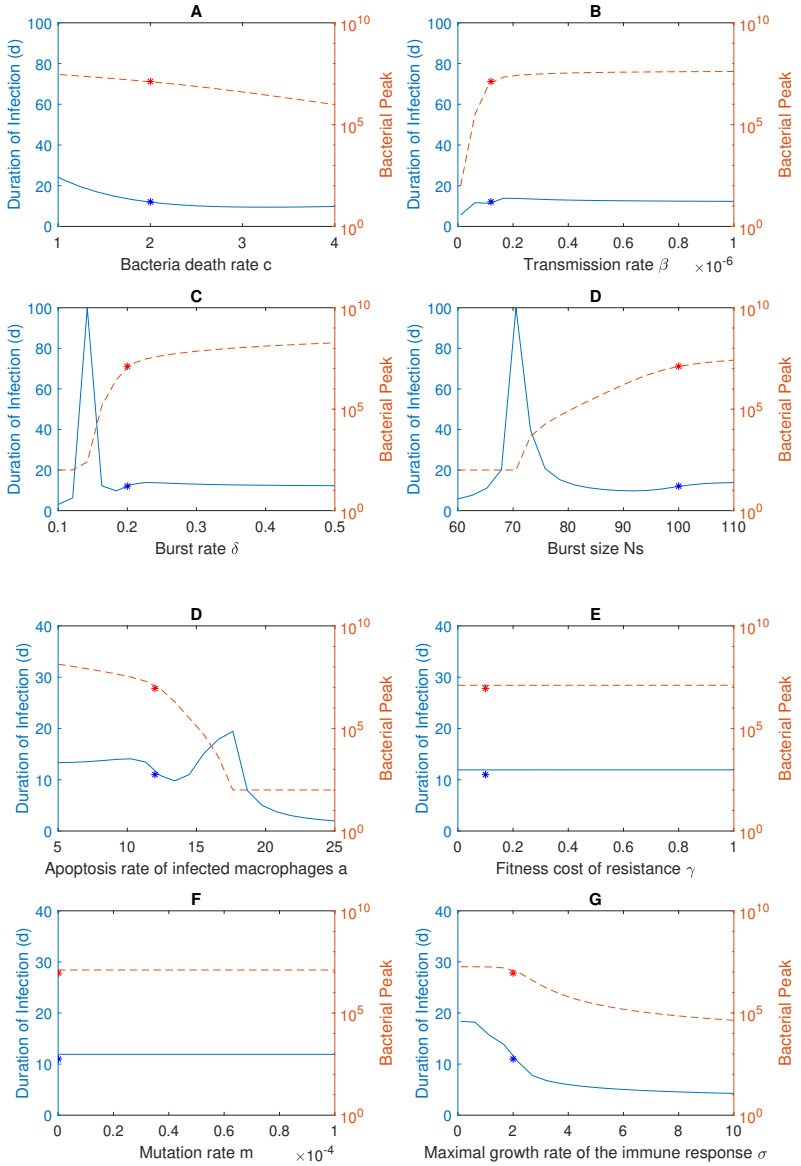


Figure 2.11: Infection outcomes dependent on one parameter. Duration of infection and Bacterial peak depending on the value of the parameter. The simulations ran over 100 days. Blue line characterizes the duration of the infection, while blue dot is the parameter combination default for acute disease (Table 2.1). Dashed red line describes the bacterial peak with the red dot representing the default parameters for acute infection.

We find regions of chronic infection in burst size N_s and burst rate δ parameters, with high peaks of the duration of infection. A possible explanation is that these infection profiles do not stimulate the immunity sufficiently. In those parameter values, bacteria population is low and does not grow sufficiently to activate the immune system. This results in a chronic infection that lasts up until the 100 days of simulation. We observe by the peak of bacteria that the infection does not grow from the initial conditions (100). The net growth rate of this type of bacteria will be approximately zero, therefore, they are not growing nor being cleared. These represent cases when the host will not feel any symptoms, although they will not clear

the infection. Transmission potential of these cases is considerably high, being also taken into account in the spread of infectious diseases. The fact that the behaviour of burst size and burst rate graphs are very close to one another is explained by the role they play in the model, since they both represent how the extracellular bacteria are generated. Mutation rate m and fitness cost γ of these mutations, in the range considered and under other parameter values assumed, do not shift the system into different outcome measures. Increasing the death rate of infected macrophages a and growth rate of the immune response σ, we observe a faster clearance with an inferior peak as expected.

Our simulations seem to indicate that burst size and burst rate must be optimal from the point of the view of the pathogen. If there is not enough burst to fuel the infection, the infection does not grow. However, if there is too much burst, there will be a quicker stimulation of immunity and/or depletion of the resources (macrophages), also leading to clearance of the infection. Therefore, there must be a balance between the infection of macrophages and the stimulation of immunity in order to have a growing and maximally prolonged infection.

2.6 Conclusions

The main processes responsible for the infection outcome derive from growth and death processes of bacteria within host, where the presence and absence of immunity is important. Competition between strains determines how much diversity can be sustained, and target cell limitation constrains the maximal growth potential of the microbial population. When there is no immunity present, the main processes that control bacteria are target cell limitation, intracellular proliferation and extracellular death rate. However, the presence of immunity adds a new negative feedback that is caused by the interaction between the pathogen and the immune action which increases the opportunity for stable clearance.

In the absence of immunity (E=0), extracellular population is higher than intracellular population for the range of parameters chosen and it is mainly defined by the death rates of both populations. In the absence of treatment, susceptible population will win competition over resistant population since the fitness cost is higher than the mutation rate. Otherwise, it would be the resistant bacteria dominating the infection, although it is a rare event.

We find that infection must satisfy critical parameter values in order to persist in the host. Only very specific parameter combinations make the bacteria balance its transmission potential and infection rates together with the death rates to create an optimal harmony that allow the bacteria to remain in the host. If there is too much proliferation it will kill the host too fast, if there is not enough it will not persist either. So an intermediate rate of growth is optimal to prolong the chances of survival within the host.

Ultimately, target cell limitation constrains the progression of the infection, and immunity is responsible for bringing the infection towards final clearance. The interplay between all these processes is expected to become even more complex under external intervention, such as drug treatment.

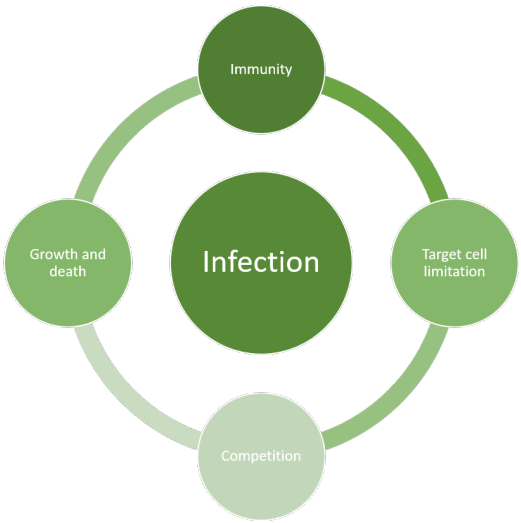


Figure 2.12: Main processes responsible for infection outcomes.

Chapter 3

Dynamics with treatment

3.1 Introduction

In the previous chapter, we explored the model without the presence of treatment. Also, we studied analytically the behaviour of the bacterial infections. This was important, in order to understand the equilibria and their critical conditions. Nonetheless, this behaviour does not reflect the short time scale dynamics of the infection, namely in the acute infection, where the infection grows and gets cleared. Looking into the transient dynamics up to a fixed stimulation time, we focus on the application of antimicrobial treatments to infection and its positive or negative consequences. This knowledge is useful in the design for the best possible treatment for bacterial infections.

After their discovery, antibiotics use in medicine and agriculture was followed by resistance emergence that led to higher risks for clinical care. This still happens nowadays. It is known that the lag time until drug-resistance evolution varies across different drugs and pathogens, resulting in a reduced effectiveness of each drug after this event occurs. Understanding and preventing resistance emergence through administration of treatment is urgently necessary. Generally, to slow the evolution of resistance, we want to reduce the global use of antibiotics to reduce the selective pressure. However, it must be balanced with treatment benefits, such as patient health and transmission potential. This creates a trade-off between treatment doses and duration between resistance potential and patient health. The traditional way to administer medication is to use it when the patient needs, applying antibiotics as aggressively as possible, using the highest dose until the pathogen is eliminated (Ehrlich, 1913). Nonetheless, there is now empirical evidence that suggests that reducing the dosage or the length of treat-

ment may slow the spread of resistance (Huijben et al., 2013) (Geli et al., 2012). These new insights suggest the drug therapy should aim to optimize clinical outcomes, but not necessarily to clear the infection. The role of immunity in infection clearance and resistance management is gaining more attention in recent years, and many studies highlight the need for a synergy between antibiotic treatment and host natural defenses (Gjini and Brito, 2016).

3.2 Antibiotic treatment in acute infections

Extrapolating to the reality, usually there is a treatment administered in any infection that causes severe damage to the host. This is preceded by a physician's appointment who evaluates the situation and proposes an adequate treatment. Thus, when the patient goes to the hospital with an acute bacterial infection, frequently it is applied a 7-day treatment with a fixed dosage. With this in mind, we want to study and evaluate the different treatments we can apply to patients, as well as infection dynamics that they produce.

We implement in the model the antimicrobial treatment acting on the extracellular bacterial population, with the parameter $A_m > 0$, antibiotic killing rate per day. The half-life of the bacteria, time that takes the bacteria population to become half the size, varies with the antibiotic killing rate administered. For example, when $A_m = 1$, the half-life of bacteria is 16.6 hours. For $A_m = 5$ it is 3.12 hours. Consequently, for $A_m = 10$, we have a reduction to 1.66 hours. In this calculation, it is neglected the effect of immunity and growth, so the real value will not be as linear.

Treatment acts only against susceptible bacteria, since resistant bacteria are assumed to have acquired full resistance against this drug (HLR). We assume that the application of the therapy is dependent on the extracellular bacteria present. When pathogen reaches the symptom threshold ($B_s + B_r = \Omega$) level, treatment begins. First, the duration of treatment is fixed with 7 day treatment duration. Then, we test the impact of other treatment durations with summary measures of infection. Dose is fixed throughout the treatment and it is set as a parameter also. Ω in this chapter varies between 10^3, 10^4, 10^5, 10^6 or peak ($\frac{dB}{dt} = 0$).

The ultimate goal is to clear faster, reduce pathology to the patient and to use ideally as little amount of drug as possible. The dynamics of the infection undergoing antimicrobial treatment are described in Figure 3.1. Effective treatment reduces the bacterial population and stops resistance emergence. Immunity is not stimulated and clearance is achieved within 8 days.

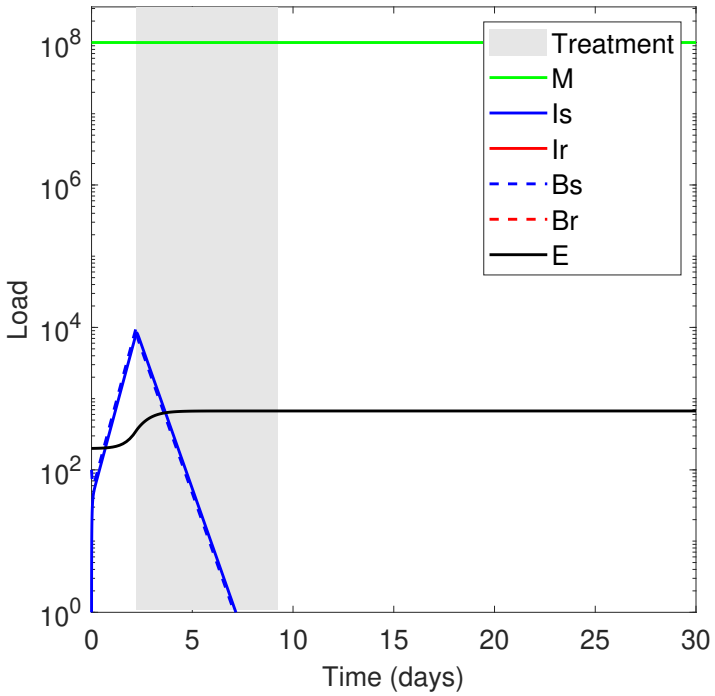


Figure 3.1: Dynamics of a successful 7 day treatment. Blue lines refer to susceptible bacteria populations. Red lines refer to resistant bacteria populations. Dashed lines representing the extracellular populations, while solid lines represent the intracellular populations (infected macrophages). Green line reflects the population of uninfected macrophages. Black line shows the growth of immunity. Grey patch represents when the treatment was applied. Treatment was applied when extracellular populations reached 10^4. Here, the antibiotic killing rate per day is 10 ($A_m = 10$).

3.3 Treatment dynamics with 7 days using multiple doses

Typically, when treatment is used in acute infections, stimulation of immunity either is completely prevented or it takes longer to happen. This may lead to the rise of a stronger population of resistant bacteria (both intracellular and extracellular) if the treatment is strong enough. After a few days, immunity gets stimulated and is responsible to produce clearance of infection of both populations. Possible relapse of the susceptible bacteria population can happen if the treatment is not efficient. There are cases, as shown in Figure 3.2, in which antibiotic treatment will lead to future relapses, prolonging the infection. There can still be relapses with resistance bacteria, depending on the antibiotic killing rate and symptom threshold.

Low dose of antibiotic killing rate produces more growing of bacteria, and, therefore, higher stimulation of immunity. Clearance happens during treatment essentially through immunity activity. On the other hand, a higher dose produces less growth of bacteria. Immunity will not have sufficient strength to clear the infection during treatment, which will cause a relapse of sensible bacteria. Relapse can be even bigger if we increase the dose, since immunity will be less

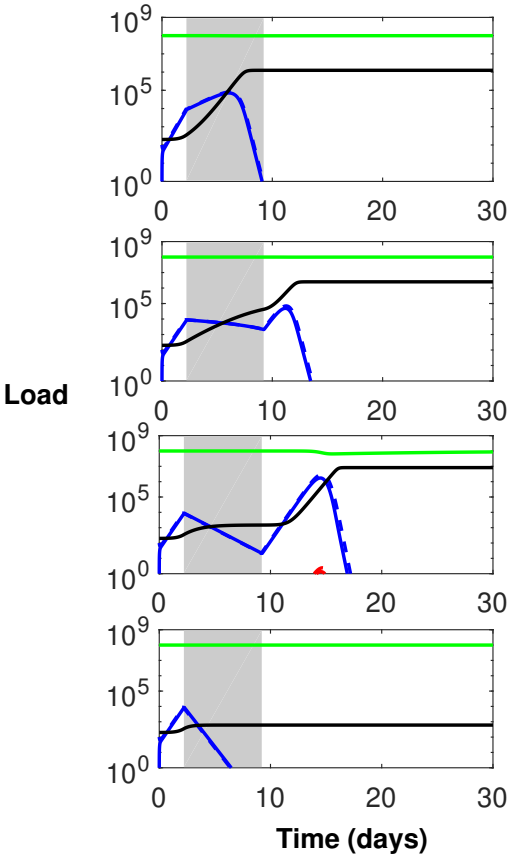


Figure 3.2: 7-day treatment dynamics with multiple antibiotic killing rates of an acute infection over 30 days. Antibiotic dose is growing from top to bottom, from 4 to 20 ($[A = 4, B = 8, C = 12, D = 20]$). Blue lines refer to susceptible bacteria populations. Red lines refer to resistant bacteria populations. Dashed lines representing the extracellular populations, while solid lines represent the intracellular populations (infected macrophages). Green line reflects the population of uninfected macrophages. Black line shows the growth of immunity. Grey patch represents when the treatment was applied. Treatment was applied when extracellular populations reached $1000(\Omega = 10^3)$. Parameters of simulations are described in Table 2.1.

and less stimulated during treatment. This phenomenon has been investigated in other studies(Stromberg and Antia, 2011). The highest dose will be efficient, since clearance is achieved during treatment through the application of antibiotic, although with the risk of toxicity to the patient.

3.4 Comparison of the treatment dynamics in 3, 7 and 14 days duration therapies

Alternative therapies to bacterial infections are starting being debated by scientists and physicians together. They consist of different combinations of durations and antimicrobial concentrations (Onakpoya et al., 2018) (Dawson-Hahn et al., 2017). Considering this, we explored

more thoroughly a 3 day treatment duration representing small duration therapies and 14 day treatment duration, which represents long duration therapies options. In light of this reality, we observe the dynamics of the distinct therapy options. We take into account real biological doses between sub-inhibitory and supra-inhibitory for bacterial populations and we assess the diverse outcomes they generate (Figure 3.3). To achieve bacterial decline, smaller doses are needed if we start the treatment later. This happens due to the contribution of immune response that actively fights the infection together with the antibiotic. When the treatment onset is later, there is more immunity built in the host. Therefore, the joint action of the antibiotic and immunity is stronger.

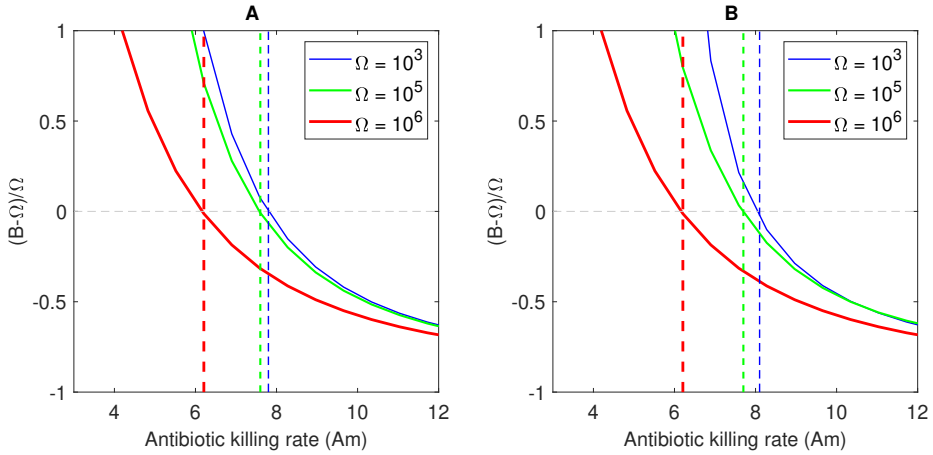


Figure 3.3: Minimum inhibitory concentration dose for the infection comparing pre and post-treatment bacterial levels.B refers to the instantaneous bacteria when the treatment ends. A) For a 3 day treatment duration. B) For a 7 day treatment duration. In A), the minimum inhibitory killing rate for Ω = 10e3 is 7.8; for Ω = 10e5 it is 7.6 and for Ω = 10e6 it is 6.2. In B), the minimum inhibitory killing rate for Ω = 10e3 the minimum inhibitory killing rate is 8.1; for Ω = 10e5 it is 7.7 and for Ω = 10e6 it is 6.2.

Thus, we observe distinct outcomes for each combination of antibiotic killing rates and duration. Three-day treatment duration produce worse consequences when higher antibiotic killing rates are applied (Figure 3.4). Moving to a longer treatment duration, such as 14 days, we see that low doses will benefit the host in reducing the infection duration and peak. However, antibiotic concentration administered is very high considering the other therapy options and it can be still administered during the clearance phase, possibly affecting the host microbiota. In moderate to higher killing rates across treatment durations, we find regimes of doses that will exhibit relapses. In longer therapies, and at later stage, we observe fully resistant relapses, leading to infections that are harder to clear via antibiotics. Three-day treatment duration selects considerably less resistant bacteria.

To summarize the different combinations of therapy outcomes and to quantify the differences of treatment outcomes also according to the infection profile, we calculated the duration

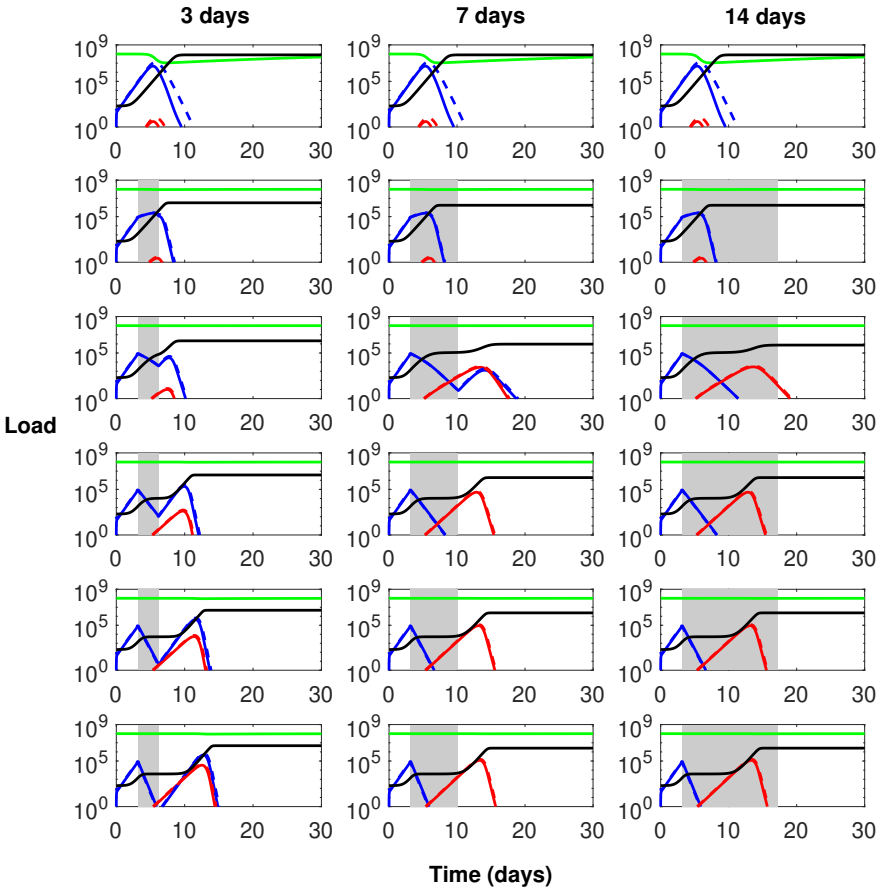


Figure 3.4: Treatment dynamics of an acute infection over 30 days for different combinations of antibiotic killing rates and duration treatments. First column is a 3-day treatment. Second column is a 7-day treatment. Third column is a 14-day treatment. Antibiotic killing rate is growing from top to bottom, $A_m \in [0, 20]$ with 6 discrete values (0,4,8,12,16,20). Top row refers to a no-treatment situation. Blue lines refer to susceptible bacteria populations. Red lines refer to resistant bacteria populations. Dashed lines representing the extracellular populations, while solid lines represent the intracellular populations (infected macrophages). Green line reflects the population of uninfected macrophages. Black line shows the growth of immunity. Grey patch represents when the treatment was applied. Treatment was applied when extracellular populations reached 100000 ($\Omega = 10^5$). Parameters of simulations are described in Table 2.1.

of infection for each combination of dose, duration and type of infection (e.g. intracellular death rate parameter value, a). We used this parameter because it is the main driver, based on the sensitivity results for duration of infection (section 2.4). After this, we compare infection duration to a no-treatment situation. We observe an identical infection duration for 7 day and 14 day treatment (Figure 3.5). On the contrary, there is a distinct pattern for the small duration treatment. There is more improvement using higher durations.

We find regimes that produce better results for the 3 distinct therapies. Aggressive dosage and low doses benefit the host. Moderate doses produce relapses and prolong the infection. Taking into account the fact that the parameter value used for acute infection of

intracellular death rate is 12, we actually find a similar pattern in the 3 different therapies. Sub-inhibitory doses will reduce the duration, as well as aggressive doses.

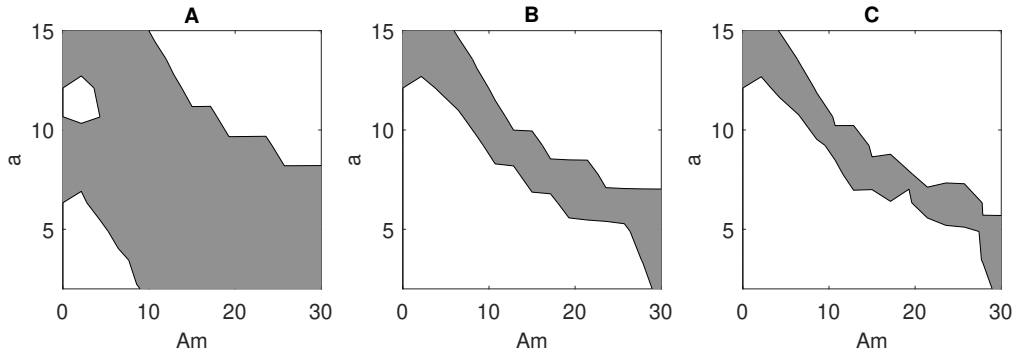


Figure 3.5: Relative duration of acute infections for different treatment-infection parameter combinations over 30 days. A) 3 day treatment. B) 7 day treatment. C) 14 day treatment. Grey illustrates the area where the treatment prolongs the infection when compared with the no treatment case ($A_m = 0$). White zones are the cases when treatment shortens infection duration. $\Omega = 10^3$.

3.4.1 Treatment combinations of doses and duration

In light of these findings, we want to focus on regimes that aggravate the infection and regimes that offer benefits to the human host. Hence, we combine possible doses and durations for acute infection therapies (Figure 3.6). We detect fast clearance in combinations of high doses and duration. On the other hand, using intermediate and inhibitory antibiotic killing rates (Am = [5,10]) and long treatment durations, we notice longer infection periods, due to lack of immunity stimulation at the end of treatment. This will cause a possible relapse.

Immunity plays an effective role in the infection dynamics, being a key element regarding clearance. Therefore, stimulation of immunity must be correctly balanced with administration of different antibiotic doses and duration. In case this fails, there will be possible relapses with susceptible or resistant bacteria, affecting the host's health and contributing for the generation and proliferation of resistance strains in hospitals or cities.

We notice that there are different treatments that achieve the same outcome regarding duration of infection. That is the case of high doses and short duration, and low doses and higher duration combinations. There is several alternatives to achieve the same duration of infection.

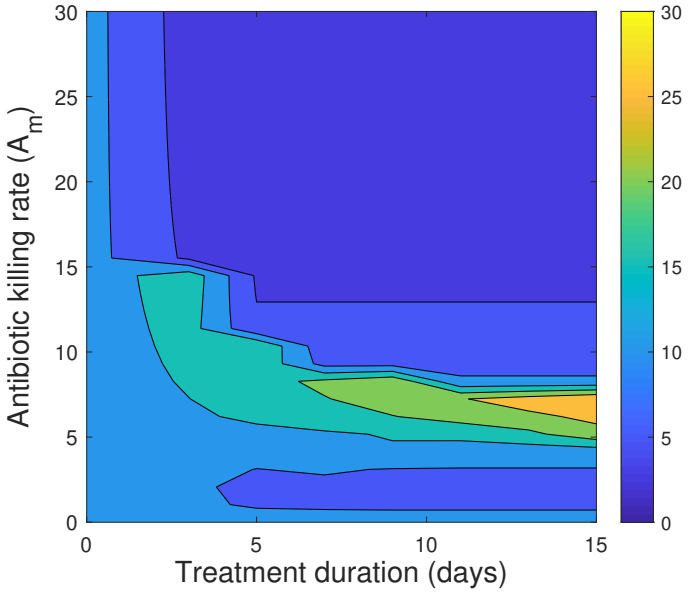


Figure 3.6: Map of the duration of infection produced by antibiotic treatment. Duration of an acute infection with different combinations of antibiotic dose and treatment duration over 30 days observation. $\Omega = 10^4$. Parameters as in Table 2.1. The worst case is when A_m = Minimum inhibitory killing rate (Figure 3.3).

3.4.2 Role of symptom threshold

Until now, we used mainly different symptom threshold for the administration of treatment. This difference in Ω makes the figures in the previous section not comparable. When the patient goes to the hospital, we can have multiple stages of infection. The in-host bacteria load can be low, intermediate or high, depending on each person's symptoms and inflammation. With this in mind, we apply the same combinations of dose and duration of the Figure 3.6 for different Ω (10^3, 10^5, peak) and we calculate the duration of infection (3.7A) as well as resistance burden (3.7B), which is the total number of resistant bacteria present throughout the infection, described as:

$$Br_{tot} = \int_0^{T_{ext}} B_r(t)dt.$$

We find that if we apply the treatment later ($\Omega = peak$) the outcomes are more predictable. Figure 3.7 shows less variation using this range of doses and durations. Additionally, when we administer very early, we produce less resistance bacteria and, on average, reduced duration of the infection. On the contrary, intermediate symptom thresholds ($\Omega = 10^5$) produce higher infection periods and higher resistance burden. This is provoked by the lack of synergy between antibiotics and immunity. In these cases, the antibiotic benefits the resistance strains in the competition with the susceptible strain. Treatment does not affect the resistant bacteria, leading

to relapses. This produces a longer infection period and higher resistance burden, by selecting this strain in the competition between these two populations.

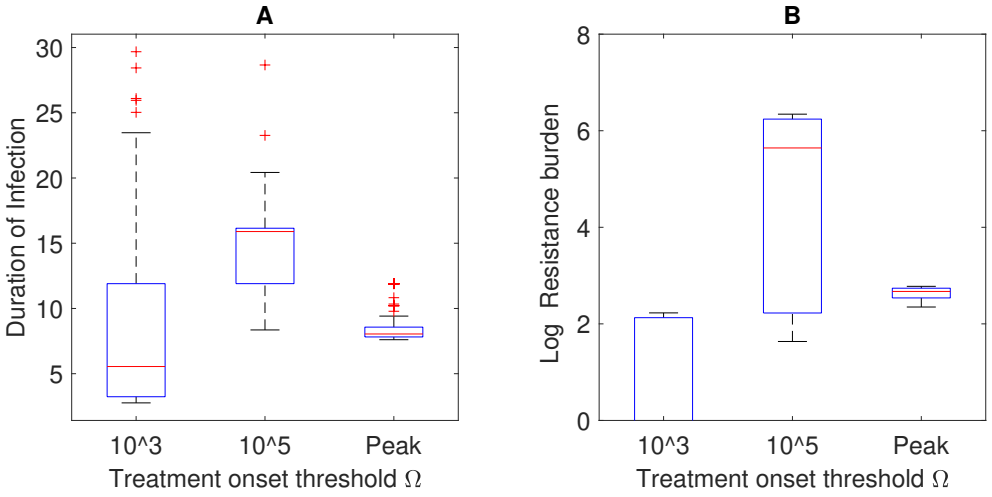


Figure 3.7: Comparison of treatments applied in different symptom thresholds. Treatment onset threshold affects infection outcomes across [A_m, duration] combinations. $A_m \in [1, 30]$. $Dur \in [1, 15]$. Peak is consider when $\frac{dB}{dt} = 0$ Applying treatment at higher pathogen loads is more predictive of the infection outcomes, when compared to lower loads. Maximum resistance is selected in medium treatment onset thresholds, since immunity is not always stimulated.

3.5 Conclusion

Intervention is essential to clear the infection faster, reducing the bacterial load and resistance. Antibiotic treatment provides effective clearance of the infection if well administered. However, treatment can also prolong the infection and lead to relapses.

Different combinations of therapies lead to similar infection outcomes. For example, high antibiotic killing rates combined with low duration provides the same duration of infection as a long duration and low antibiotic killing rate. Different strategies can be used to reach the same outcome. In addition, the same therapy, given at different points of infection (Ω), can give very different outcomes. Prediction of outcomes by the administration of treatment is highly complex.

To achieve clearance, the key aspect is to maintain balance between the antibiotic administered and stimulation of immunity. The treatment must provide effective depletion of bacteria still leaving room for immunity to grow, preventing future relapses. Combined with the previous study, intracellular and extracellular compartments gain an extra role in managing and controlling infections by being the targets of both antibiotic and immune stimulation.

In the next chapter, we will focus specifically on the comparison between short and long duration treatment and take an in-depth look in many aspects of infection outcomes.

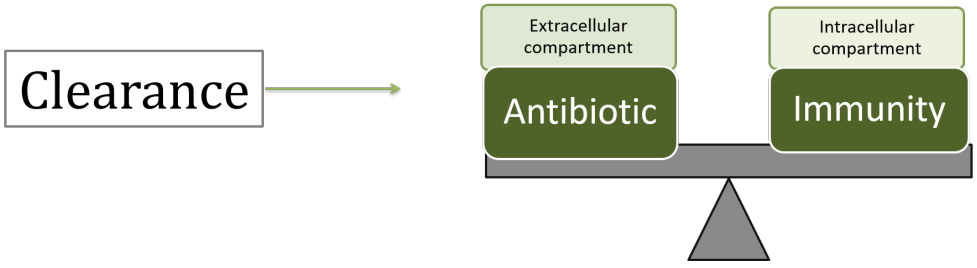


Figure 3.8: How to achieve clearance. Collaboration of antibiotic treatment and stimulation of immunity is crucial to fight the infection and prevent it from relapsing.

Chapter 4

Comparing short- and long-duration treatment

In general, antibiotic treatment regimens consist primarily of two variables: the dose and the duration of treatment. The traditional treatment regimens usually consist of a fixed dose administered for a specified duration. Optimization studies are used to determine the dose and duration that produce the best infection resolution. However, one limitation of this approach is that it depends on the infection criteria used. Nowadays, traditional treatment regimens may be effective although they are not the optimal duration or dose combination for most of the patients. Also, alternative therapies such as adaptive treatment have been explored (Gjini and Brito, 2016) providing new insights on managing resistance and optimization of treatment.

Misuse of antimicrobial drugs, as well as its overuse are linked to numerous consequences such as toxicity, selection of resistant bacteria, patient compliance and even financial costs for hospitals. These issues become more pertinent if we take into account the fact that there is a high prevalence of antibiotic prescription in hospitals that can reach up to 50% of all hospitalized patients. Thus, reducing antimicrobial drugs prescription while obtaining optimal results is the main goal of clinicians.

Given the pressure to reduce all these consequences of overuse of antimicrobial drugs, short-course therapy for many infection diseases is very tempting. There all several studies comparing single dose therapy to standard therapy, as well as short-course treatments to long or traditional ones. All show deeply mixed results, with the general trend not finding significant differences between treatments. For pneumonia (Furlan et al., 2018), bacterial sinusitis (Falagas et al., 2009), *Pseudomonas aeruginosa* lung infection in patients with cystic fibrosis

(CF) (Elborn et al., 2016), *Helicobacter pylori* infection (Usta et al., 2008), urinary tract infection (Berger, 2006), acute pyelonephritis (Onakpoya et al., 2018) (Dawson-Hahn et al., 2017) and *Escherichia coli* bloodstream infection (BSI) (Giannella et al., 2018) there is no significant differences between longer treatments and short treatments, taking into account relapses or mortality. These studies reinforce the need for further investigation on optimality and alternative therapies, mainly short-courses.

Here, we focus on the detailed comparison between two different therapies duration: 3-day treatment duration and 7-day treatment duration and we study different outcome measures represented by pathogen and resistance burden, duration of infection and damage to the host. We also want to answer questions on treatment optimality, for resistance, damage and duration whenever possible. For example, it is not clear which infection and health parameters should we optimize with treatment and if these can be optimized simultaneously (Figure 4.2).

With this in mind, we evaluate the system 7 days post-treatment (7 d.p.t.) closer to clinical reality and compare the efficacy of the short and long treatments considering instantaneous measures (Figure 4.1), cumulative measures and summary measures of the treated infection. This is different from previous chapters where our horizon of observation was 30 days. All parameters are assumed fixed at the values in Table 2.1, unless otherwise stated.

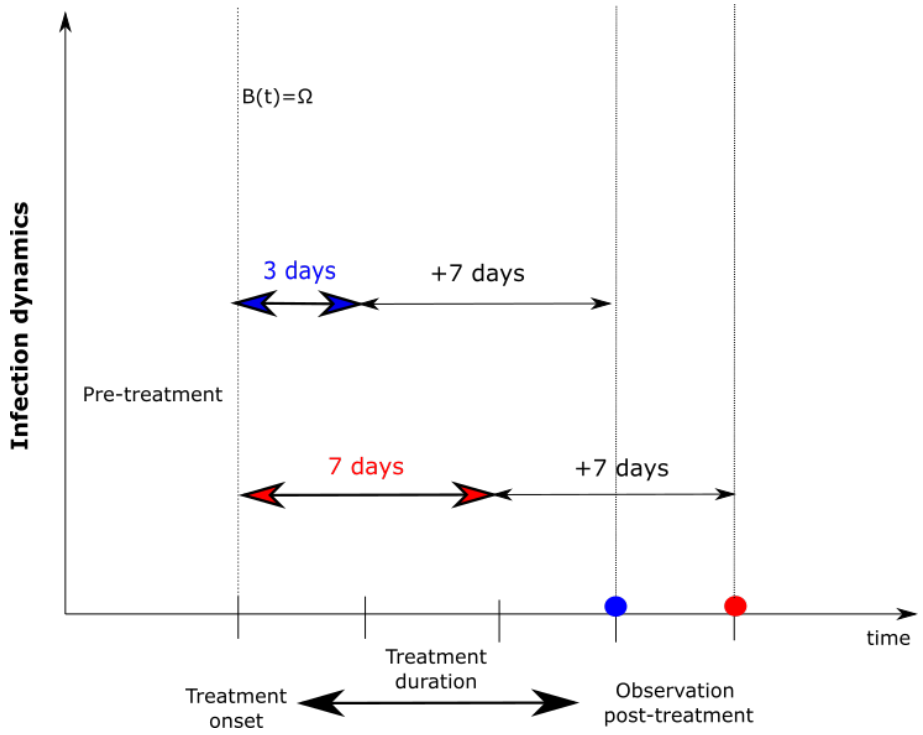


Figure 4.1: Diagram of the modelling framework in this chapter study. Description of the treatment administration and effectiveness evaluation adopted in this chapter to be closer to what happens in clinical trials.

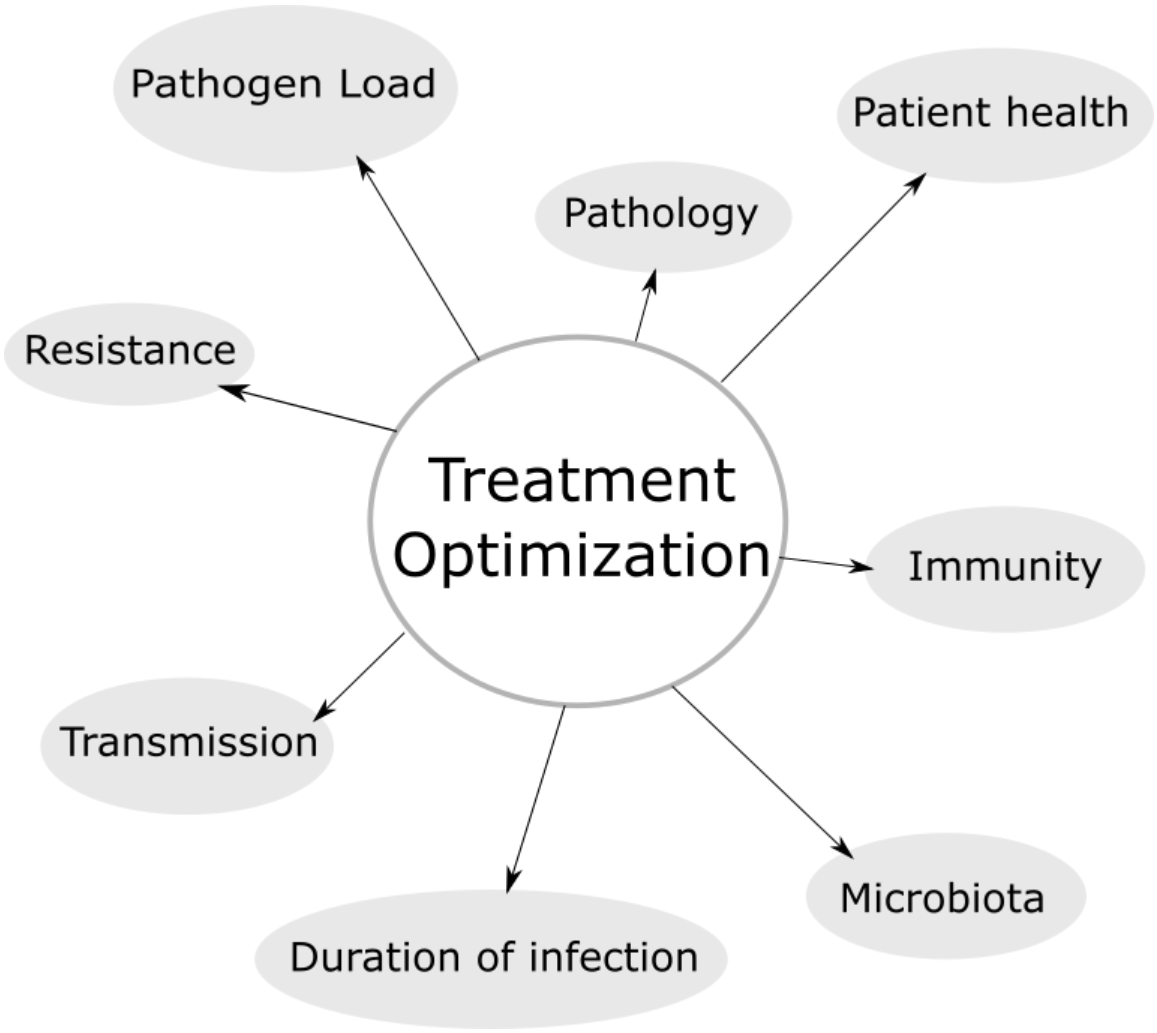


Figure 4.2: Possible treatment optimization measures.

4.1 Methods

4.1.1 Treatment measures and infection outcomes

Applying treatment to the infection, we are simulating data using different antibiotic killing rates that vary from 1 to 20 ($A_m \in [1, 20]$), comparing two types of treatment: 3-day duration and 7-day duration. We combine dose-duration values and we test over different symptom thresholds Ω. They intend to represent early treatment ($\Omega = 10^3$), intermediate treatment ($\Omega = 10^5$) and late treatment ($\Omega = 10^6$). During the infection process, we keep track of several instantaneous measures, such as pathogen density $B(t)$, resistant density $B_r(t)$ and pathology due to bacteria $H^B(t)$, immunity $H^E(t)$ and macrophages $H^M(t)$. Pathology represents the damage the host suffers from the infection. This measure represents one way to track the health of the patient.

$$H^B(t) = 1 - \left(\frac{B_{ext}}{B(t)}\right)^g, \qquad H^E(t) = 1 - \left(\frac{E_0}{E(t)}\right)^g, \qquad H^M(t) = 1 - \left(\frac{K}{M(t)}\right)^g, \tag{4.1}$$

where g is a sensitivity parameter, here chosen equal to 0.1, that scales the importance of the population for host health. The pathology is calculated from the deviation from 'homoeostasis' of these cell populations: extinction threshold for bacteria; initial level of immunity and carrying capacity for macrophages, which represent a healthy host. The instantaneous pathology measures usually follow the dynamics of population they track (see Figure 4.3 A). Both instantaneous and cumulative pathology caused by immunity increases with the spread of infection, since immunity is only growing. Treatment prevents damage to the host, by affecting directly the bacteria, and indirectly immunity and macrophages. There will be less immune stimulation and therefore less damage. Also, there is less consumption of macrophages, leading to reduced pathology (Figure 4.3 C). Initially, the damage caused by the infection is mainly due to the presence of bacteria. As the infection progresses, immunity grows and starts to damage the host. In the end, immune cells are still present on the host's body, causing some damage, while the bacteria are extinct. A successful treatment can reduce significantly the damage caused by both bacteria and immunity.

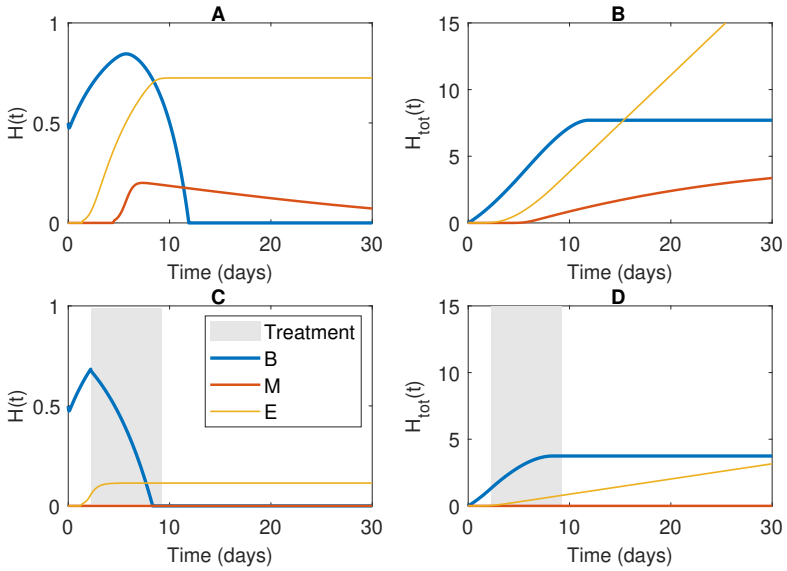


Figure 4.3: Pathology dynamics during an acute infection regarding bacteria (B), immunity (E) and macrophages (M) damage to the host. A) Instantaneous pathology without treatment. B) Cumulative pathology without treatment. C) Instantaneous pathology with treatment. D) Cumulative pathology with treatment. A) and B) refer to the dynamics described in Figure 2.2. C) and D) refer to the dynamics described in Figure 3.1.

We also model different infection summary measures, such as duration of infection,

pathogen burden and resistance burden which will capture the total infection history in terms of bacteria present from the beginning of the infection until the measure point T. Similarly, cumulative pathology is derived as an area under the curve from the equations of the instant pathology, given by the integrals:

$$H^B_{tot}(T) = \int_0^T H^B(t)dt, \qquad H^E_{tot}(T) = \int_0^T H^E(t)dt, \qquad H^M_{tot}(T) = \int_0^T H^M(t)dt. \tag{4.2}$$

4.2 3-day and 7-day mean treatment dynamics

In order to better understand the effect of duration of treatment in the infection dynamics, we calculated the mean dynamics for a range of A_m ([1,20]) by finding the mean of each population at each point of simulation. This intends to show a typical infection dynamic under treatment assuming that all antibiotic killing rates in this range are uniformly distributed in the population of treated patients.

The main qualitative differences in the dynamics of treated infection with long and short treatment are illustrated in Figure 4.4 and summary measure in Figure A.3. The mean infection dynamics with 3 and 7 days treatment duration shows a clear sign, throughout the different treatment onsets, that the 7-day duration treatment selects more resistance, increases the infection duration and stimulates less immunity. We can state, on average, that we can reduce the application of antibiotics in time (from 7 to 3 days), and still effectively clear the infection, without selecting resistance. The contribution of immunity in this case is essential.

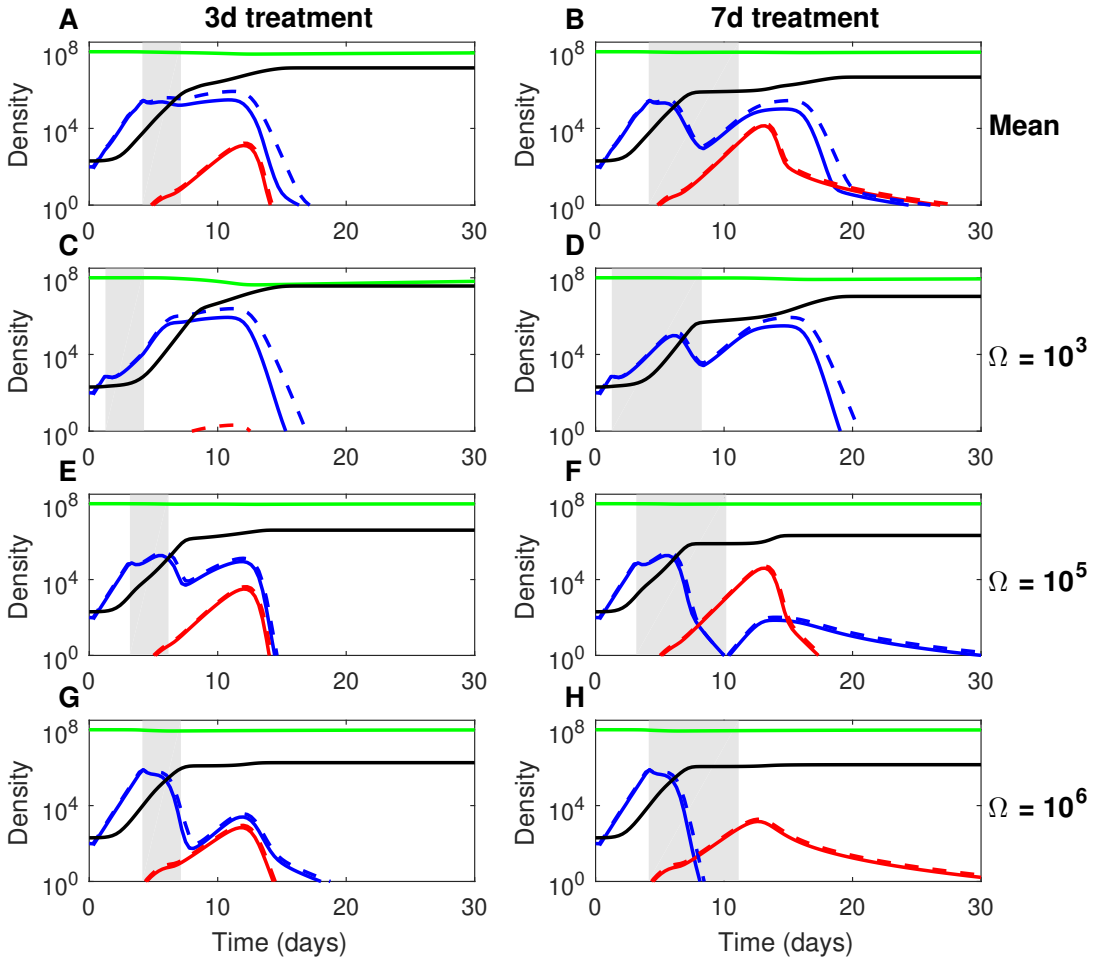


Figure 4.4: Typical infection dynamics under 3-day and 7-day treatment as a function of the symptom threshold Ω. The treatment onset and duration (denoted by the vertical gray lines) affects in major qualitative and quantitative ways the net dynamics obtained with antibiotic treatment. Here we illustrate the average over many simulations of treatment at doses in the range $A_m \in [1, 20]$. Equivalent to assuming that all these antibiotic killing rates are uniformly distributed in the population of treated patients. For quantitative comparison see Figure 4.6. For summary measures of these dynamics see Figure A.3.

4.3 Quantifying differences in treatment outcomes and the importance of treatment onset

We found previously that different treatment durations produce distinct treatment outcomes. In this section, we look to quantify the differences looking at pathology, duration of infection, resistance and pathogen burden and infection resolution.

Regarding pathology (Figure 4.5), for higher antibiotic killing rates, the pattern is constant, with 3-day duration presenting less pathology overall. For lower antibiotic killing rates,

there are regimes where 3-day treatment continues to be successful in reducing pathology. However there are also present situations where 7-day treatment works considerable better. Moreover, 3-day and 7-day treatment duration present different scenarios across different symptom thresholds. When we increase the time we administrate the antibiotic (the symptom threshold), we shift the system into a more predictable result, narrowing the outcomes. We end up observing that treatment options in late treatment will mostly cause an increase in the pathology when we consider the bacteriological measure. If we increase the symptom threshold to a very late state, treatment may even cause more pathology than no-treatment situation. In summary, low doses seem optimal regarding pathology overall for bacteria damage. However, if we apply the treatment in an early stage, high doses will be more effective in both treatment regimes. Also, 7-day treatment duration is frequently able to generate more pathology when compared to a no-treatment situation. This does not occur with 3-day treatment duration.

In terms of bacterial pathology, the reason 3-day treatment duration produces less damage than a longer period of treatment is that for shorter treatments low doses will decrease the growth but allow immunity to grow and high doses will effectively kill the infection fast. However, if we apply 7-day duration in the same conditions, with low doses we also decrease the growth but we select longer for resistance bacteria, leading to possible relapses. High doses will have the similar effect of 3 days, but with a cost of damage to the host's microbiota due to the administration of higher antibiotic total killing at the end of treatment.

We also compare other measures of infection that take into account the history of all infection from time 0 up to 30 days (Figure 4.6). We compare 3-day versus 7-day treatment in the dimensions of infection duration (Figure 4.6A), bacterial burden (Figure 4.6B) and resistance burden (Figure 4.6C) for different treatment onsets Ω. Each dot represents a distinct antibiotic killing rate used between 1 and 20.

It is possible to identify statistically differences between 3-day treatment duration and 7-day treatment duration. Overall, the test showed that 7-day duration leads to more favourable treatment outcomes. Applying a Mann-Whitney test to simulations in Figure 4.6A comparing medians of distributions across 3-day and 7-day outcomes revealed that infection duration is significantly lower for 3-day treatment when applied at intermediate Ω (p-value=4.7e-24), but when applied at early onset, 7-day treatment leads to lower infection duration (p-value=2.1e-6). The 3-day treatment led to significantly higher bacterial burden at low to intermediate Ω (p-value= 1.1e-19 for early, p-value=2.7e-42 for intermediate), described in Figure 4.6B. For resistance selection the Mann-Whitney test confirmed significant superiority of the 7-day treat-

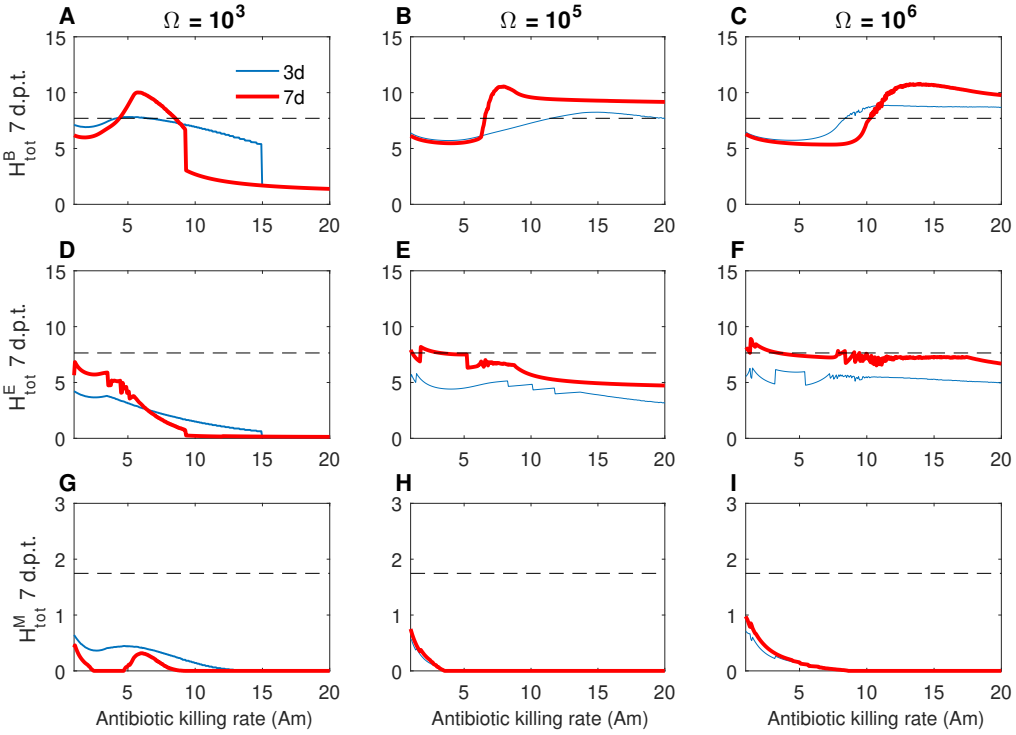


Figure 4.5: Cumulative pathology outcomes for different treatments, 7 days after the end of therapy. The dashed line represents the pathology in the case of no-treatment. Top row: pathology due to bacterial growth. Middle row: Pathology due to immune response activation. Bottom row: Pathology due to macrophage depletion. (A, D, G) $\Omega = 10^3$ and treatment starts at very low bacterial levels. (B, E, H) $\Omega = 10^5$ and treatment starts at higher bacterial levels. (C,F, I) $\Omega = 10^6$ and treatment is triggered at very high bacterial levels. $A_m = [1, 20]$.

ment for low Ω (early treatment) giving less resistance than 3-day duration (p-value=8.3e-29), while for intermediate treatment timing, the superiority was reversed, making 3-day treatment better (p-value=6.1e-12). However, if it is too late ($\Omega = 10^6$), the two treatments present similar distribution for all infection measures (Figure 4.6C).

Concerning early and intermediate applications, there are significant differences in superiority. The 3-day treatment produces lower results on infection duration and resistance burden when applied at intermediate stages of infection. On the other hand, the 7-day treatment duration has better outcomes for every measure when applied earlier and for pathogen load when applied at intermediate stages. In addition, 7-day treatment duration also produces better outcomes for the duration of the infection when applied at late stages when compared to 3-day treatment duration outcomes. There is an overall superiority of the longer treatment for this wide range of antibiotic killing rates.

After comparing cumulative infection summary measures, we also consider instantaneous measure for comparing 3-day and 7-day treatment, namely the bacteriologic outcome at

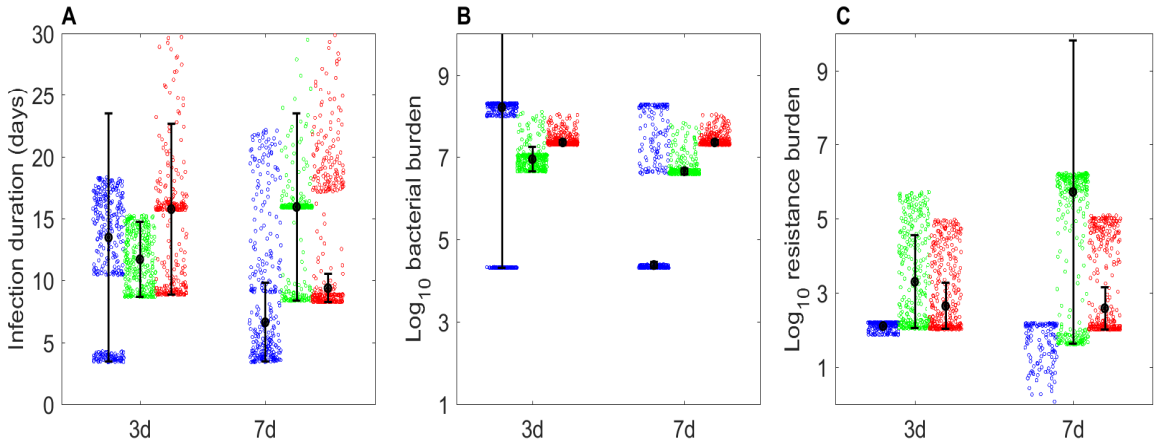


Figure 4.6: Summary outcomes of infection compared for 3-day and 7-day treatment at different Ω. A) Infection duration B) Total bacterial burden C) Total resistance burden. Each dot represent a different dose from $A_m \in [1, 20]$, colored by symptom threshold (blue, green, red: $\Omega = 10^3$, 10^5, 10^6) for two distinct treatment durations (3 days and 7 days).

7 days post treatment (Figure 4.7). We check the bacterial load present, 7 days after the treatment ends. We designate "infection resolution" the situation when the bacteria does not exceed the initial inoculum. Otherwise, we say treatment has failed.

Considering the bacterial load 7 days post-treatment, low antibiotic killing rates always work for both cases (3- and 7-day treatment duration) for all symptom thresholds. When we move to intermediate A_m, there are regimes where it actually favours 7 days and where it favours 3 days. This is a blurred area, although the outcomes can be similar. This supports the idea of reducing the treatment duration, since we are considering 4 days before in absolute terms as described in Figure 4.1. High doses will work effectively in both cases when the treatment onset is reduced. When we increase the time of application of treatment, high doses won't have the same outcome as before. They will mostly select resistance in 3 days and 7 days.

For early treatment, there is 47% clearance with 3-day treatment duration, and 83% with 7-day treatment duration. Moving to intermediate Ω, there is a shift for both treatments. Low treatment duration achieves a 77% clearance rate, and long treatment duration achieves 93%. When we apply the treatment at a late stage of infection (high Ω), 3-day treatment duration produces almost the same clearance rate as before with 70% and long treatment clears 87%. We should emphasize that not all treatment failures defined by instantaneous presence of bacteria are equivalent, some correspond to a situation that is getting better over time, infection on the way to clearance, some correspond to a worsening situation, infection tending towards a relapse. Once again, 7-day treatment duration shows clear advantage overall. Nonetheless, there are regimes that 3 day treatment duration is better or leads to the same outcome. These

are the cases of low doses to intermediate ones.

It is hard to optimize a treatment that is superior for all infection outcomes. We must be careful in what infection outcomes we consider relevant and we have to start defining priorities on what we should minimize.

4.4 The case of non-independence of dose and duration of treatment

Until this point, we have always considered the same antibiotic killing rate for the different treatment duration. This will cause overall less antibiotic administered in the short treatments when compared to the longer treatments. In this section, we study an alternative case: preserving the same intensity of antibiotic treatment when comparing long and short duration. This means that the same total antibiotic will be administered throughout the treatments, whether is 3 or 7 days only that in 3-day treatment the per-day killing rate will be higher and 7-day treatment relatively lower. We compare the short treatment against long treatment, but now with this constraint.

$$Dose \quad x \quad Duration = constant(intensity) \tag{4.3}$$

This constraint follows some comparative treatments in clinical trials, when higher doses are applied in short treatments and lower doses in long treatments, to keep the total amount of killing similar (Huttner et al., 2018). We analysed the same infection characteristics and we produce the same figures, displayed in Appendix A. The antibiotic killing doses adopted for the long treatment (7 days) are $A_m \in [0, 12]$. For the short treatment(3 days), it was adopted $A_m \in [0, 28]$.

The comparison between the two scenarios show several differences. Regarding the mean dynamics (Figure A.4), 7-day treatment duration produce similar infection dynamics, when keeping the intensity fixed. However, 3-day treatment duration display distinct features. It selects more resistance, and immunity is less stimulated overall. When considering early application of treatment, we obtained better results. Short treatment produces a smaller peak of susceptible bacteria. Nevertheless, if we consider the later applications of treatment, we find worse scenarios for the mean intensity dynamics. Resistance is selected, reflected in an higher peak of resistant bacteria.

Moving to pathology (Figure A.5), we detect that for the same intensity, pathology produced by macrophages and immunity is higher for the longer treatment overall. This contrasts with the pathology produced by the pathogen. In this case, we find three different scenarios depending on the treatment onset. For early applications, long treatment is superior for low intensities. For intermediate to higher intensities, shorter treatment is better, demonstrating exceptional results in reducing pathology. For intermediate onsets, half of the intensities (low to intermediate) show superiority of long treatment. The other half benefits shorter treatments. At the end, late treatment onsets produce higher pathology in shorter treatments. In this case, longer treatments wins overall. We observe that, although there are regimes when longer treatment has an advantage, there are others which cause substantial damage to the host, even higher than the no-treatment case display by the dotted line.

Relative to the outcome measures (Figure A.6), 7-day treatment duration produces better results when comparing the means of each measure. However, if we look at the worst possible cases, they are also produced by longer treatments. On intermediate and late onsets, it is more beneficial if there is a longer treatment application. On early onsets there are not significant differences between treatments. We reinforce the idea that despite 7 day treatment duration displays better results for the means, they are also responsible for the worst cases (higher duration of infection and pathogen burden). This has an impact on the physician's choice.

Finally, infection resolution 7 days post treatment change considerably. There are meaningful changes between the dependence or independence of dose and duration combination. When considering low treatment onsets, if we fix the total amount of drug administered, we have similar percentage for infection resolution for both treatments. On later treatment onsets, we see a considerable change, increasing the success of longer treatment for almost the double. On the other hand, if we administer the same antibiotic killing rate with different durations, we see the opposite pattern. Low treatment onsets show a clear distance between success of treatment, while later treatment onsets bring balance to both therapy values of infection resolution (Figure 4.7).

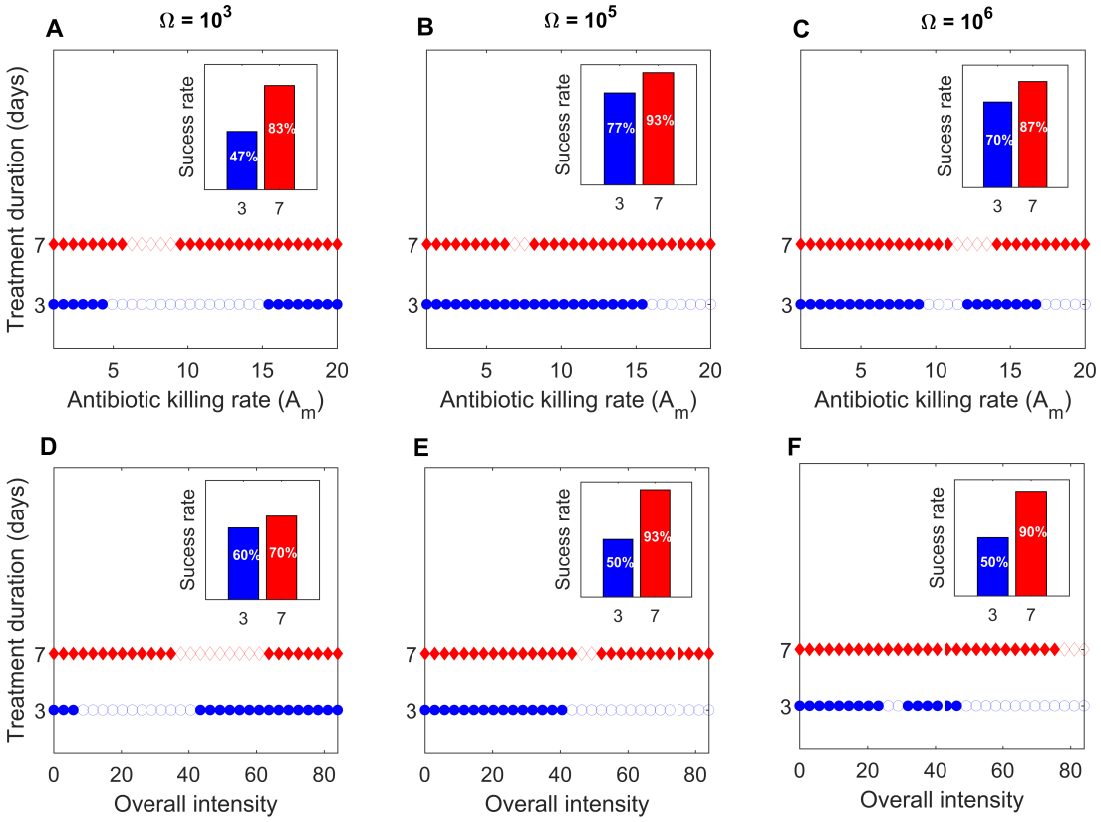


Figure 4.7: Infection resolution (instantaneous measure) at 7 days post-treatment for 3-day and 7-day duration at different Ω and treatment criteria. A) and D) $\Omega = 10^3$, B) and E) 10^5, C)and F) 10^6. Diamonds and red: 7-day treatment outcome, circles and blue: 3-day treatment outcome. Empty symbols: Bacteria population above the initial inoculum ($B(t) > B(0)$) at 7-day post treatment. Filled symbols: Bacteria population below the initial inoculum ($B(t) > B(0)$) at 7-day post treatment. Proportion of treatment successes across all the dose range for the three Ω values are described on bar plots, where treatment success is defined as $B(t) < B_0$ at observation point post-treatment. A), B) and C) x axis define the antibiotic killing rate used as the D), E) and F) maintain the total intensity constant on both treatments (A_m x Dur).

Infection resolution is, therefore, dependent on the criteria we define, and the infection outcome that we measure (see diagram in Figure 4.1). Scientists together with physicians struggle to find the optimal treatment for a patient. This fact can be explained by the difficulty on not only defining a reliable criteria but also, as we previously saw, to optimize for multiple infection outcomes with the same treatment. New challenges on optimization of treatment or personalized medicine emerge and models may provide answers to the current questions.

4.5 The challenge of finding an optimal treatment

Now, starting with the basic question: is it possible to find an optimal treatment for a given host? Until now we restricted to only 3- and 7-day duration. In this section we allow for any combination of dose and duration to be used and compare infection outcomes. However, searching for an optimal dose-duration combination is a challenging goal. The optimal treatment will differ from host to host and what infection criteria we use. It depends also on treatment timing: for our default parameter combinations, it seems that short and more aggressive treatment is the best when applied early in infection, but later in infection, long and milder treatment is preferable (Figure 4.7). However, for infection burden minimization, the best treatment does not depend on timing, it's usually short and aggressive. For resistance minimization, if treatment is applied late, the best regime would be short duration and low dose, while if treatment is applied earlier, the duration should be higher. In summary, treatment timing together with the infection criteria we want to optimize are two extremely important parameters to take into account when we choose the treatment not only for population level(Uecker and Bonhoeffer, 2018) but also for the within-host level.

Following this idea, we examine different infection criteria for the optimal treatment given 2 different hosts: one with stronger immune activation($k = 10^3$) and other with lower immune activation ($k = 10^4$). The infection criteria were duration of infection, bacterial burden, resistance burden and pathology. The optimal treatment for each case will be the therapy that minimizes the infection criteria and the one that involves the least use of antibiotic administration to the patient (minimum intensity). Adding to this, we separate the time of treatment (Ω) by early(10^3) and late (10^6) and we find distinct optimal treatments.

We find no single optimal treatment for all the criteria (Figure 4.8). Also, we do not observe the same optimal treatments for different treatment onsets. There is always a different optimal treatment if the timing of administration changes relative to infection course. There is a general pattern where more developed infections tend to shift the optimal treatment into a longer duration and reduced antibiotic killing rate, even reducing the overall used antibiotic (intensity).

To find the optimal treatment even for a given host becomes a difficult task. Often we are not able to optimize simultaneously for different infection outcome features. In this case, we may follow the application of a milder and longer treatment for patients with developed infections and more aggressive and shorter treatments for patients with recent infections.

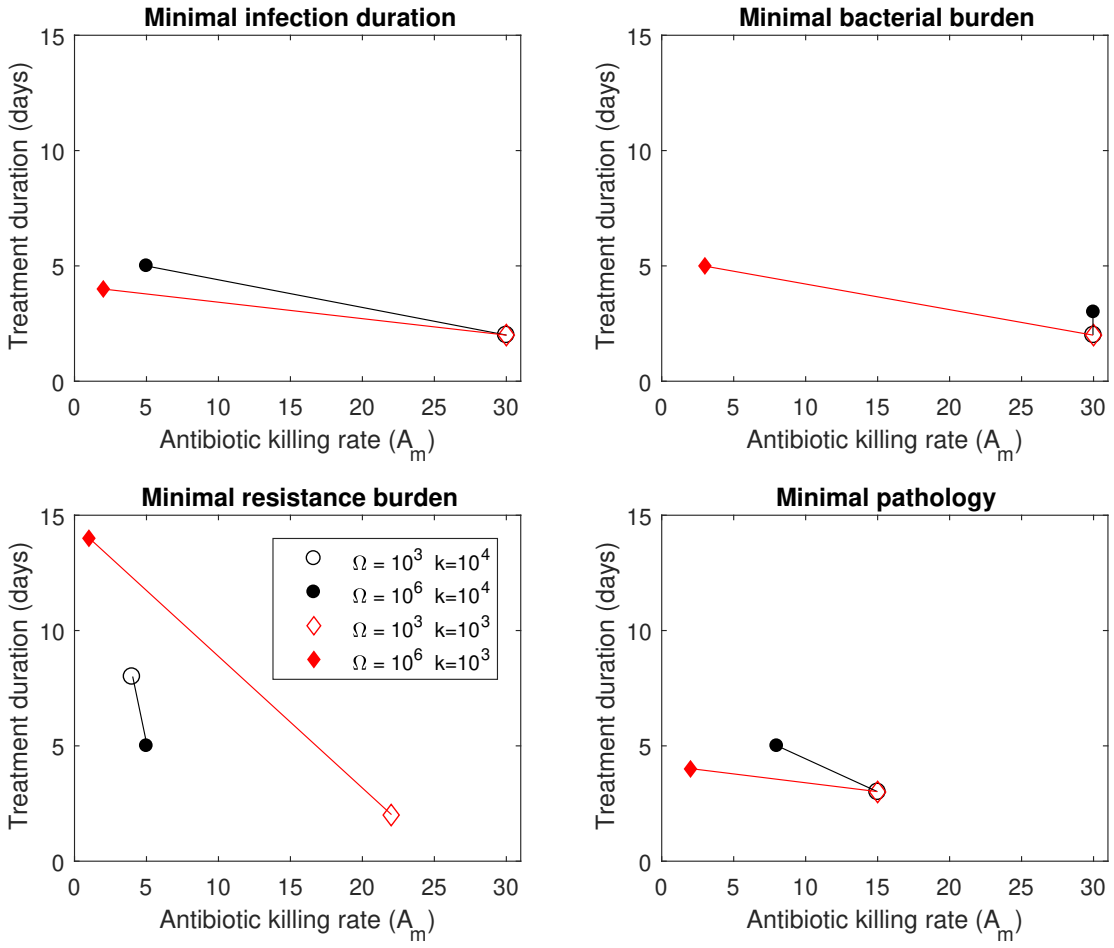


Figure 4.8: Optimal treatment for different parameter combination. For 2 different hosts: one with stronger immune activation($k = 10^3$, red) and other with lower immune activation ($k = 10^4$, black). Infection outcome criteria: A)Duration of infection, B)Bacterial burden, C)Resistance burden and D)Pathology. The optimal treatment minimizes the infection criteria and the total amount of the drug. Non-filled symbols refer to early onsets ($\Omega = 10^3$)) and filled symbols refer to late onsets ($\Omega = 10^6$)).

4.6 Conclusions

The optimal treatment is never easy to find. It depends on the symptom threshold (Ω), treatment duration and antibiotic killing rate per day. Also, it depends on the host and infection profile. We focused specifically on Ω dependence since it controls bacteria, immunity and resistance levels at the beginning of treatment. Different patients will receive treatment at different points of the infection. Depending on the conditions, we find through simulations that optimal treatment varies.

Overall, 7-day treatment duration is more efficient than 3-day. However, we find many cases when 3-day treatment is non-inferior and even superior to 7-day treatment. Therefore, there is room for the 3-day treatment to play a role in optimizing the treatments for acute infections. Aggressive treatment (high doses) can work effectively when applied in early stages of the infection. In middle stages of the infection, high doses may not work, and the solution may be low to moderate doses. We observed that 3-day duration treatment has significant lower infection resolution in low treatment onsets when compared to the 7-day duration treatment. However, when we increase the treatment onset, we raise the infection resolution in both cases, producing similar success in intermediate to late treatment onsets.

When fixing the total amount of drug used, overall we find significant new results that favour again 7-day treatment duration. Although, 3-day treatment duration continues to be viable for early applications of treatment. Aggressive intensities still work effectively when applied in early stages of infection, losing its effectiveness when used at later stages of infection. Infection resolution is higher for 3-day treatment duration when we keep the intensity fixed on early onsets. These data reinforce the idea of viable alternative therapies, namely, shorter treatment durations.

Optimality of treatment is constrained to the criteria we choose. In addition, timing of treatment onset is crucial. Thus, to find the best possible treatment for a given host is nowadays a challenging task.

Chapter 5

Discussion

Mathematical models help us understand natural phenomena and to predict them. We do have to keep in our mind that the model is a simplification of the reality, but it gives us useful insights on the mechanisms leading to a specific phenomenon and can guide us to put optimization principles in practice.

In this work, we explored intracellular infection using a mathematical model with particular features such as the existence of two compartments (intracellularly and extracellularly) and presence of adaptive immunity. We observe that infection outcome is very sensitive to the presence or absence of immunity. In immunodeficient patients, the infection depends on the target cell limitation, together with the competition between strains and it is very likely to persist. On the other hand, when immunity is active, the infection is cleared, and can only persist if the window of activation of immunity is limited, or its final level is sub-optimal. In acute infection scenarios, the peak and duration of infection are subject to balance between pathogen growth and stimulation of immunity. There will be critical infection rate for each infection persisting longer in the host.

In order to fight the bacterial infection and limit the pathology related to it, intervention in the form of antimicrobial treatment is essential. To clear the infection and reduce the host's damage from the bacteria are the main goals of current therapies, aiming for the use of the least amount of antibiotic that can be effective. We can achieve that, if the treatment is well administered, or we can suffer from a prolonged infection with possible resistance relapses.

Nowadays, there are recommended prescriptions for bacterial infections, namely 7-day treatment duration for acute infections, supported by several studies. However, recent clinical trials show that we can achieve the same result while applying a shorter course antibiotic therapy.

In chapter 3, we show that different combinations of therapies (dose-duration) may lead to similar infection outcomes. We can use these strategies to select treatment for different hosts with specific infections. We have to look for maximum stimulation of immunity even with the application of treatment, since it is mainly immunity the responsible for clearance of the infection.

Lastly, we show that shorter-course treatments are viable alternatives to longer treatments. In chapter 4, we dive in a deep comparison between 7-day treatment duration and 3-day treatment duration. We find overall superiority of 7-day duration treatment as expected. However, we also find many cases when 3-day treatment is as beneficial or even more so than 7-day treatment. Application of a milder and longer treatment for patients with developed infections is beneficial. Aggressive and shorter treatments for patients with recent infections are also preferable. We notice that the comparison in success rate between short and long treatment depends on when the treatment is administered and how the comparison is made, by fixing the amount of killing per unit of time, or by fixing total killing.

As far as optimization is concerned, it is difficult (if not impossible) to minimize all infection outcomes with a single treatment. The variance of patients characteristics does not allow to find a single best therapy; it depends on the symptom threshold, treatment duration and antibiotic killing rate, in addition to the hosts and infection profile. Therefore, optimizing antimicrobial therapies for patients will require better clinical quantification of these parameters, especially those related to immune response. In addition, more experimental studies on understanding how treatments drive resistance and clearance are essential to continue to pursue the goal of personalised medicine: to administer the best possible therapy at the lowest cost for the patient and society.

Bibliography

Anderson, R. M. and May, R. M. (1991). *Infectious Diseases of Humans: Dynamics and Control*. Oxford Science Publications.

Andersson, D. I. and Levin, B. R. (1999). The biological cost of antibiotic resistance. *Current Opinion in Microbiology*, 2(5):489–493.

Ankomah, P. and Levin, B. R. (2014). Exploring the collaboration between antibiotics and the immune response in the treatment of acute, self-limiting infections. *Proceedings of the National Academy of Sciences*, 111(23):8331–8338.

Austin, D. J., Kristinsson, K. G., and Anderson, R. M. (1999). The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. *Proceedings of the National Academy of Sciences*, 96(3):1152–1156.

Berger, R. E. (2006). Duration of antibacterial treatment for uncomplicated urinary tract infection in women: Commentary. *Journal of Urology*, 175(3):968.

Bull, J. J., Vegge, C. S., Schmerer, M., Chaudhry, W. N., and Levin, B. R. (2014). Phenotypic resistance and the dynamics of bacterial escape from phage control. *PLoS ONE*, 9(4):1–10.

Cannavó, F. (2012). Sensitivity analysis for volcanic source modeling quality assessment and model selection. *Computers and Geosciences*, 44:52–59.

Casadevall, A. and anne Pirofski, L. (2018). What is a host? Attributes of individual susceptibility. *Infection and Immunity*, 86(2):1–12.

Chaudhry, W. N., Concepcion-Acevedo, J., Park, T., Andleeb, S., Bull, J. J., and Levin, B. R. (2017). Synergy and order effects of antibiotics and phages in killing pseudomonas aeruginosa biofilms. *PLoS ONE*, 12(1):1–16.

Cicchese, J. M., Pienaar, E., Kirschner, D. E., and Linderman, J. J. (2017). Applying Opti-

mization Algorithms to Tuberculosis Antibiotic Treatment Regimens. *Cellular and Molecular Bioengineering*, 10(6):523–535.

Ciupe, S. M. and Heffernan, J. M. (2017). In-host modeling. *Infectious Disease Modelling*, 2(2):188–202.

Coates, J., Park, B. R., Le, D., Şimşek, E., Chaudhry, W., and Kim, M. (2018). Antibiotic-induced population fluctuations and stochastic clearance of bacteria. *eLife*, 7:1–26.

Colijn, C. and Cohen, T. (2015). How competition governs whether moderate or aggressive treatment minimizes antibiotic resistance. *eLife*, 4(September2015):1–29.

Colijn, C., Jones, N., Johnston, I. G., Yaliraki, S., and Barahona, M. (2017). Toward precision healthcare: Context and mathematical challenges. *Frontiers in Physiology*, 8(MAR):1–10.

Cookson, B. T., Deatherage, B. L., and Cummings, L. A. (2009). Adaptive Immune Responses during Salmonella Infection. *EcoSal Plus*, 3(2).

Corthésy, B. (2010). Role of secretory immunoglobulin A and secretory component in the protection of mucosal surfaces. *Future Microbiology*, 5(5):817–829.

D'Agata, E. M., Magal, P., Olivier, D., Ruan, S., and Webb, G. F. (2007). Modeling antibiotic resistance in hospitals: The impact of minimizing treatment duration. *Journal of Theoretical Biology*, 249(3):487–499.

Dawson-Hahn, E. E., Mickan, S., Onakpoya, I., Roberts, N., Kronman, M., Butler, C. C., and Thompson, M. J. (2017). Short-course versus long-course oral antibiotic treatment for infections treated in outpatient settings: A review of systematic reviews. *Family Practice*, 34(5):511–519.

Domínguez-Hüttinger, E., Boon, N. J., Clarke, T. B., and Tanaka, R. J. (2017a). Mathematical modeling of Streptococcus pneumoniae colonization, invasive infection and treatment. *Frontiers in Physiology*, 8(MAR):1–14.

Domínguez-Hüttinger, E., Boon, N. J., Clarke, T. B., and Tanaka, R. J. (2017b). Mathematical modelling of colonization, invasive infection and treatment of Streptococcus pneumoniae. *Frontiers in Physiology*, 8(115):1–8.

D'Orazio, S. E., Halme, D. G., Ploegh, H. L., and Starnbach, M. N. (2003a). Class Ia MHC-deficient BALB/c mice generate CD8+ T cell-mediated protective immunity against Listeria monocytogenes infection. *Journal of immunology (Baltimore, Md. : 1950)*, 171(1):291–8.

D'Orazio, S. E., Halme, D. G., Ploegh, H. L., and Starnbach, M. N. (2003b). Class Ia MHC-deficient BALB/c mice generate CD8+ T cell-mediated protective immunity against Listeria monocytogenes infection. *Journal of immunology (Baltimore, Md. : 1950)*, 171(1):291–8.

Edelson, B. T., Cossart, P., and Unanue, E. R. (1999). Cutting edge: paradigm revisited: antibody provides resistance to Listeria infection. *Journal of immunology (Baltimore, Md. : 1950)*, 163(8):4087–4090.

Ehrlich, P. (1913). Address in Pathology, ON CHEMIOTHERAPY: Delivered before the Seventeenth International Congress of Medicine. *British medical journal*, 2(2746):353–9.

Elborn, J. S., Vataire, A. L., Fukushima, A., Aballea, S., Khemiri, A., Moore, C., Medic, G., and Hemels, M. E. (2016). Comparison of Inhaled Antibiotics for the Treatment of Chronic Pseudomonas aeruginosa Lung Infection in Patients With Cystic Fibrosis: Systematic Literature Review and Network Meta-analysis. *Clinical Therapeutics*, 38(10):2204–2226.

Falagas, M. E., Avgeri, S. G., Matthaiou, D. K., Dimopoulos, G., and Siempos, I. I. (2008). Short- versus long-duration antimicrobial treatment for exacerbations of chronic bronchitis: A meta-analysis. *Journal of Antimicrobial Chemotherapy*, 62(3):442–450.

Falagas, M. E., Karageorgopoulos, D. E., Grammatikos, A. P., and Matthaiou, D. K. (2009). Effectiveness and safety of short vs. long duration of antibiotic therapy for acute bacterial sinusitis: A meta-analysis of randomized trials. *British Journal of Clinical Pharmacology*, 67(2):161–171.

Furlan, L., Erba, L., Trombetta, L., Sacco, R., Colombo, G., Casazza, G., Solbiati, M., Montano, N., Marta, C., Sbrojavacca, R., Perticone, F., Corazza, G. R., and Costantino, G. (2018). Short- vs long-course antibiotic therapy for pneumonia: a comparison of systematic reviews and guidelines for the SIMI Choosing Wisely Campaign. *Internal and Emergency Medicine*, (0123456789).

Gaivão, M., Dionisio, F., and Gjini, E. (2017). Transmission Fitness in Co-colonization and the Persistence of Bacterial Pathogens. *Bulletin of Mathematical Biology*, 79(9):2068–2087.

Ganusov, V. V. (2016a). Physical and mathematical modeling in experimental papers: achieving robustness of mathematical modeling studies. *ArXiv*, (3):6–8.

Ganusov, V. V. (2016b). Strong inference in mathematical modeling: A method for robust science in the twenty-first century. *Frontiers in Microbiology*, 7(JUL).

Ganusov, V. V., Bergstrom, C. T., Antia, R., and Ergstrom, C. A. R. L. T. B. (2002). Within-Host Population Dynamics and the Evolution of Microparasites in a Heterogeneous Host Population Within-Host Population Dynamics and the Evolution of Microparasites in a Heterogeneous Host Population. *Evolution*, 56(2):213–223.

Geli, P., Laxminarayan, R., Dunne, M., and Smith, D. L. (2012). "One-Size-Fits-All"? Optimizing Treatment Duration for Bacterial Infections. *PLoS ONE*, 7(1).

Giannella, M., Pascale, R., Toschi, A., Ferraro, G., Graziano, E., Furii, F., Bartoletti, M., Tedeschi, S., Ambretti, S., Lewis, R. E., and Viale, P. (2018). Treatment duration for Escherichia coli bloodstream infection and outcomes: retrospective single-centre study. *Clinical Microbiology and Infection*, 24(10):1077–1083.

Gjini, E. and Brito, P. H. (2016). Integrating Antimicrobial Therapy with Host Immunity to Fight Drug-Resistant Infections: Classical vs. Adaptive Treatment. *PLoS Computational Biology*, 12(4):1–34.

Gould, I. M. (2008). The epidemiology of antibiotic resistance. *International Journal of Antimicrobial Agents*, 32(SUPPL. 1):2–9.

Gould, I. M. (2009). Antibiotic resistance: the perfect storm. *International Journal of Antimicrobial Agents*, 34(SUPPL. 3):S2–S5.

Grassly, N. C. and Fraser, C. (2008). Mathematical models of infectious disease transmission. *Nature Reviews Microbiology*, 6(6):477–487.

Guleria, I. and Pollard, J. W. (2001a). Aberrant macrophage and neutrophil population dynamics and impaired Th1 response to Listeria monocytogenes in colony-stimulating factor 1-deficient mice. *Infection and Immunity*, 69(3):1795–1807.

Guleria, I. and Pollard, J. W. (2001b). Aberrant macrophage and neutrophil population dynamics and impaired Th1 response to Listeria monocytogenes in colony-stimulating factor 1-deficient mice. *Infection and Immunity*, 69(3):1795–1807.

Harty, J. T. and Bevan, M. J. (1999). Responses of CD8+ T cells to intracellular bacteria. *Current Opinion in Immunology*, 11:89–93.

Hoebe, K., Janssen, E., and Beutler, B. (2004). The interface between innate and adaptive immunity. *Nature Immunology*, 5(10):971–974.

Huijben, S., Bell, A. S., Sim, D. G., Tomasello, D., Mideo, N., Day, T., and Read, A. F. (2013). Aggressive Chemotherapy and the Selection of Drug Resistant Pathogens. *PLoS Pathogens*, 9(9).

Huttner, A., Kowalczyk, A., Turjeman, A., Babich, T., Brossier, C., Eliakim-Raz, N., Kosiek, K., De Tejada, B. M., Roux, X., Shiber, S., Theuretzbacher, U., Von Dach, E., Yahav, D., Leibovici, L., Godycki-Ćwirko, M., Mouton, J. W., and Harbarth, S. (2018). Effect of 5-day Nitrofurantoin vs single-dose fosfomycin on clinical resolution of uncomplicated lower urinary tract infection in women a randomized clinical trial. *JAMA - Journal of the American Medical Association*, 319(17):1781–1789.

Johnson, P. J. T. and Levin, B. R. (2013). Pharmacodynamics, Population Dynamics, and the Evolution of Persistence in Staphylococcus aureus. *PLoS Genetics*, 9(1).

Karageorgopoulos, D. E., Valkimadi, P. E., Kapaskelis, A., Rafailidis, P. I., and Falagas, M. E. (2009). Short versus long duration of antibiotic therapy for bacterial meningitis: A meta-analysis of randomised controlled trials in children. *Archives of Disease in Childhood*, 94(8):607–614.

Karslake, J., Maltas, J., Brumm, P., and Wood, K. B. (2016). Population Density Modulates Drug Inhibition and Gives Rise to Potential Bistability of Treatment Outcomes for Bacterial Infections. *PLoS Computational Biology*, 12(10):1–21.

Kaufmann, S. H. and Ladel, C. H. (1994). Role of T Cell Subsets in Immunity against Intracellular Bacteria: Experimental Infections of Knock-Out Mice with Listeria monocytogenes and Mycobacterium bovis BCG. *Immunobiology*, 191(4-5):509–519.

Kepler, T. B. and Perelson, A. S. (1998). Drug concentration heterogeneity facilitates the evolution of drug resistance. *Proceedings of the National Academy of Sciences*, 95(20):11514–11519.

Kikuchi, T., Kobayashi, T., Gomi, K., Suzuki, T., Tokue, Y., Watanabe, A., and Nukiwa, T. (2004). Dendritic cells pulsed with live and dead Legionella pneumophila elicit distinct immune responses. *J.Immunol.*, 172(3):1727–1734.

Kirschner, D., Pienaar, E., Marino, S., and Linderman, J. J. (2017). A review of computational and mathematical modeling contributions to our understanding of Mycobacterium tuberculosis within-host infection and treatment. *Current Opinion in Systems Biology*, 3:170–185.

Kouyos, R. D., Metcalf, C. J. E., Birger, R., Klein, E. Y., zur Wiesch, P. A., Ankomah, P., Arinaminpathy, N., Bogich, T. L., Bonhoeffer, S., Brower, C., Chi-Johnston, G., Cohen, T., Day, T., Greenhouse, B., Huijben, S., Metlay, J., Mideo, N., Pollitt, L. C., Read, A. F., Smith, D. L., Standley, C., Wale, N., and Grenfell, B. (2014). The path of least resistance: Aggressive or moderate treatment? *Proceedings of the Royal Society B: Biological Sciences*, 281(1794).

Levin, B. R., Baquero, F., Ankomah, P. P., and McCall, I. C. (2017). Phagocytes, Antibiotics, and Self-Limiting Bacterial Infections. *Trends in Microbiology*, 25(11):878–892.

Levin, B. R. and Bull, J. J. (2004). Population and evolutionary dynamics of phage therapy. *Nature Reviews Microbiology*, 2(2):166–173.

Levin, B. R., Concepción-Acevedo, J., and Udekwu, K. I. (2014). Persistence: a copacetic and parsimonious hypothesis for the existence of non-inherited resistance to antibiotics. *Curr Opin Microbiol.*, (0):18–21.

Levin, B. R., Perrot, V., and Walker, N. (2000). Compensatory mutations, antibiotic resistance and the population genetics of adaptive evolution in bacteria. *Genetics*, 154(3):985–997.

Levin, B. R. and Rozen, D. E. (2006). Non-inherited antibiotic resistance. *Nature Reviews Microbiology*, 4(7):556–562.

Levin-Reisman, I., Ronin, I., Gefen, O., Braniss, I., Shoresh, N., and Balaban, N. Q. (2017). Antibiotic tolerance facilitates the evolution of resistance. *Science*, 355(6327):826–830.

Lin, R., Xu, X., Li, Y., Sun, J., Fan, Z., Jiang, Q., Huang, F., Zhou, H., Nie, D., Guo, Z., Mao, Y., Xiao, Y., and Liu, Q. (2014). Comparison of long-term and short-term administration of itraconazole for primary antifungal prophylaxis in recipients of allogeneic hematopoietic stem cell transplantation: A multicenter, randomized, open-label trial. *Transplant Infectious Disease*, 16(2):286–294.

Llewelyn, M. J., Fitzpatrick, J. M., Darwin, E., Sarahtonkin-Crine, Gorton, C., Paul, J., Peto, T. E., Yardley, L., Hopkins, S., and Walker, A. S. (2017). The antibiotic course has had its day. *BMJ (Online)*, 358(box 1):1–5.

Lutters, M. and Vogt-Ferrier, N. B. (2008). Antibiotic duration for treating uncomplicated, symptomatic lower urinary tract infections in elderly women. *Cochrane Database of Systematic Reviews*, (3).

Mantovani, A., Cassatella, M. A., Costantini, C., and Jaillon, S. (2011). Neutrophils in the activation and regulation of innate and adaptive immunity. *Nature Reviews Immunology*, 11(8):519–531.

Mercado, R., Vijh, S., Allen, S. E., Kerksiek, K., Pilip, I. M., and Pamer, E. G. (2000a). Early Programming of T Cell Populations Responding to Bacterial Infection. *The Journal of Immunology*, 165(12):6833–6839.

Mercado, R., Vijh, S., Allen, S. E., Kerksiek, K., Pilip, I. M., and Pamer, E. G. (2000b). Early Programming of T Cell Populations Responding to Bacterial Infection. *The Journal of Immunology*, 165(12):6833–6839.

Michael, M., Hodson, E. M., Craig, J. C., Martin, S., and Moyer, V. A. (2003). Short versus standard duration oral antibiotic therapy for acute urinary tract infection in children. *Cochrane Database of Systematic Reviews*, (1).

Onakpoya, I. J., Walker, A. S., Tan, P. S., Spencer, E. A., Gbinigie, O. A., Cook, J., Llewelyn, M. J., and Butler, C. C. (2018). Overview of systematic reviews assessing the evidence for shorter versus longer duration antibiotic treatment for bacterial infections in secondary care. *PLoS ONE*, 13(3):1–20.

Oreskes, N., Shrader-Frechette, K., and Belitz, K. (1994). Verification,validation, and confirmation of numerical models in the earth sciences. *Science*, 263(5147):641–647.

Perelson, A. S. (2002). Modelling viral and immune system dynamics. *Nature Reviews Immunology*, 2(1):28–36.

Pessoa, D., Souto-Maior, C., Gjini, E., Lopes, J. S., Ceña, B., Codeço, C. T., and Gomes, M. G. M. (2014). Unveiling Time in Dose-Response Models to Infer Host Susceptibility to Pathogens. *PLoS Computational Biology*, 10(8).

Roach, D. R., Leung, C. Y., Henry, M., Morello, E., Singh, D., Di Santo, J. P., Weitz, J. S., and Debarbieux, L. (2017). Synergy between the Host Immune System and Bacteriophage Is Essential for Successful Phage Therapy against an Acute Respiratory Pathogen. *Cell Host and Microbe*, 22(1):38–47.e4.

Shlaes, D. M. and Spellberg, B. (2012). Overcoming the challenges to developing new antibiotics. *Current Opinion in Pharmacology*, 12(5):522–526.

Sommer, M. O., Munck, C., Toft-Kehler, R. V., and Andersson, D. I. (2017). Prediction of antibiotic resistance: Time for a new preclinical paradigm? *Nature Reviews Microbiology*, 15(11):689–696.

Stolbrink, M., Amiry, J., and Blakey, J. D. (2018). Does antibiotic treatment duration affect the outcomes of exacerbations of asthma and COPD? A systematic review. *Chronic Respiratory Disease*, 15(3):225–240.

Stromberg, S. P. and Antia, R. (2011). Vaccination by delayed treatment of infection. *Vaccine*, 29(52):9624–9631.

Torres, B. Y., Oliveira, J. H. M., Thomas Tate, A., Rath, P., Cumnock, K., and Schneider, D. S. (2016). Tracking Resilience to Infections by Mapping Disease Space. *PLoS Biology*, 14(4):1–19.

Uecker, H. and Bonhoeffer, S. (2018). Antibiotic treatment protocols revisited : The challenges of a conclusive assessment by mathematical modeling. *Biorxiv*, pages 1–20.

Usta, Y., Saltik-Temizel, I. N., Demir, H., Uslu, N., Ozen, H., Gurakan, F., and Yuce, A. (2008). Comparison of short- and long-term treatment protocols and the results of second-line quadruple therapy in children with Helicobacter pylori infection. *Journal of Gastroenterology*, 43(6):429–433.

Vázquez-boland, J. a., Kuhn, M., Berche, P., Chakraborty, T., Domi, G., González-zorn, B., and Wehland, J. (2001). Listeria pathogenesis and molecular virulence determinants listeria pathogenesis and molecular virulence determinants. *Clin. Microbiol. Rev.*, 14(3):584–640.

Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *P & T : A peer-reviewed journal for formulary management (2015)*, 40(4):277–83.

Warsinske, H. C., DiFazio, R. M., Linderman, J. J., Flynn, J. A. L., and Kirschner, D. E. (2017). Identifying mechanisms driving formation of granuloma-associated fibrosis during Mycobacterium tuberculosis infection. *Journal of Theoretical Biology*, 429:1–17.

Warsinske, H. C., Wheaton, A. K., Kim, K. K., Linderman, J. J., Moore, B. B., and Kirschner, D. E. (2016). Computational modeling predicts simultaneous targeting of fibroblasts and epithelial cells is necessary for treatment of pulmonary fibrosis. *Frontiers in Pharmacology*, 7(JUN).

Weissman, J. L., Holmes, R., Barrangou, R., Moineau, S., Fagan, W. F., Levin, B., and Johnson, P. L. (2018). Immune loss as a driver of coexistence during host-phage coevolution. *ISME Journal*, 12(2):585–597.

Woolhouse, M., W. J. D. E. C. B. &. L. B. R. (2002). Biological and biomedical implications of the co-ecolution of pathogens and their hosts. *Nature genetics*, 32(december):569–577.

World Health Organization (2018). Global Tuberculosis Report. Technical report.

Appendix A

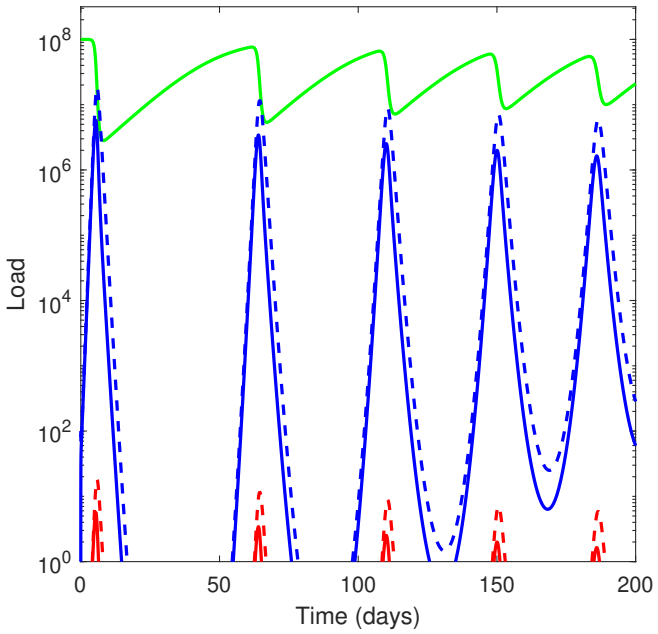


Figure A.1: Model dynamics for very low extinction threshold in the absence of immunity. Dampling oscillations tend to a persistence equilibrium. Blue lines refer to susceptible bacteria populations. Red lines refer to resistant bacteria populations. Dashed lines representing the extracellular populations, while solid lines represent the intracellular populations (infected macrophages). Green line reflects the population of uninfected macrophages. $B_{ext} = 10^{-}10$. Simulation run with the default parameters from table 2.1. Although we expected persistence analytically, real infections will not show the same pattern, since there is immediate extinction when the bacterial population is very low. Therefore, the clearance regime predicted from analysis in figure 2.7 is conservative (smaller than in the numerical simulations).

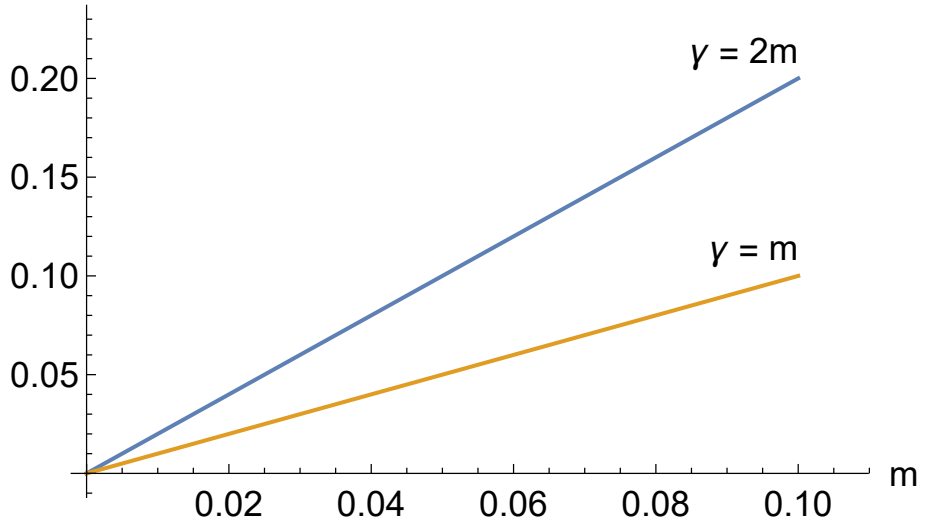


Figure A.2: Conditions for coexistence of bacteria populations in the γ vs m space. Above the blue line we are in a zone where there is coexistence of both bacteria populations, with susceptible bacteria dominating over the resistant bacteria. While between the two lines, there is again coexistence although resistant population dominates. Below the yellow line, there is only existence of resistant population, with the clearance of susceptible populations. This conditions where extracted from analysis without immunity in a coexistence equilibrium state.

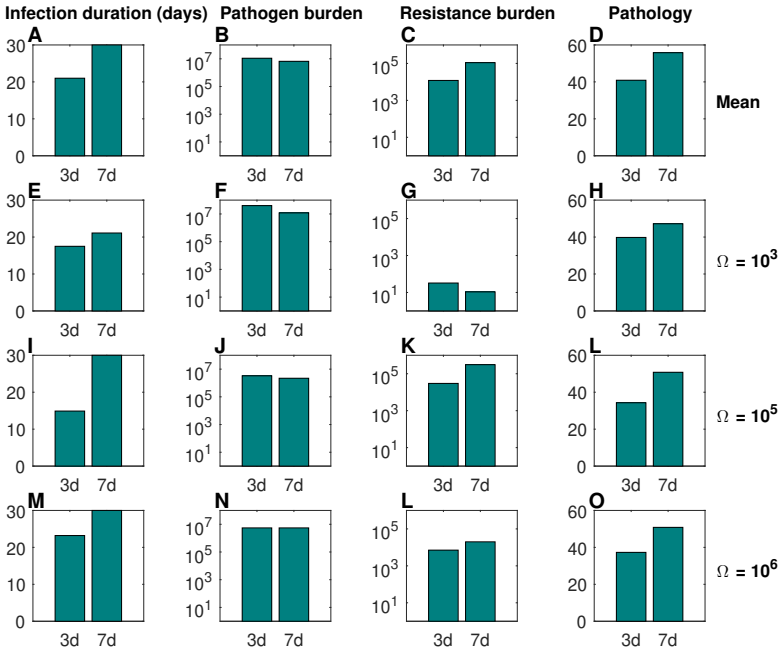


Figure A.3: Summary measures for the average dynamics of 3 and 7 day treatment duration. This measures are generated from the dynamics of the infections represented in the Figure 4.3 of the main text. A) Infection duration of the mean dynamics for 3 and 7 day treatment duration. B) Pathogen burden of the mean dynamics for the 3 and 7 day treatment duration. C) Resistance burden of the mean dynamics for the 3 and 7 day treatment duration. D) Cumulative pathology of the mean dynamics for the 3 and 7 day treatment duration. Second , third and fourth line are refering to only specific symptom threshold, 10e3, 10e5 and 10e6, respectively.

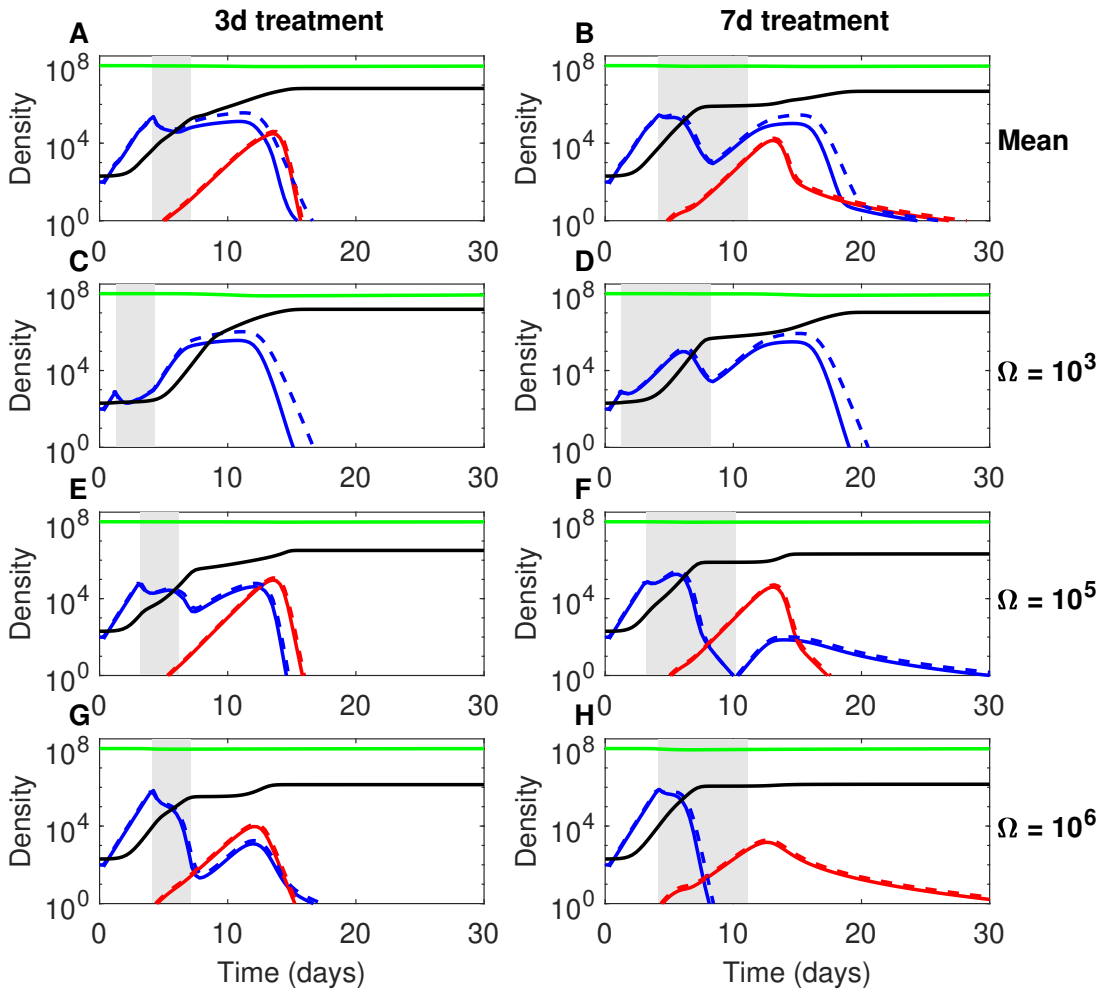


Figure A.4: Typical infection dynamics under 3-day and 7-day treatment as a function of the symptom threshold Ω when the total amount of drug is kept fixed. The treatment onset and duration (denoted by the vertical gray lines) affects in major qualitative and quantitative ways the net dynamics obtained with antibiotic treatment. Here we illustrate the average over many simulations of treatment at intensity in the range $Intensity \in [1, 84]$. Equivalent to assuming that all these intensities are uniformly distributed in the population of treated patients.

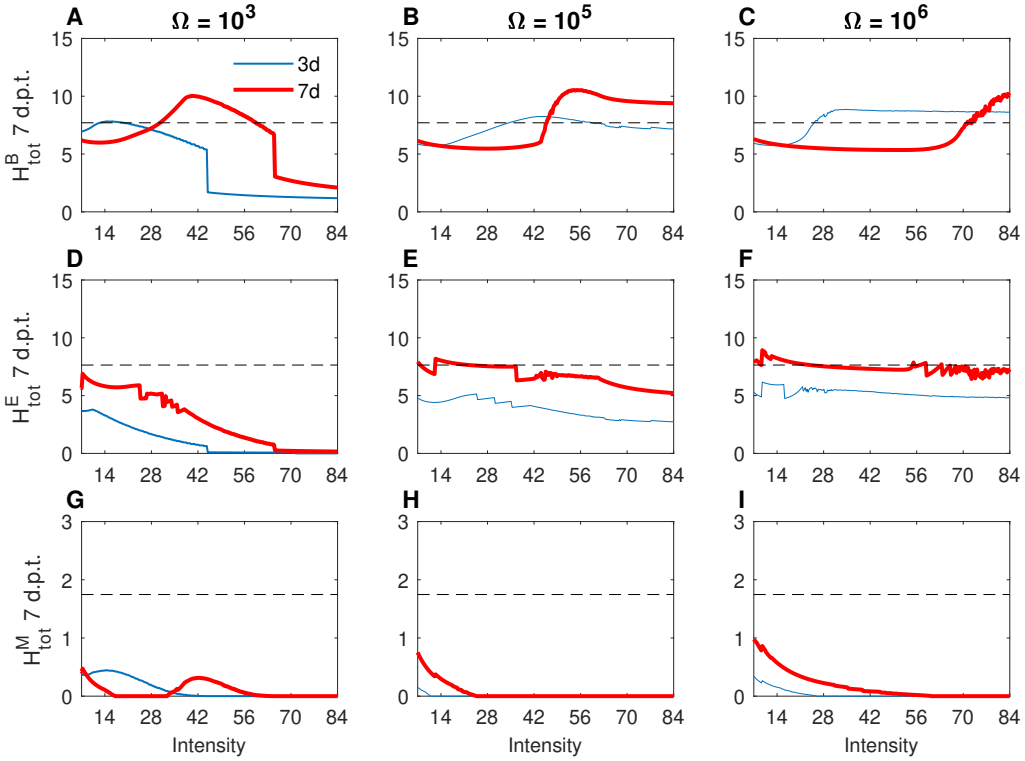


Figure A.5: Cumulative pathology outcomes for different treatments, 7-days after the end of therapy keeping the total intensity fixed. The dashed line represents the pathology in the case of no-treatment. Top row: pathology due to bacterial growth. Middle row: Pathology due to immune response activation. Bottom row: Pathology due to macrophage depletion. (A, D, G) $\Omega = 10^3$ and treatment starts at very low bacterial levels. (B, E, H) $\Omega = 10^5$ and treatment starts at higher bacterial levels. (C,F, I) $\Omega = 10^6$ and treatment is triggered at very high bacterial levels.

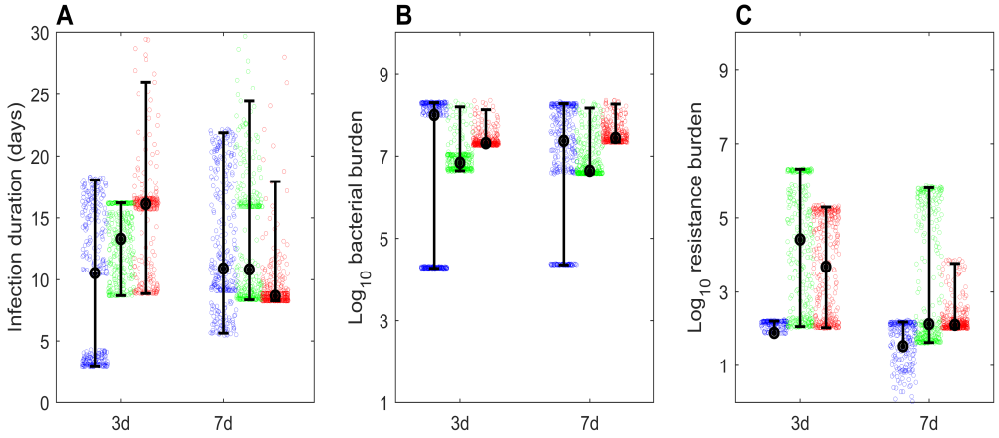


Figure A.6: Summary outcomes of infection compared for 3-day and 7-day treatment at different Ω keeping the total intensity fixed. A) Infection duration B) Total bacterial burden C) Total resistance burden. Each dot represent a different dose from $A_m \in [1, 20]$, coloured by symptom threshold (blue, green, red: $\Omega = 10^3$, 10^5, 10^6) for two distinct treatment durations (3 days and 7 days).

MODEL WITH ONE TYPE OF BACTERIA AND NO IMMUNITY (3 EQUATIONS)

```
Clear[r, K, beta, delta, a, Ns, c]
```
apaga
```
ptot = Bs;
eq1 = r * M * (1 - M / K) - beta * M * ptot;
eq2 = beta * M * Bs - Is * (delta + a);
eq3 = Ns * Is * delta - beta * M * Bs - (c  + Am) * Bs;
Am = 0;
eqlist = FullSimplify[Solve[{eq1 == 0, eq2 == 0, eq3 == 0}, {M, Is, Bs}]]
```
simplifica compl··· resolve
```
Jac1 = D[eq1, {{M, Is, Bs}}];
```
derivada
```
Jac2 = D[eq2, {{M, Is, Bs}}];
```
derivada
```
Jac3 = D[eq3, {{M, Is, Bs}}];
```
derivada
```
Jac = {Jac1, Jac2, Jac3};
```

$$\left\{\{M \to 0, Is \to 0, Bs \to 0\}, \{M \to K, Is \to 0, Bs \to 0\}, \left\{M \to -\frac{c\,(a+\text{delta})}{\text{beta}\,(a+\text{delta}-\text{delta}\,Ns)},\right.\right.$$
$$Is \to -\left((c\,((a+\text{delta})\,(c+\text{beta}\,K)-\text{beta}\,\text{delta}\,K\,Ns)\,r)\,/\,(\text{beta}^2\,K\,(a+\text{delta}-\text{delta}\,Ns)^2)\right),$$
$$\left.\left.Bs \to ((a+\text{delta})\,(c+\text{beta}\,K)-\text{beta}\,\text{delta}\,K\,Ns)\,r)\,/\,(\text{beta}^2\,K\,(a+\text{delta}-\text{delta}\,Ns))\right\}\right\}$$

```
MatrixForm[Jac]
```
forma de matriz
```
Jac1 = Jac /. eqlist[[1]];
Jac2 = Jac /. eqlist[[2]];
Jac3 = Jac /. eqlist[[3]];
FullSimplify[Eigenvalues[Jac1]]
```
autovalores

$$\begin{pmatrix} -\text{beta}\,Bs - \frac{M\,r}{K} + \left(1-\frac{M}{K}\right) r & 0 & -\text{beta}\,M \\ \text{beta}\,Bs & -a-\text{delta} & \text{beta}\,M \\ -\text{beta}\,Bs & \text{delta}\,Ns & -c-\text{beta}\,M \end{pmatrix}$$

$$\{-c, -a-\text{delta}, r\}$$

{-c, -a - delta, r}
The trivial equilibrium is unstable because r is positive.

$$\{-c, -a-\text{delta}, r\}$$

```
eigs2 = FullSimplify[Eigenvalues[Jac2]]
```
simplifica compl··· autovalores

$$\left\{\frac{1}{2}\left(-a-c-\text{delta}-\text{beta}\,K-\sqrt{\left((a-c+\text{delta}-\text{beta}\,K)^2+4\,\text{beta}\,\text{delta}\,K\,Ns\right)}\right),\right.$$
$$\left.\frac{1}{2}\left(-a-c-\text{delta}-\text{beta}\,K+\sqrt{\left((a-c+\text{delta}-\text{beta}\,K)^2+4\,\text{beta}\,\text{delta}\,K\,Ns\right)}\right), -r\right\}$$

```
Reduce[eigs2[[2]] < 0 && a > 0 && c > 0 && delta > 0 && beta > 0 && K > 0 && Ns > 0 ]
```

reduz

$$\text{delta} > 0 \,\&\&\, a > 0 \,\&\&\, c > 0 \,\&\&\, K > 0 \,\&\&\, \text{beta} > 0 \,\&\&\, 0 < Ns < \frac{a\,c + c\,\text{delta} + a\,\text{beta}\,K + \text{beta}\,\text{delta}\,K}{\text{beta}\,\text{delta}\,K}$$

Carrying capacity – equilibrium for macrophages,
and no infection is stable if : Ns <
((a c + c delta + a beta K + beta delta K) / (beta delta K)).

```
Reduce[(a - c + delta - beta K)^2 + 4 beta delta K Ns < 0 &&
  a > 0 && c > 0 && delta > 0 && beta > 0 && K > 0 && Ns > 0]
```

reduz

```
False
```

The no - infection equilibrium is not reached through oscillations, because the expression inside the square root cannot be negative. Thus, this is a node.

```
eigs3 = FullSimplify[Eigenvalues[Jac3]]
```

simplifica compl··· autovalores

$$\Big\{\text{Root}\Big[a^3 c^2 r + 3 a^2 c^2 \text{delta}\, r + 3 a c^2 \text{delta}^2 r + c^2 \text{delta}^3 r + a^3 \text{beta}\, c K r + 3 a^2 \text{beta}\, c \text{delta}\, K r + 3 a \text{beta}\, c \text{delta}^2 K r + \text{beta}\, c \text{delta}^3 K r - a^2 c^2 \text{delta}\, \text{Ns}\, r - 2 a c^2 \text{delta}^2 \text{Ns}\, r - c^2 \text{delta}^3 \text{Ns}\, r - 2 a^2 \text{beta}\, c \text{delta}\, K \text{Ns}\, r - 4 a \text{beta}\, c \text{delta}^2 K \text{Ns}\, r - 2 \text{beta}\, c \text{delta}^3 K \text{Ns}\, r + a \text{beta}\, c \text{delta}^2 K \text{Ns}^2 r + \text{beta}\, c \text{delta}^3 K \text{Ns}^2 r + \left(-a^3 c r + a^2 c^2 r - 3 a^2 c \text{delta}\, r + 2 a c^2 \text{delta}\, r - 3 a c \text{delta}^2 r + c^2 \text{delta}^2 r - c \text{delta}^3 r + a^2 \text{beta}\, c K r + 2 a \text{beta}\, c \text{delta}\, K r + \text{beta}\, c \text{delta}^2 K r + a^2 c \text{delta}\, \text{Ns}\, r + a c^2 \text{delta}\, \text{Ns}\, r + 2 a c \text{delta}^2 \text{Ns}\, r + c^2 \text{delta}^2 \text{Ns}\, r + c \text{delta}^3 \text{Ns}\, r - a \text{beta}\, c \text{delta}\, K \text{Ns}\, r - \text{beta}\, c \text{delta}^2 K \text{Ns}\, r\right) \#1 + \left(a^3 \text{beta}\, K + 3 a^2 \text{beta}\, \text{delta}\, K + 3 a \text{beta}\, \text{delta}^2 K + \text{beta}\, \text{delta}^3 K - 2 a^2 \text{beta}\, \text{delta}\, K \text{Ns} - a \text{beta}\, c \text{delta}\, K \text{Ns} - 4 a \text{beta}\, \text{delta}^2 K \text{Ns} - \text{beta}\, c \text{delta}^2 K \text{Ns} - 2 \text{beta}\, \text{delta}^3 K \text{Ns} + a \text{beta}\, \text{delta}^2 K \text{Ns}^2 + \text{beta}\, c \text{delta}^2 K \text{Ns}^2 + \text{beta}\, \text{delta}^3 K \text{Ns}^2 - a^2 c r - 2 a c \text{delta}\, r - c \text{delta}^2 r + a c \text{delta}\, \text{Ns}\, r + c \text{delta}^2 \text{Ns}\, r\right) \#1^2 + \left(a^2 \text{beta}\, K + 2 a \text{beta}\, \text{delta}\, K + \text{beta}\, \text{delta}^2 K - 2 a \text{beta}\, \text{delta}\, K \text{Ns} - 2 \text{beta}\, \text{delta}^2 K \text{Ns} + \text{beta}\, \text{delta}^2 K \text{Ns}^2\right) \#1^3 \&,\, 1\Big],$$

$$\text{Root}\Big[a^3 c^2 r + 3 a^2 c^2 \text{delta}\, r + 3 a c^2 \text{delta}^2 r + c^2 \text{delta}^3 r + a^3 \text{beta}\, c K r + 3 a^2 \text{beta}\, c \text{delta}\, K r + 3 a \text{beta}\, c \text{delta}^2 K r + \text{beta}\, c \text{delta}^3 K r - a^2 c^2 \text{delta}\, \text{Ns}\, r - 2 a c^2 \text{delta}^2 \text{Ns}\, r - c^2 \text{delta}^3 \text{Ns}\, r - 2 a^2 \text{beta}\, c \text{delta}\, K \text{Ns}\, r - 4 a \text{beta}\, c \text{delta}^2 K \text{Ns}\, r - 2 \text{beta}\, c \text{delta}^3 K \text{Ns}\, r + a \text{beta}\, c \text{delta}^2 K \text{Ns}^2 r + \text{beta}\, c \text{delta}^3 K \text{Ns}^2 r + \left(-a^3 c r + a^2 c^2 r - 3 a^2 c \text{delta}\, r + 2 a c^2 \text{delta}\, r - 3 a c \text{delta}^2 r + c^2 \text{delta}^2 r - c \text{delta}^3 r + a^2 \text{beta}\, c K r + 2 a \text{beta}\, c \text{delta}\, K r + \text{beta}\, c \text{delta}^2 K r + a^2 c \text{delta}\, \text{Ns}\, r + a c^2 \text{delta}\, \text{Ns}\, r + 2 a c \text{delta}^2 \text{Ns}\, r + c^2 \text{delta}^2 \text{Ns}\, r + c \text{delta}^3 \text{Ns}\, r - a \text{beta}\, c \text{delta}\, K \text{Ns}\, r - \text{beta}\, c \text{delta}^2 K \text{Ns}\, r\right) \#1 + \left(a^3 \text{beta}\, K + 3 a^2 \text{beta}\, \text{delta}\, K + 3 a \text{beta}\, \text{delta}^2 K + \text{beta}\, \text{delta}^3 K - 2 a^2 \text{beta}\, \text{delta}\, K \text{Ns} - a \text{beta}\, c \text{delta}\, K \text{Ns} - 4 a \text{beta}\, \text{delta}^2 K \text{Ns} - \text{beta}\, c \text{delta}^2 K \text{Ns} - 2 \text{beta}\, \text{delta}^3 K \text{Ns} + a \text{beta}\, \text{delta}^2 K \text{Ns}^2 + \text{beta}\, c \text{delta}^2 K \text{Ns}^2 + \text{beta}\, \text{delta}^3 K \text{Ns}^2 - a^2 c r - 2 a c \text{delta}\, r - c \text{delta}^2 r + a c \text{delta}\, \text{Ns}\, r + c \text{delta}^2 \text{Ns}\, r\right) \#1^2 + \left(a^2 \text{beta}\, K + 2 a \text{beta}\, \text{delta}\, K + \text{beta}\, \text{delta}^2 K - 2 a \text{beta}\, \text{delta}\, K \text{Ns} - 2 \text{beta}\, \text{delta}^2 K \text{Ns} + \text{beta}\, \text{delta}^2 K \text{Ns}^2\right) \#1^3 \&,\, 2\Big],$$

$$\text{Root}\Big[a^3 c^2 r + 3 a^2 c^2 \text{delta}\, r + 3 a c^2 \text{delta}^2 r + c^2 \text{delta}^3 r + a^3 \text{beta}\, c K r + 3 a^2 \text{beta}\, c \text{delta}\, K r + 3 a \text{beta}\, c \text{delta}^2 K r + \text{beta}\, c \text{delta}^3 K r - a^2 c^2 \text{delta}\, \text{Ns}\, r - 2 a c^2 \text{delta}^2 \text{Ns}\, r - c^2 \text{delta}^3 \text{Ns}\, r - 2 a^2 \text{beta}\, c \text{delta}\, K \text{Ns}\, r - 4 a \text{beta}\, c \text{delta}^2 K \text{Ns}\, r - 2 \text{beta}\, c \text{delta}^3 K \text{Ns}\, r + a \text{beta}\, c \text{delta}^2 K \text{Ns}^2 r + \text{beta}\, c \text{delta}^3 K \text{Ns}^2 r + \left(-a^3 c r + a^2 c^2 r - 3 a^2 c \text{delta}\, r + 2 a c^2 \text{delta}\, r - 3 a c \text{delta}^2 r + c^2 \text{delta}^2 r - c \text{delta}^3 r + a^2 \text{beta}\, c K r + 2 a \text{beta}\, c \text{delta}\, K r + \text{beta}\, c \text{delta}^2 K r + a^2 c \text{delta}\, \text{Ns}\, r + a c^2 \text{delta}\, \text{Ns}\, r + 2 a c \text{delta}^2 \text{Ns}\, r + c^2 \text{delta}^2 \text{Ns}\, r + c \text{delta}^3 \text{Ns}\, r - a \text{beta}\, c \text{delta}\, K \text{Ns}\, r - \text{beta}\, c \text{delta}^2 K \text{Ns}\, r\right) \#1 + \left(a^3 \text{beta}\, K + 3 a^2 \text{beta}\, \text{delta}\, K + 3 a \text{beta}\, \text{delta}^2 K + \text{beta}\, \text{delta}^3 K - 2 a^2 \text{beta}\, \text{delta}\, K \text{Ns} - a \text{beta}\, c \text{delta}\, K \text{Ns} - 4 a \text{beta}\, \text{delta}^2 K \text{Ns} - \text{beta}\, c \text{delta}^2 K \text{Ns} - 2 \text{beta}\, \text{delta}^3 K \text{Ns} + a \text{beta}\, \text{delta}^2 K \text{Ns}^2 + \text{beta}\, c \text{delta}^2 K \text{Ns}^2 + \text{beta}\, \text{delta}^3 K \text{Ns}^2 - a^2 c r - 2 a c \text{delta}\, r - c \text{delta}^2 r + a c \text{delta}\, \text{Ns}\, r + c \text{delta}^2 \text{Ns}\, r\right) \#1^2 + \left(a^2 \text{beta}\, K + 2 a \text{beta}\, \text{delta}\, K + \text{beta}\, \text{delta}^2 K - 2 a \text{beta}\, \text{delta}\, K \text{Ns} - 2 \text{beta}\, \text{delta}^2 K \text{Ns} + \text{beta}\, \text{delta}^2 K \text{Ns}^2\right) \#1^3 \&,\, 3\Big]\Big\}$$

Bs /. eqlist[[3]]

$$\frac{\left(\left(a + \text{delta}\right)\left(c + \text{beta}\, K\right) - \text{beta}\, \text{delta}\, K \text{Ns}\right) r}{\text{beta}^2 K \left(a + \text{delta} - \text{delta}\, \text{Ns}\right)}$$

```
condExist = Reduce[ r > 0 && a > 0 && c > 0 && delta > 0 &&
```
reduz
```
    beta > 0 && K > 0 && Ns > 0 && -(c (a + delta))/(beta (a + delta - delta Ns)) > 0 &&
    -((c ((a + delta) (c + beta K) - beta delta K Ns) r)/(beta^2 K (a + delta - delta Ns)^2)) >
     0 && (((a + delta) (c + beta K) - beta delta K Ns) r)/(beta^2 K (a + delta - delta Ns)) >
     0, {Ns, K, beta, delta, a, c, r}]
```

$$Ns > 1 \&\& K > 0 \&\& beta > 0 \&\& delta > 0 \&\& 0 < a < -delta + delta\, Ns \&\& 0 < c < \frac{-a\, beta\, K - beta\, delta\, K + beta\, delta\, K\, Ns}{a + delta} \&\& r > 0$$

```
Reduce[condExist && eigs3[[1]] < 0 && eigs3[[2]] < 0 && eigs3[[3]] < 0]
```
reduz

```
$Aborted
```

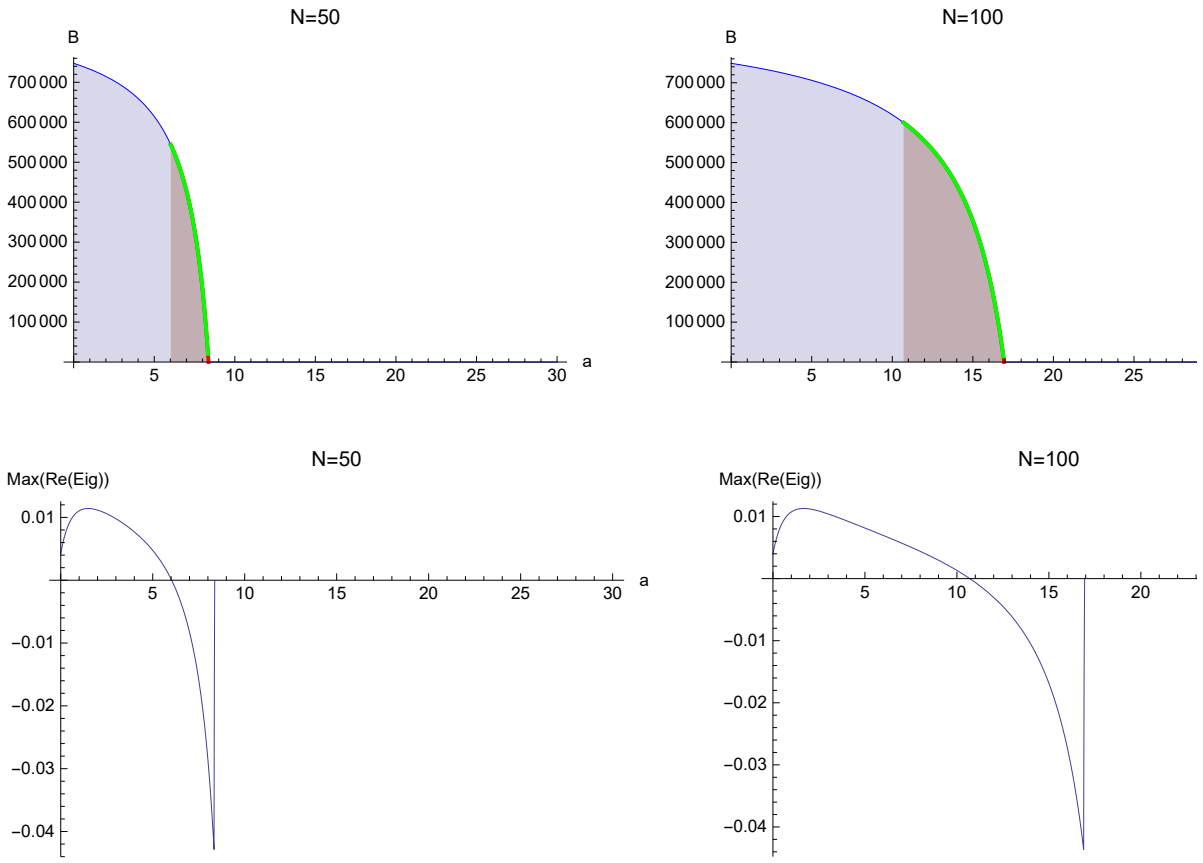
```
Reduce[condExist && Ns < (a c + c delta + a beta K + beta delta K)/(beta delta K)]
```
reduz

```
False
```

The clearance equilibrium always exists. When the clearance equilibrium is stable, the persistence equilibrium does not exist and, when the clearance equilibrium is unstable, the persistence equilibrium exists.

Stability analysis at persistence equilibrium.

```
r = 0.09;
K = 10^8;
beta = 1.2 * 10^(-7);
delta = 0.2;
Ns = 50;
c = 2;
region3 = condExist && Max[Re[Eigenvalues[Jac3]]] < 0 &&
                       má·· p··· autovalores
    Max[Im[Eigenvalues[Jac3]]] > 0 && Bs /. eqlist[[3]] ;
        p··· autovalores
region2 = condExist && Max[Re[Eigenvalues[Jac3]]] < 0 && Bs /. eqlist[[3]] ;
                       má·· p··· autovalores
region1 = condExist  && Bs /. eqlist[[3]] ;
g1 = Plot[{region1, region2, region3},
     gráfico
   {a, 0, 30}, PlotStyle → {Blue, {Red, Thick}, {Green, Thick}},
               estilo do gráfico azul  ve··· espesso verde  espesso
   Filling → Bottom, AxesLabel -> {"a ", "B"}, PlotLabel → "N=50"];
   coloração inferior legenda dos eixos              etiqueta de gráf·· valor numérico
g3 = Plot[condExist && Max[Re[Eigenvalues[Jac3]]], {a, 0, 30},
     gráfico               má·· p·· autovalores
   AxesLabel -> {"a", "Max(Re(Eig)) "}, PlotLabel → "N=50"];
   legenda dos eixos       má·· parte real       etiqueta de gráf··· valor numérico
r = 0.09;
K = 10^8;
beta = 1.2 * 10^(-7);
delta = 0.2;
Ns = 100;
c = 2;
g4 = Plot[condExist && Max[Re[Eigenvalues[Jac3]]],
     gráfico               má·· p·· autovalores
   {a, 0, 30}, AxesLabel -> {"a", "Max(Re(Eig)) "}, PlotLabel → "N=100"];
               legenda dos eixos       má·· parte real       etiqueta de gráf·· valor numérico
region3 = condExist && Max[Re[Eigenvalues[Jac3]]] < 0 &&
                       má·· p··· autovalores
    Max[Im[Eigenvalues[Jac3]]] > 0 && Bs /. eqlist[[3]] ;
    má·· p··· autovalores
region2 = condExist && Max[Re[Eigenvalues[Jac3]]] < 0 && Bs /. eqlist[[3]] ;
                       má·· p··· autovalores
region1 = condExist  && Bs /. eqlist[[3]] ;
g2 = Plot[{region1, region2, region3},
     gráfico
   {a, 0, 30}, PlotStyle → {Blue, {Red, Thick}, {Green, Thick}},
               estilo do gráfico azul  ve··· espesso verde  espesso
   Filling → Bottom, AxesLabel -> {"a ", "B"}, PlotLabel → "N=100"];
   coloração inferior legenda dos eixos              etiqueta de gráf·· valor numérico
GraphicsRow[{Show[g1], Show[g2]}]
linha de gráficos  mostra  mostra
GraphicsRow[{Show[g3], Show[g4]}]
linha de gráficos  mostra  mostra

(*in blue we show the existence of the equilibrium (can be stable or unstable),
in red, we show the stability of the equilibrium when it exists,
in green are those stable equilibria manifested through oscillations*)
```



We can clearly see three different possibilities for the equilibrium of persistence. In blue, it is unstable, green means it is stable manifested through oscillations and in red it is also stable but without oscillations.

MODEL WITH 2 BACTERIA SUB-POPULATIONS

```
ptot = Bs + Br;
eq1 = r * M * (1 - M / K) - beta * M * ptot;
eq2 = beta * M * Bs - Is * (delta + a);
eq3 = beta * M * Br - Ir * (delta + a);
eq4 = Ns * (1 - m) * Is * delta - beta * M * Bs - (c ) * Bs;
eq5 = Ns * (1 - gamma) * Ir * delta + m * Ns * Is * delta - beta * M * Br - (c) * Br;
eqlist = FullSimplify[
          simplifica completamente
  Solve[{eq1 == 0, eq2 == 0, eq3 == 0, eq4 == 0, eq5 == 0}, {M, Is, Ir, Bs, Br}]]
  resolve
```

$$\{\{M \to 0, Is \to 0, Ir \to 0, Bs \to 0, Br \to 0\}, \{M \to K, Is \to 0, Ir \to 0, Bs \to 0, Br \to 0\},$$
$$\{M \to -\frac{c\,(a+delta)}{beta\,(a+delta+delta\,(-1+gamma)\,Ns)}, Is \to 0,$$
$$Ir \to -\left(\left(c\,\left((a+delta)\,(c+beta\,K)+beta\,delta\,(-1+gamma)\,K\,Ns\right)\,r\right)\Big/\left(beta^2\,K\,(a+delta+delta\,(-1+gamma)\,Ns)^2\right)\right),$$
$$Bs \to 0, Br \to \frac{\left((a+delta)\,(c+beta\,K)+beta\,delta\,(-1+gamma)\,K\,Ns\right)\,r}{beta^2\,K\,(a+delta+delta\,(-1+gamma)\,Ns)}\},$$
$$\{M \to -\frac{c\,(a+delta)}{beta\,(a+delta+delta\,(-1+m)\,Ns)},$$
$$Is \to -\left(\left(c\,(gamma-m)\,\left((a+delta)\,(c+beta\,K)+beta\,delta\,K\,(-1+m)\,Ns\right)\,r\right)\Big/\left(beta^2\,gamma\,K\,(a+delta+delta\,(-1+m)\,Ns)^2\right)\right),$$
$$Ir \to -\frac{c\,m\,\left((a+delta)\,(c+beta\,K)+beta\,delta\,K\,(-1+m)\,Ns\right)\,r}{beta^2\,gamma\,K\,(a+delta+delta\,(-1+m)\,Ns)^2},$$
$$Bs \to \left((gamma-m)\,\left((a+delta)\,(c+beta\,K)+beta\,delta\,K\,(-1+m)\,Ns\right)\,r\right)\Big/\left(beta^2\,gamma\,K\,(a+delta+delta\,(-1+m)\,Ns)\right),$$
$$Br \to \frac{m\,\left((a+delta)\,(c+beta\,K)+beta\,delta\,K\,(-1+m)\,Ns\right)\,r}{beta^2\,gamma\,K\,(a+delta+delta\,(-1+m)\,Ns)}\}\}$$

$$\left\{\{M \to 0,\ Is \to 0,\ Ir \to 0,\ Bs \to 0,\ Br \to 0\},\right.$$
$$\{M \to K,\ Is \to 0,\ Ir \to 0,\ Bs \to 0,\ Br \to 0\},$$
$$\left\{M \to -\frac{c\,(a+delta)}{beta\,(a+delta+delta\,(-1+gamma)\,Ns)},\ Is \to 0,\right.$$
$$Ir \to -\left(\left(c\,\left((a+delta)\,(c+beta\,K)+beta\,delta\,(-1+gamma)\,K\,Ns\right)r\right)\Big/\left(beta^2\,K\,(a+delta+delta\,(-1+gamma)\,Ns)^2\right)\right),$$
$$Bs \to 0,\ Br \to \left.\frac{\left((a+delta)\,(c+beta\,K)+beta\,delta\,(-1+gamma)\,K\,Ns\right)r}{beta^2\,K\,(a+delta+delta\,(-1+gamma)\,Ns)}\right\},$$
$$\left\{M \to -\frac{c\,(a+delta)}{beta\,(a+delta+delta\,(-1+m)\,Ns)},\right.$$
$$Is \to -\left(\left(c\,(gamma-m)\,\left((a+delta)\,(c+beta\,K)+beta\,delta\,K\,(-1+m)\,Ns\right)r\right)\Big/\left(beta^2\,gamma\,K\,(a+delta+delta\,(-1+m)\,Ns)^2\right)\right),$$
$$Ir \to -\frac{c\,m\,\left((a+delta)\,(c+beta\,K)+beta\,delta\,K\,(-1+m)\,Ns\right)r}{beta^2\,gamma\,K\,(a+delta+delta\,(-1+m)\,Ns)^2},$$
$$Bs \to \left((gamma-m)\,\left((a+delta)\,(c+beta\,K)+beta\,delta\,K\,(-1+m)\,Ns\right)r\right)\Big/\left(beta^2\,gamma\,K\,(a+delta+delta\,(-1+m)\,Ns)\right),$$
$$Br \to \left.\frac{m\,\left((a+delta)\,(c+beta\,K)+beta\,delta\,K\,(-1+m)\,Ns\right)r}{beta^2\,gamma\,K\,(a+delta+delta\,(-1+m)\,Ns)}\right\}\}$$

Jacobian Matrix

```
row1 = D[eq1, {{M, Is, Ir, Bs, Br}}];
       derivada
row2 = D[eq2, {{M, Is, Ir, Bs, Br}}];
       derivada
row3 = D[eq3, {{M, Is, Ir, Bs, Br}}];
       derivada
row4 = D[eq4, {{M, Is, Ir, Bs, Br}}];
       derivada
row5 = D[eq5, {{M, Is, Ir, Bs, Br}}];
       derivada
Jac = {row1, row2, row3, row4, row5};

MatrixForm[Jac]
forma de matriz
Jac1 = Jac /. eqlist[[1]]
Jac2 = Jac /. eqlist[[2]]
Jac3 = Jac /. eqlist[[3]]
Jac4 = Jac /. eqlist[[4]]
```

$$\begin{pmatrix} -beta\,(Br+Bs)-\frac{M\,r}{K}+\left(1-\frac{M}{K}\right)r & 0 & 0 & -beta\,M & -beta\,M \\ beta\,Bs & -a-delta & 0 & beta\,M & 0 \\ beta\,Br & 0 & -a-delta & 0 & beta\,M \\ -beta\,Bs & delta\,(1-m)\,Ns & 0 & -c-beta\,M & 0 \\ -beta\,Br & delta\,m\,Ns & delta\,(1-gamma)\,Ns & 0 & -c-beta\,M \end{pmatrix}$$

$$\{\{r, 0, 0, 0, 0\}, \{0, -a - \text{delta}, 0, 0, 0\}, \{0, 0, -a - \text{delta}, 0, 0\},$$
$$\{0, \text{delta}\,(1 - m)\,Ns, 0, -c, 0\}, \{0, \text{delta}\,m\,Ns, \text{delta}\,(1 - \text{gamma})\,Ns, 0, -c\}\}$$

$$\{\{-r, 0, 0, -\text{beta}\,K, -\text{beta}\,K\}, \{0, -a - \text{delta}, 0, \text{beta}\,K, 0\}, \{0, 0, -a - \text{delta}, 0, \text{beta}\,K\},$$
$$\{0, \text{delta}\,(1 - m)\,Ns, 0, -c - \text{beta}\,K, 0\}, \{0, \text{delta}\,m\,Ns, \text{delta}\,(1 - \text{gamma})\,Ns, 0, -c - \text{beta}\,K\}\}$$

$$\{\{\frac{c\,(a + \text{delta})\,r}{\text{beta}\,K\,(a + \text{delta} + \text{delta}\,(-1 + \text{gamma})\,Ns)} -$$
$$\frac{((a + \text{delta})\,(c + \text{beta}\,K) + \text{beta}\,\text{delta}\,(-1 + \text{gamma})\,K\,Ns)\,r}{\text{beta}\,K\,(a + \text{delta} + \text{delta}\,(-1 + \text{gamma})\,Ns)} +$$
$$\left(1 + \frac{c\,(a + \text{delta})}{\text{beta}\,K\,(a + \text{delta} + \text{delta}\,(-1 + \text{gamma})\,Ns)}\right) r, 0, 0,$$
$$\frac{c\,(a + \text{delta})}{a + \text{delta} + \text{delta}\,(-1 + \text{gamma})\,Ns}, \frac{c\,(a + \text{delta})}{a + \text{delta} + \text{delta}\,(-1 + \text{gamma})\,Ns}\},$$
$$\{0, -a - \text{delta}, 0, -\frac{c\,(a + \text{delta})}{a + \text{delta} + \text{delta}\,(-1 + \text{gamma})\,Ns}, 0\},$$
$$\{\frac{((a + \text{delta})\,(c + \text{beta}\,K) + \text{beta}\,\text{delta}\,(-1 + \text{gamma})\,K\,Ns)\,r}{\text{beta}\,K\,(a + \text{delta} + \text{delta}\,(-1 + \text{gamma})\,Ns)},$$
$$0, -a - \text{delta}, 0, -\frac{c\,(a + \text{delta})}{a + \text{delta} + \text{delta}\,(-1 + \text{gamma})\,Ns}\},$$
$$\{0, \text{delta}\,(1 - m)\,Ns, 0, -c + \frac{c\,(a + \text{delta})}{a + \text{delta} + \text{delta}\,(-1 + \text{gamma})\,Ns}, 0\},$$
$$\{-\frac{((a + \text{delta})\,(c + \text{beta}\,K) + \text{beta}\,\text{delta}\,(-1 + \text{gamma})\,K\,Ns)\,r}{\text{beta}\,K\,(a + \text{delta} + \text{delta}\,(-1 + \text{gamma})\,Ns)}, \text{delta}\,m\,Ns,$$
$$\text{delta}\,(1 - \text{gamma})\,Ns, 0, -c + \frac{c\,(a + \text{delta})}{a + \text{delta} + \text{delta}\,(-1 + \text{gamma})\,Ns}\}\}$$

$$\left\{\left\{\frac{c\,(a+\text{delta})\,r}{\text{beta}\,K\,(a+\text{delta}+\text{delta}\,(-1+m)\,Ns)}+\left(1+\frac{c\,(a+\text{delta})}{\text{beta}\,K\,(a+\text{delta}+\text{delta}\,(-1+m)\,Ns)}\right)r-\right.\right.$$
$$\text{beta}\left(\left((\text{gamma}-m)\,\left((a+\text{delta})\,(c+\text{beta}\,K)+\text{beta}\,\text{delta}\,K\,(-1+m)\,Ns\right)r\right)\Big/\right.$$
$$\left(\text{beta}^2\,\text{gamma}\,K\,(a+\text{delta}+\text{delta}\,(-1+m)\,Ns)\right)+$$
$$\left.\left.\frac{m\,\left((a+\text{delta})\,(c+\text{beta}\,K)+\text{beta}\,\text{delta}\,K\,(-1+m)\,Ns\right)r}{\text{beta}^2\,\text{gamma}\,K\,(a+\text{delta}+\text{delta}\,(-1+m)\,Ns)}\right),\right.$$
$$0,\,0,\,\frac{c\,(a+\text{delta})}{a+\text{delta}+\text{delta}\,(-1+m)\,Ns},\,\frac{c\,(a+\text{delta})}{a+\text{delta}+\text{delta}\,(-1+m)\,Ns}\Big\},$$
$$\Big\{\left((\text{gamma}-m)\,\left((a+\text{delta})\,(c+\text{beta}\,K)+\text{beta}\,\text{delta}\,K\,(-1+m)\,Ns\right)r\right)\Big/$$
$$\left(\text{beta}\,\text{gamma}\,K\,(a+\text{delta}+\text{delta}\,(-1+m)\,Ns)\right),$$
$$-a-\text{delta},\,0,\,-\frac{c\,(a+\text{delta})}{a+\text{delta}+\text{delta}\,(-1+m)\,Ns},\,0\Big\},$$
$$\left\{\frac{m\,\left((a+\text{delta})\,(c+\text{beta}\,K)+\text{beta}\,\text{delta}\,K\,(-1+m)\,Ns\right)r}{\text{beta}\,\text{gamma}\,K\,(a+\text{delta}+\text{delta}\,(-1+m)\,Ns)},\,0,\right.$$
$$\left.-a-\text{delta},\,0,\,-\frac{c\,(a+\text{delta})}{a+\text{delta}+\text{delta}\,(-1+m)\,Ns}\right\},$$
$$\Big\{-\left(\left((\text{gamma}-m)\,\left((a+\text{delta})\,(c+\text{beta}\,K)+\text{beta}\,\text{delta}\,K\,(-1+m)\,Ns\right)r\right)\Big/\right.$$
$$\left.\left(\text{beta}\,\text{gamma}\,K\,(a+\text{delta}+\text{delta}\,(-1+m)\,Ns)\right)\right),$$
$$\text{delta}\,(1-m)\,Ns,\,0,\,-c+\frac{c\,(a+\text{delta})}{a+\text{delta}+\text{delta}\,(-1+m)\,Ns},\,0\Big\},$$
$$\left\{-\frac{m\,\left((a+\text{delta})\,(c+\text{beta}\,K)+\text{beta}\,\text{delta}\,K\,(-1+m)\,Ns\right)r}{\text{beta}\,\text{gamma}\,K\,(a+\text{delta}+\text{delta}\,(-1+m)\,Ns)},\,\text{delta}\,m\,Ns,\right.$$
$$\left.\text{delta}\,(1-\text{gamma})\,Ns,\,0,\,-c+\frac{c\,(a+\text{delta})}{a+\text{delta}+\text{delta}\,(-1+m)\,Ns}\right\}\Big\}$$

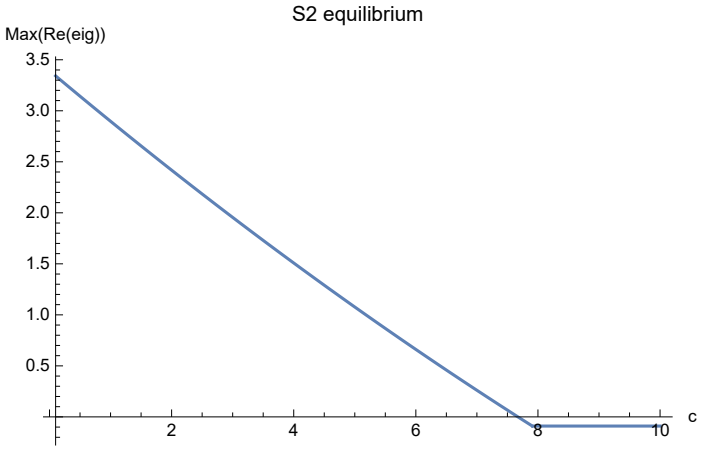
eigenvalues at each equilibrium to determine stability

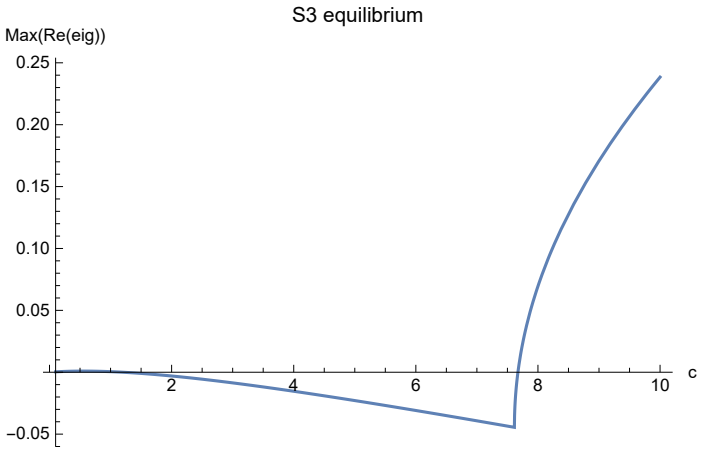
```
eig1 = Eigenvalues[Jac1]
eig2 = Eigenvalues[Jac2]
eig3 = Eigenvalues[Jac3]
eig4 = FullSimplify[Eigenvalues[Jac4]]
```

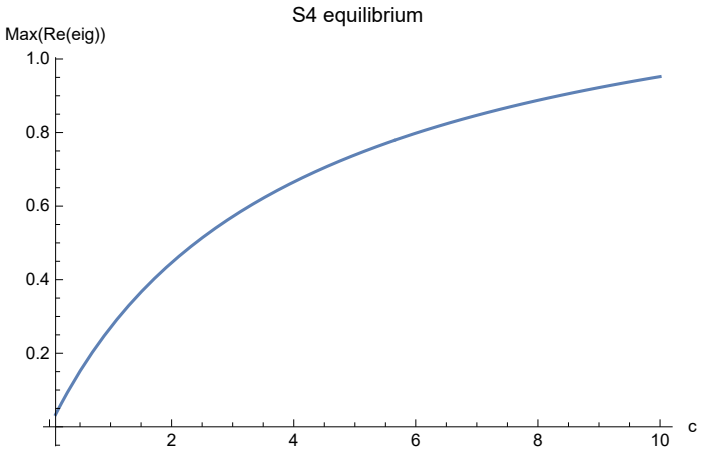
$$\left\{\frac{1}{2}\left(-a-c-\text{delta}-\text{beta}\,K-\sqrt{\left((a+c+\text{delta}+\text{beta}\,K)^2-4\left(a\,c+c\,\text{delta}+a\,\text{beta}\,K+\text{beta}\,\text{delta}\,K-\text{beta}\,\text{delta}\,K\,Ns+\text{beta}\,\text{delta}\,\text{gamma}\,K\,Ns\right)\right)}\right),\right.$$
$$\frac{1}{2}\left(-a-c-\text{delta}-\text{beta}\,K+\sqrt{\left((a+c+\text{delta}+\text{beta}\,K)^2-4\left(a\,c+c\,\text{delta}+a\,\text{beta}\,K+\text{beta}\,\text{delta}\,K-\text{beta}\,\text{delta}\,K\,Ns+\text{beta}\,\text{delta}\,\text{gamma}\,K\,Ns\right)\right)}\right),$$
$$\frac{1}{2}\left(-a-c-\text{delta}-\text{beta}\,K-\sqrt{\left((a+c+\text{delta}+\text{beta}\,K)^2-4\left(a\,c+c\,\text{delta}+a\,\text{beta}\,K+\text{beta}\,\text{delta}\,K-\text{beta}\,\text{delta}\,K\,Ns+\text{beta}\,\text{delta}\,K\,m\,Ns\right)\right)}\right),$$
$$\left.\frac{1}{2}\left(-a-c-\text{delta}-\text{beta}\,K+\sqrt{\left((a+c+\text{delta}+\text{beta}\,K)^2-4\left(a\,c+c\,\text{delta}+a\,\text{beta}\,K+\text{beta}\,\text{delta}\,K-\text{beta}\,\text{delta}\,K\,Ns+\text{beta}\,\text{delta}\,K\,m\,Ns\right)\right)}\right),\ -r\right\}$$

```
r = 0.09;
K = 10^8;
beta = 1.2 * 10^(-7);
delta = 0.2;
a = 12;
gamma = 0.1;(*c=2;*)
Ns = 100;
m = 10^(-7);
eig1 = Eigenvalues[Jac1];
eig2 = Eigenvalues[Jac2];
eig3 = Eigenvalues[Jac3];
eig4 = FullSimplify[Eigenvalues[Jac4]];

ss = {{M → 0, Is → 0, Ir → 0, Bs → 0, Br → 0},
    {M → K, Is → 0, Ir → 0, Bs → 0, Br → 0},
    {M → -(c (a + delta))/(beta (a + delta + delta (-1 + gamma) Ns)), Is → 0,
     Ir → -((c ((a + delta) (c + beta K) + beta delta (-1 + gamma) K Ns) r)/
        (beta^2 K (a + delta + delta (-1 + gamma) Ns)^2)),
     Bs → 0, Br → (((a + delta) (c + beta K) + beta delta (-1 + gamma) K Ns) r)/
```

```
    (beta^2 K (a + delta + delta (-1 + gamma) Ns))},
  {M → -(c (a + delta)) / (beta (a + delta + delta (-1 + m) Ns)),
   Is → -((c (gamma - m) ((a + delta) (c + beta K) + beta delta K (-1 + m) Ns) r) /
      (beta^2 gamma K (a + delta + delta (-1 + m) Ns)^2)),
   Ir → -((c m ((a + delta) (c + beta K) + beta delta K (-1 + m) Ns) r) /
      (beta^2 gamma K (a + delta + delta (-1 + m) Ns)^2)),
   Bs → ((gamma - m) ((a + delta) (c + beta K) + beta delta K (-1 + m) Ns) r) /
     (beta^2 gamma K (a + delta + delta (-1 + m) Ns)),
   Br → (m ((a + delta) (c + beta K) + beta delta K (-1 + m) Ns) r) /
     (beta^2 gamma K (a + delta + delta (-1 + m) Ns))}};
(*Check that the equilibria exist: {M/.ss[[1]]≥0,Is/.ss[[1]]≥0,
  verifica
   Ir/.ss[[1]]≥0,Bs/.ss[[1]]≥0,Br/.ss[[1]]≥0}...*)
(*Check which of the equilibria is stable for that variation in a
  verifica
 given parameter (e.g. c), by plotting the maximum real part,
if it is negative, that equilibrium is stable*)

Plot[Max[Re[eig2]], {c, 0.1, 10},
 grá··  má··  parte real
 AxesLabel → {"c", "Max(Re(eig))"}, PlotLabel → "S2 equilibrium"]
                  má··  parte real      etiqueta de gráfico
Plot[Max[Re[eig3]], {c, 0.1, 10}, AxesLabel → {"c", "Max(Re(eig))"},
      má··  parte real                 legenda dos eixos      má··  parte real
 PlotLabel → "S3 equilibrium"]
 etiqueta de gráfico
Plot[Max[Re[eig4]], {c, 0.1, 10}, AxesLabel → {"c", "Max(Re(eig))"},
      má··  parte real                 legenda dos eixos      má··  parte real
 PlotLabel → "S4 equilibrium"]
 etiqueta de gráfico
```








```
eig1 = Eigenvalues[Jac1];
```
autovalores

```
{-c, -c, -a - delta, -a - delta, r}
```

{-c, -c, -a - delta, -a - delta, r}

```
eig2 = Eigenvalues[Jac2]
```
autovalores

$$\left\{\frac{1}{2}\left(-a-c-\text{delta}-\text{beta}\,K-\sqrt{\left((a+c+\text{delta}+\text{beta}\,K)^2-4\left(a\,c+c\,\text{delta}+a\,\text{beta}\,K+\text{beta}\,\text{delta}\,K-\text{beta}\,\text{delta}\,K\,Ns+\text{beta}\,\text{delta}\,\text{gamma}\,K\,Ns\right)\right)}\right),\right.$$
$$\frac{1}{2}\left(-a-c-\text{delta}-\text{beta}\,K+\sqrt{\left((a+c+\text{delta}+\text{beta}\,K)^2-4\left(a\,c+c\,\text{delta}+a\,\text{beta}\,K+\text{beta}\,\text{delta}\,K-\text{beta}\,\text{delta}\,K\,Ns+\text{beta}\,\text{delta}\,\text{gamma}\,K\,Ns\right)\right)}\right),$$
$$\frac{1}{2}\left(-a-c-\text{delta}-\text{beta}\,K-\sqrt{\left((a+c+\text{delta}+\text{beta}\,K)^2-4\left(a\,c+c\,\text{delta}+a\,\text{beta}\,K+\text{beta}\,\text{delta}\,K-\text{beta}\,\text{delta}\,K\,Ns+\text{beta}\,\text{delta}\,K\,m\,Ns\right)\right)}\right),$$
$$\left.\frac{1}{2}\left(-a-c-\text{delta}-\text{beta}\,K+\sqrt{\left((a+c+\text{delta}+\text{beta}\,K)^2-4\left(a\,c+c\,\text{delta}+a\,\text{beta}\,K+\text{beta}\,\text{delta}\,K-\text{beta}\,\text{delta}\,K\,Ns+\text{beta}\,\text{delta}\,K\,m\,Ns\right)\right)}\right),\ -r\right\}$$

Resistance equilibrium is stable when the infection is strong enough to proliferate in the host, but not too strong to kill. This means that when this equilibrium exists it is not always stable. There are regimes when it is unstable, namely for very slow or very fast infections. On the other hand, coexistence equilibrium seems to always unstable when exists. Resistance persistence equilibrium, which is the worst scenario, is the only case whether we can have a stable persistence equilibrium in the host. However, it is dependent on the fitness cost being lower than the mutation rate.

Analysis of the full model

```
Clear[r, K, beta, delta, a, Ns, c]
apaga
ptot = Bs + Br;
eq1 = r * M * (1 - M / K) - beta * M * ptot;
eq2 = beta * M * Bs - Is * (delta + a + v * E1);
eq3 = beta * M * Br - Ir * (delta + a + v * E1);
eq4 = Ns * (1 - m) * Is * delta - beta * M * Bs - (c  + Am) * Bs;
Am  = 0;
eq5 = Ns * (1 - gamma) * Ir * delta + m * Ns * Is * delta - beta * M  * Br - (c) * Br;
eq6 = sigma * E1 * ptot / (ptot + k);
eqlist = FullSimplify[Solve[
          simplifica compl···resolve
    {eq1 == 0, eq2 == 0, eq3 == 0, eq4 == 0, eq5 == 0, eq6 == 0}, {M, Is, Ir, Bs, Br, E1}]]
```

$$\left\{\left\{M \to 0,\ Ir \to \frac{Is}{-1+gamma},\ Bs \to -\frac{delta\ Is\ (-1+m)\ Ns}{c},\right.\right.$$
$$\left.Br \to \frac{delta\ Is\ (-1+m)\ Ns}{c},\ E1 \to -\frac{a+delta}{v}\right\},\ \{M \to 0,\ Is \to 0,\ Ir \to 0,\ Bs \to 0,\ Br \to 0\},$$
$$\{M \to K,\ Is \to 0,\ Ir \to 0,\ Bs \to 0,\ Br \to 0\},\ \{M \to 0,\ Is \to 0,\ Ir \to 0,\ Bs \to 0,\ Br \to 0,\ E1 \to 0\},$$
$$\{M \to K,\ Is \to 0,\ Ir \to 0,\ Bs \to 0,\ Br \to 0,\ E1 \to 0\},$$
$$\left\{M \to -\frac{c\ (a+delta)}{beta\ (a+delta+delta\ (-1+gamma)\ Ns)},\ Is \to 0,\right.$$
$$Ir \to -\frac{c\ ((a+delta)\ (c+beta\ K)+beta\ delta\ (-1+gamma)\ K\ Ns)\ r}{beta^2\ K\ (a+delta+delta\ (-1+gamma)\ Ns)^2},\ Bs \to 0,$$
$$\left.Br \to \frac{((a+delta)\ (c+beta\ K)+beta\ delta\ (-1+gamma)\ K\ Ns)\ r}{beta^2\ K\ (a+delta+delta\ (-1+gamma)\ Ns)},\ E1 \to 0\right\},$$
$$\left\{M \to -\frac{c\ (a+delta)}{beta\ (a+delta+delta\ (-1+m)\ Ns)},\right.$$
$$Is \to -\frac{c\ (gamma-m)\ ((a+delta)\ (c+beta\ K)+beta\ delta\ K\ (-1+m)\ Ns)\ r}{beta^2\ gamma\ K\ (a+delta+delta\ (-1+m)\ Ns)^2},$$
$$Ir \to -\frac{c\ m\ ((a+delta)\ (c+beta\ K)+beta\ delta\ K\ (-1+m)\ Ns)\ r}{beta^2\ gamma\ K\ (a+delta+delta\ (-1+m)\ Ns)^2},$$
$$Bs \to \frac{(gamma-m)\ ((a+delta)\ (c+beta\ K)+beta\ delta\ K\ (-1+m)\ Ns)\ r}{beta^2\ gamma\ K\ (a+delta+delta\ (-1+m)\ Ns)},$$
$$\left.Br \to \frac{m\ ((a+delta)\ (c+beta\ K)+beta\ delta\ K\ (-1+m)\ Ns)\ r}{beta^2\ gamma\ K\ (a+delta+delta\ (-1+m)\ Ns)},\ E1 \to 0\right\}\}$$

Equilibrium SOLUTIONS

$\left\{M \to 0,\ Br \to \frac{delta\ Is\ (-1+m)\ Ns}{c},\ Bs \to -\frac{delta\ Is\ (-1+m)\ Ns}{c},\ Ir \to \frac{Is}{-1+gamma},\ E1 \to -\frac{a+delta}{v}\right\}$ **is unreal, because Immunity is below 0.**

{M→0,Br→0,Bs→0,Ir→0,Is→0} **Trivial equilibrium with zero immunity and zero uninfected macrophages: --> It can be reached only if bacteria outgrow their resource, driving it to extinction and themselves to extinction. (DEATH of host?)**

{M→K,Br→0,Bs→0,Ir→0,Is→0} -> **Carrying capacity equilibrium for macrophages is possible without infection. The equilibrium refers to a clearance situation or a pre-infection situation, where the macrophages are at carrying capacity. Immunity is a free variable and can have any positive value for this to be true.**

$$\left\{M \to -\frac{c\,(a+\text{delta})}{\text{beta}\,(a+\text{delta}+\text{delta}\,(-1+\text{gamma})\,Ns)},\ Br \to \frac{((a+\text{delta})\,(c+\text{beta}\,K)+\text{beta}\,\text{delta}\,(-1+\text{gamma})\,K\,Ns)\,r}{\text{beta}^2\,K\,(a+\text{delta}+\text{delta}\,(-1+\text{gamma})\,Ns)},\right.$$ **->**

$$\left. Bs \to 0,\ Ir \to -\frac{c\,((a+\text{delta})\,(c+\text{beta}\,K)+\text{beta}\,\text{delta}\,(-1+\text{gamma})\,K\,Ns)\,r}{\text{beta}^2\,K\,(a+\text{delta}+\text{delta}\,(-1+\text{gamma})\,Ns)^2},\ Is \to 0,\ E1 \to 0\right\}$$

Resistant only population persists [Worst case scenario], with Immunity = 0. This is basically a consumer-resource dynamics, maintained at a balance: not too much consumption of M, and sufficiently low mortality to keep both populations going --> chronic infection!

In this equilibrium, the ratio of intracellular vs. extracellular bacteria is given by:

$$\frac{Ir}{Br} = \frac{-c}{a+\text{delta}+\text{delta}\,(-1+\text{gamma})\,Ns},$$ **Death of the extracellular B vs generation potential**

We can have 2 cases:

- First, intracellular population is smaller, when c < delta(1-gamma)Ns-(a+delta)

-Second, intracellular population is higher, when c > delta(1-gamma)Ns-(a+delta)

$$\left\{M \to -\frac{c\,(a+\text{delta})}{\text{beta}\,(a+\text{delta}+\text{delta}\,(-1+m)\,Ns)},\ Br \to \frac{m\,((a+\text{delta})\,(c+\text{beta}\,K)+\text{beta}\,\text{delta}\,K\,(-1+m)\,Ns)\,r}{\text{beta}^2\,\text{gamma}\,K\,(a+\text{delta}+\text{delta}\,(-1+m)\,Ns)},\right.$$ **-> Coexis-**

$$Bs \to \frac{(\text{gamma}-m)\,((a+\text{delta})\,(c+\text{beta}\,K)+\text{beta}\,\text{delta}\,K\,(-1+m)\,Ns)\,r}{\text{beta}^2\,\text{gamma}\,K\,(a+\text{delta}+\text{delta}\,(-1+m)\,Ns)},$$

$$Ir \to -\frac{c\,m\,((a+\text{delta})\,(c+\text{beta}\,K)+\text{beta}\,\text{delta}\,K\,(-1+m)\,Ns)\,r}{\text{beta}^2\,\text{gamma}\,K\,(a+\text{delta}+\text{delta}\,(-1+m)\,Ns)^2},$$

$$Is \to -\frac{c\,(\text{gamma}-m)\,((a+\text{delta})\,(c+\text{beta}\,K)+\text{beta}\,\text{delta}\,K\,(-1+m)\,Ns)\,r}{\text{beta}^2\,\text{gamma}\,K\,(a+\text{delta}+\text{delta}\,(-1+m)\,Ns)^2},\ E1 \to 0\Big\}$$

tence of bacteria population equilibrium again without immunity

In this equilibrium, $\frac{Ir}{Br} = \frac{Ir+Is}{Br+Bs} = \frac{-c}{a+\text{delta}+\text{delta}\,(-1+m)\,Ns}$,

$\frac{Is}{Ir} = \frac{Bs}{Br} = \frac{\text{gamma}-m}{m}$, **which means that the ratio of extracellular populations and intracellular is mantained at the same level, depending only on the cost of fitness by mutations and the**

mutation rate itself.
In addition, susceptible populations will always be higher than the resistant ones because the fitnes cost of resistance gamma is typically higher than the mutation probability. Another important information is that the rate of intracellular bacteria should be lower in this equilibrium than in Resistant-Only equilibrium, when comparing with extracellular populations, because m < gamma.

Conditions for Coexistance to exist:

1)gamma > m , for the rate to be bigger than zero and positive!

2) $\frac{gamma}{m}$ **< 2 Br > Bs**

3) $\frac{gamma}{m}$ **> 2, Bs > Br**

gamma is responsible for the cost of fitness, so if it is high enough, resistant won't be able to grow larger than the susceptible population. This has an inverse relation with mutation, the higher the mutation rate, the higher capacity of resistant to compete with susceptible.

$$\frac{M* \text{ in resistance}}{M* \text{ in coexistance}} = \frac{a+delta+delta\ (-1+gamma)\ Ns}{a+delta+delta\ (-1+m)\ Ns}$$

$$M^* = \left(\frac{a+delta}{beta}\right)* \frac{Ir*}{Br*}$$

$$\frac{Ir* \text{ in resistance}}{Is* + Ir* \text{ in coexistance}} =$$

$$\left(((a + delta)\ (c + beta\ K) + beta\ delta\ (-1 + gamma)\ K\ Ns)\ (a + delta + delta\ (-1 + m)\ Ns)^2 \right) \Big/ \left((a + delta + delta\ (-1 + gamma)\ Ns)^2\ ((a + delta)\ (c + beta\ K) + beta\ delta\ K\ (-1 + m)\ Ns) \right)$$

$$\frac{Br* \text{ in resistance}}{Bs* + Br* \text{ in coexistance}} =$$

$$\begin{aligned}&((a+\text{delta})\ (c+\text{beta}\,K)+\\&\quad\text{beta}\,\text{delta}\ (-1+\text{gamma})\ K\,Ns)\\&\quad(a+\text{delta}+\text{delta}\ (-1+m)\ Ns))\ /\\&((a+\text{delta}+\text{delta}\ (-1+\text{gamma})\ Ns)\\&\quad((a+\text{delta})\ (c+\text{beta}\,K)+\\&\quad\text{beta}\,\text{delta}\,K\ (-1+m)\ Ns))\end{aligned}$$

Values that variables assume in equilibrium

```
{Br, Bs, E1, Ir, Is, M} /. eqlist
```

$$\begin{aligned}&\Big\{\Big\{\frac{\text{delta Is}\ (-1+m)\ Ns}{c},\ -\frac{\text{delta Is}\ (-1+m)\ Ns}{c},\ -\frac{a+\text{delta}}{v},\ \frac{Is}{-1+\text{gamma}},\ Is,\ 0\Big\},\\&\{0,\ 0,\ E1,\ 0,\ 0,\ 0\},\ \{0,\ 0,\ E1,\ 0,\ 0,\ K\},\ \{0,\ 0,\ 0,\ 0,\ 0,\ 0\},\\&\{0,\ 0,\ 0,\ 0,\ 0,\ K\},\ \Big\{\frac{((a+\text{delta})\ (c+\text{beta}\,K)+\text{beta}\,\text{delta}\ (-1+\text{gamma})\ K\,Ns)\ r}{\text{beta}^2\,K\ (a+\text{delta}+\text{delta}\ (-1+\text{gamma})\ Ns)},\\&0,\ 0,\ -\frac{c\ ((a+\text{delta})\ (c+\text{beta}\,K)+\text{beta}\,\text{delta}\ (-1+\text{gamma})\ K\,Ns)\ r}{\text{beta}^2\,K\ (a+\text{delta}+\text{delta}\ (-1+\text{gamma})\ Ns)^2},\\&0,\ -\frac{c\ (a+\text{delta})}{\text{beta}\ (a+\text{delta}+\text{delta}\ (-1+\text{gamma})\ Ns)}\Big\},\\&\Big\{\frac{m\ ((a+\text{delta})\ (c+\text{beta}\,K)+\text{beta}\,\text{delta}\,K\ (-1+m)\ Ns)\ r}{\text{beta}^2\,\text{gamma}\,K\ (a+\text{delta}+\text{delta}\ (-1+m)\ Ns)},\\&((\text{gamma}-m)\ ((a+\text{delta})\ (c+\text{beta}\,K)+\text{beta}\,\text{delta}\,K\ (-1+m)\ Ns)\ r)\ /\\&(\text{beta}^2\,\text{gamma}\,K\ (a+\text{delta}+\text{delta}\ (-1+m)\ Ns)),\ 0,\\&-\frac{c\,m\ ((a+\text{delta})\ (c+\text{beta}\,K)+\text{beta}\,\text{delta}\,K\ (-1+m)\ Ns)\ r}{\text{beta}^2\,\text{gamma}\,K\ (a+\text{delta}+\text{delta}\ (-1+m)\ Ns)^2},\\&-\Big((c\ (\text{gamma}-m)\ ((a+\text{delta})\ (c+\text{beta}\,K)+\text{beta}\,\text{delta}\,K\ (-1+m)\ Ns)\ r)\ /\\&\quad(\text{beta}^2\,\text{gamma}\,K\ (a+\text{delta}+\text{delta}\ (-1+m)\ Ns)^2)\Big),\ -\frac{c\ (a+\text{delta})}{\text{beta}\ (a+\text{delta}+\text{delta}\ (-1+m)\ Ns)}\Big\}\Big\}\end{aligned}$$

Jacobian Matrix

```
Jac1 = D[eq1, {{M, Is, Ir, Bs, Br, E1}}];
```
derivada
```
Jac2 = D[eq2, {{M, Is, Ir, Bs, Br, E1}}];
```
derivada
```
Jac3 = D[eq3, {{M, Is, Ir, Bs, Br, E1}}];
```
derivada
```
Jac4 = D[eq4, {{M, Is, Ir, Bs, Br, E1}}];
```
derivada
```
Jac5 = D[eq5, {{M, Is, Ir, Bs, Br, E1}}];
```
derivada
```
Jac6 = D[eq6, {{M, Is, Ir, Bs, Br, E1}}];
```
derivada
```
Jac = {Jac1, Jac2, Jac3, Jac4, Jac5, Jac6};

MatrixForm[Jac]
```
forma de matriz
```
Jac1 = Jac /. eqlist[[1]];
Jac2 = Jac /. eqlist[[2]];
Jac3 = Jac /. eqlist[[3]];
Jac4 = Jac /. eqlist[[4]];
Jac5 = Jac /. eqlist[[5]];
Jac6 = Jac /. eqlist[[6]];
```

$$\begin{pmatrix} -\text{beta}\,(\text{Br}+\text{Bs}) - \frac{\text{M r}}{\text{K}} + \left(1-\frac{\text{M}}{\text{K}}\right)\text{r} & 0 & 0 & -\text{beta M} \\ \text{beta Bs} & -\text{a} - \text{delta} - \text{E1 v} & 0 & \text{beta M} \\ \text{beta Br} & 0 & -\text{a} - \text{delta} - \text{E1 v} & 0 \\ -\text{beta Bs} & \text{delta}\left(1-\text{m}\right)\text{Ns} & 0 & -\text{c} - \text{beta M} \\ -\text{beta Br} & \text{delta m Ns} & \text{delta}\left(1-\text{gamma}\right)\text{Ns} & 0 \\ 0 & 0 & 0 & -\frac{(\text{Br}+\text{Bs})\ \text{E1 sigma}}{(\text{Br}+\text{Bs}+\text{k})^2} + \frac{\text{E1 sigma}}{\text{Br}+\text{Bs}+\text{k}} \end{pmatrix}$$

```
TableForm[%14]
```
forma de tabela

$-\text{beta}\,(\text{Br}+\text{Bs}) - \frac{\text{M r}}{\text{K}} + \left(1-\frac{\text{M}}{\text{K}}\right)\text{r}$	0	0	$-\text{beta M}$
beta Bs	$-\text{a} - \text{delta} - \text{E1 v}$	0	beta M
beta Br	0	$-\text{a} - \text{delta} - \text{E1 v}$	0
$-\text{beta Bs}$	$\text{delta}\left(1-\text{m}\right)\text{Ns}$	0	$-\text{c} - \text{beta M}$
$-\text{beta Br}$	delta m Ns	$\text{delta}\left(1-\text{gamma}\right)\text{Ns}$	0
0	0	0	$-\frac{(\text{Br}+\text{Bs})\ \text{E1 sigma}}{(\text{Br}+\text{Bs}+\text{k})^2} + \frac{\text{E1}}{\text{B}}$

Eigenvalues at each equilibrium

Eigenvalues[Jac1]

autovalores

Eigenvalues[Jac2]

autovalores

Eigenvalues[Jac3]

autovalores

Eigenvalues[Jac4]

autovalores

Eigenvalues[Jac5]

autovalores

Eigenvalues[Jac6]

autovalores

$$\left\{0, 0, 0, r, \frac{c^3\, k\, v - c^3\, \text{gamma}\, k\, v}{c^2\, \left(-1 + \text{gamma}\right)\, k\, v}, \frac{c^3\, k\, v - c^3\, \text{gamma}\, k\, v}{c^2\, \left(-1 + \text{gamma}\right)\, k\, v}\right\}$$

$$\{0, -c, -c, r, -a - \text{delta} - E1\, v, -a - \text{delta} - E1\, v\}$$

$$\left\{0, -r, \frac{1}{2\, k}\left(-a\, k - c\, k - \text{delta}\, k - \text{beta}\, k\, K - E1\, k\, v - \sqrt{\left(\left(a\, k + c\, k + \text{delta}\, k + \text{beta}\, k\, K + E1\, k\, v\right)^2 - 4\left(a\, c\, k^2 + c\, \text{delta}\, k^2 + a\, \text{beta}\, k^2\, K + \text{beta}\, \text{delta}\, k^2\, K - \text{beta}\, \text{delta}\, k^2\, K\, Ns + \text{beta}\, \text{delta}\, \text{gamma}\, k^2\, K\, Ns + c\, E1\, k^2\, v + \text{beta}\, E1\, k^2\, K\, v\right)\right)}\right),\right.$$

$$\frac{1}{2\, k}\left(-a\, k - c\, k - \text{delta}\, k - \text{beta}\, k\, K - E1\, k\, v + \sqrt{\left(\left(a\, k + c\, k + \text{delta}\, k + \text{beta}\, k\, K + E1\, k\, v\right)^2 - 4\left(a\, c\, k^2 + c\, \text{delta}\, k^2 + a\, \text{beta}\, k^2\, K + \text{beta}\, \text{delta}\, k^2\, K - \text{beta}\, \text{delta}\, k^2\, K\, Ns + \text{beta}\, \text{delta}\, \text{gamma}\, k^2\, K\, Ns + c\, E1\, k^2\, v + \text{beta}\, E1\, k^2\, K\, v\right)\right)}\right),$$

$$\frac{1}{2\, k}\left(-a\, k - c\, k - \text{delta}\, k - \text{beta}\, k\, K - E1\, k\, v - \sqrt{\left(\left(a\, k + c\, k + \text{delta}\, k + \text{beta}\, k\, K + E1\, k\, v\right)^2 - 4\left(a\, c\, k^2 + c\, \text{delta}\, k^2 + a\, \text{beta}\, k^2\, K + \text{beta}\, \text{delta}\, k^2\, K - \text{beta}\, \text{delta}\, k^2\, K\, Ns + \text{beta}\, \text{delta}\, k^2\, K\, m\, Ns + c\, E1\, k^2\, v + \text{beta}\, E1\, k^2\, K\, v\right)\right)}\right),$$

$$\left.\frac{1}{2\, k}\left(-a\, k - c\, k - \text{delta}\, k - \text{beta}\, k\, K - E1\, k\, v + \sqrt{\left(\left(a\, k + c\, k + \text{delta}\, k + \text{beta}\, k\, K + E1\, k\, v\right)^2 - 4\left(a\, c\, k^2 + c\, \text{delta}\, k^2 + a\, \text{beta}\, k^2\, K + \text{beta}\, \text{delta}\, k^2\, K - \text{beta}\, \text{delta}\, k^2\, K\, Ns + \text{beta}\, \text{delta}\, k^2\, K\, m\, Ns + c\, E1\, k^2\, v + \text{beta}\, E1\, k^2\, K\, v\right)\right)}\right)\right\}$$

$$\{0, -c, -c, -a - \text{delta}, -a - \text{delta}, r\}$$

$$\left\{0,\ \frac{1}{2}\left(-a-c-\text{delta}-\text{beta}\,K-\sqrt{\left((a+c+\text{delta}+\text{beta}\,K)^2-4\left(a\,c+c\,\text{delta}+a\,\text{beta}\,K+\text{beta}\,\text{delta}\,K-\text{beta}\,\text{delta}\,K\,Ns+\text{beta}\,\text{delta}\,\text{gamma}\,K\,Ns\right)\right)}\right),\right.$$
$$\frac{1}{2}\left(-a-c-\text{delta}-\text{beta}\,K+\sqrt{\left((a+c+\text{delta}+\text{beta}\,K)^2-4\left(a\,c+c\,\text{delta}+a\,\text{beta}\,K+\text{beta}\,\text{delta}\,K-\text{beta}\,\text{delta}\,K\,Ns+\text{beta}\,\text{delta}\,\text{gamma}\,K\,Ns\right)\right)}\right),$$
$$\frac{1}{2}\left(-a-c-\text{delta}-\text{beta}\,K-\sqrt{\left((a+c+\text{delta}+\text{beta}\,K)^2-4\left(a\,c+c\,\text{delta}+a\,\text{beta}\,K+\text{beta}\,\text{delta}\,K-\text{beta}\,\text{delta}\,K\,Ns+\text{beta}\,\text{delta}\,K\,m\,Ns\right)\right)}\right),$$
$$\left.\frac{1}{2}\left(-a-c-\text{delta}-\text{beta}\,K+\sqrt{\left((a+c+\text{delta}+\text{beta}\,K)^2-4\left(a\,c+c\,\text{delta}+a\,\text{beta}\,K+\text{beta}\,\text{delta}\,K-\text{beta}\,\text{delta}\,K\,Ns+\text{beta}\,\text{delta}\,K\,m\,Ns\right)\right)}\right),\ -r\right\}$$

… 1 …

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Conditions for variable to be positive - Resistant-Only Equilibrium

```
condExist = Reduce[-(c (a + delta))/(beta (a + delta + delta (-1 + gamma) Ns)) > 0 &&
   -(c ((a + delta) (c + beta K) + beta delta (-1 + gamma) K Ns) r)/(beta^2 K (a + delta + delta (-1 + gamma) Ns)^2) > 0 &&
   ((a + delta) (c + beta K) + beta delta (-1 + gamma) K Ns) r/(beta^2 K (a + delta + delta (-1 + gamma) Ns)) > 0 &&
  c > 0 && a > 0 && beta > 0 && K > 0 && delta > 0 && 0 < m < 1 &&
  0 < gamma < 1 && sigma > 0 && r > 0 && Ns > 0 && k > 0 && v > 0,
 {c, a, beta, delta, m, gamma, sigma, r, Ns, K, k, v}]
```

reduz

$$c>0\ \&\&\ a>0\ \&\&\ \text{beta}>0\ \&\&\ \text{delta}>0\ \&\&\ 0<m<1\ \&\&$$
$$0<\text{gamma}<1\ \&\&\ \text{sigma}>0\ \&\&\ r>0\ \&\&\ Ns>\frac{-a-\text{delta}}{-\text{delta}+\text{delta}\,\text{gamma}}\ \&\&$$
$$K>\frac{-a\,c-c\,\text{delta}}{a\,\text{beta}+\text{beta}\,\text{delta}-\text{beta}\,\text{delta}\,Ns+\text{beta}\,\text{delta}\,\text{gamma}\,Ns}\ \&\&\ k>0\ \&\&\ v>0$$

For resistant-only equilibrium to exist , Ns > $\frac{-a-\text{delta}}{-\text{delta}+\text{delta}\,\text{gamma}}$ and K > $\frac{-a\,c-c\,\text{delta}}{a\,\text{beta}+\text{beta}\,\text{delta}-\text{beta}\,\text{delta}\,Ns+\text{beta}\,\text{delta}\,\text{gamma}\,Ns}$

Conditions for variables to be positive - Coexistance Equilibrium

```
condExistC = Reduce[ - c (a + delta) / (beta (a + delta + delta (-1 + m) Ns)) > 0 &&
    - ((c (gamma - m) ((a + delta) (c + beta K) + beta delta K (-1 + m) Ns) r) /
        (beta^2 gamma K (a + delta + delta (-1 + m) Ns)^2)) > 0 &&
    - (c m ((a + delta) (c + beta K) + beta delta K (-1 + m) Ns) r) /
        (beta^2 gamma K (a + delta + delta (-1 + m) Ns)^2) > 0 &&
    ((gamma - m) ((a + delta) (c + beta K) + beta delta K (-1 + m) Ns) r) /
        (beta^2 gamma K (a + delta + delta (-1 + m) Ns)) > 0 &&
    (m ((a + delta) (c + beta K) + beta delta K (-1 + m) Ns) r) /
        (beta^2 gamma K (a + delta + delta (-1 + m) Ns)) > 0 && c > 0 &&
    a > 0 && beta > 0 && K > 0 && delta > 0 && 0 < m < 1 &&
    0 < gamma < 1 && sigma > 0 && r > 0 && Ns > 0 && k > 0 && v > 0,
   {c, a, beta, delta, m, gamma, sigma, r, Ns, K, k, v}]
```

$$c > 0 \&\& a > 0 \&\& \text{beta} > 0 \&\& \text{delta} > 0 \&\& 0 < m < 1 \&\& m < \text{gamma} < 1 \&\& \text{sigma} > 0 \&\& r > 0 \&\& Ns > \frac{-a-\text{delta}}{-\text{delta}+\text{delta}\,m} \&\& K > \frac{-a\,c-c\,\text{delta}}{a\,\text{beta}+\text{beta}\,\text{delta}-\text{beta}\,\text{delta}\,Ns+\text{beta}\,\text{delta}\,m\,Ns} \&\& k > 0 \&\& v > 0$$

For the coexistance equilibrium to exist , $Ns > \frac{-a-\text{delta}}{-\text{delta}+\text{delta}\,m}$ and $K > \frac{-a\,c-c\,\text{delta}}{a\,\text{beta}+\text{beta}\,\text{delta}-\text{beta}\,\text{delta}\,Ns+\text{beta}\,\text{delta}\,m\,Ns}$

Stability Analysis

Resistant-Only Equilibrium - Stability

```
eigs5 = Simplify[Eigenvalues[Jac5]];
Reduce[eigs5[[1]] < 0 && eigs5[[2]] < 0 && eigs5[[3]] < 0 &&
  eigs5[[4]] < 0 && eigs5[[5]] < 0 && eigs5[[6]] < 0 && a > 0 &&
  c > 0 && delta > 0 && beta > 0 && K > 0 && Ns > 0 && m > 0 && gamma > 0]
```

```
False
```

Resistant equilibrium is not stable.

```
Reduce[eigs5[[1]] <= 0 && eigs5[[2]] <= 0 && eigs5[[3]] <= 0 &&
  eigs5[[4]] <= 0 && eigs5[[5]] <= 0 && eigs5[[6]] <= 0 && a > 0 &&
  c > 0 && delta > 0 && beta > 0 && K > 0 && Ns > 0 && m > 0 && gamma > 0]
```

reduz

$$
\begin{aligned}
&r \geq 0 \&\& \text{delta} > 0 \&\& a > 0 \&\& c > 0 \&\& K > 0 \&\& \\
&\text{beta} > 0 \&\& \left(\left(0 < Ns \leq \frac{a\,c + c\,\text{delta} + a\,\text{beta}\,K + \text{beta}\,\text{delta}\,K}{\text{beta}\,\text{delta}\,K} \&\& \right.\right. \\
&\quad 0 < m \leq \frac{1}{4\,\text{beta}\,\text{delta}\,K\,Ns} \left(a^2 - 2\,a\,c + c^2 + 2\,a\,\text{delta} - 2\,c\,\text{delta} + \text{delta}^2 - \right. \\
&\qquad \left. 2\,a\,\text{beta}\,K + 2\,\text{beta}\,c\,K - 2\,\text{beta}\,\text{delta}\,K + \text{beta}^2\,K^2 + 4\,\text{beta}\,\text{delta}\,K\,Ns\right) \&\& \\
&\quad 0 < \text{gamma} \leq \frac{1}{4\,\text{beta}\,\text{delta}\,K\,Ns} \left(a^2 - 2\,a\,c + c^2 + 2\,a\,\text{delta} - 2\,c\,\text{delta} + \text{delta}^2 - \right. \\
&\qquad \left.\left. 2\,a\,\text{beta}\,K + 2\,\text{beta}\,c\,K - 2\,\text{beta}\,\text{delta}\,K + \text{beta}^2\,K^2 + 4\,\text{beta}\,\text{delta}\,K\,Ns\right)\right) \;||\; \\
&\quad \left(Ns > \frac{a\,c + c\,\text{delta} + a\,\text{beta}\,K + \text{beta}\,\text{delta}\,K}{\text{beta}\,\text{delta}\,K} \&\& \right. \\
&\qquad \frac{-a\,c - c\,\text{delta} - a\,\text{beta}\,K - \text{beta}\,\text{delta}\,K + \text{beta}\,\text{delta}\,K\,Ns}{\text{beta}\,\text{delta}\,K\,Ns} \leq m \leq \\
&\qquad \frac{1}{4\,\text{beta}\,\text{delta}\,K\,Ns} \left(a^2 - 2\,a\,c + c^2 + 2\,a\,\text{delta} - 2\,c\,\text{delta} + \text{delta}^2 - \right. \\
&\qquad\quad \left. 2\,a\,\text{beta}\,K + 2\,\text{beta}\,c\,K - 2\,\text{beta}\,\text{delta}\,K + \text{beta}^2\,K^2 + 4\,\text{beta}\,\text{delta}\,K\,Ns\right) \&\& \\
&\qquad \frac{-a\,c - c\,\text{delta} - a\,\text{beta}\,K - \text{beta}\,\text{delta}\,K + \text{beta}\,\text{delta}\,K\,Ns}{\text{beta}\,\text{delta}\,K\,Ns} \leq \text{gamma} \leq \\
&\qquad \frac{1}{4\,\text{beta}\,\text{delta}\,K\,Ns} \left(a^2 - 2\,a\,c + c^2 + 2\,a\,\text{delta} - 2\,c\,\text{delta} + \text{delta}^2 - \right. \\
&\qquad\quad \left.\left.\left. 2\,a\,\text{beta}\,K + 2\,\text{beta}\,c\,K - 2\,\text{beta}\,\text{delta}\,K + \text{beta}^2\,K^2 + 4\,\text{beta}\,\text{delta}\,K\,Ns\right)\right)\right)
\end{aligned}
$$

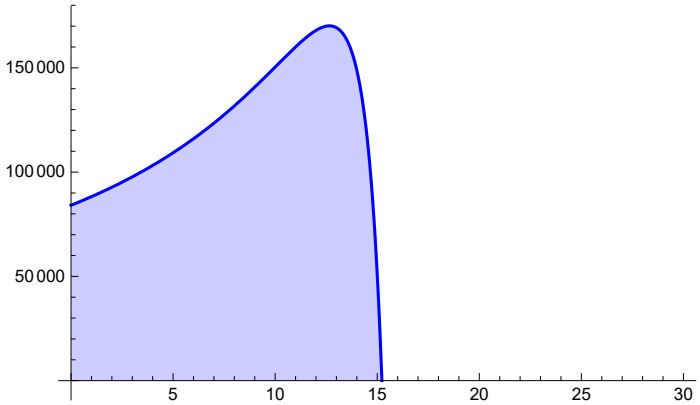
```
Reduce[
  (Sqrt[(a + c + delta + beta K)^2 - 4 (a c + c delta + a beta K + beta delta K - beta delta K Ns + beta delta gamma K Ns)] + -a -
     c - delta - beta K) < 0 && (-a - c - delta - beta K +
     Sqrt[(a + c + delta + beta K)^2 - 4 (a c + c delta + a beta K + beta delta K - beta delta K Ns + beta delta K m Ns)]) < 0]
```

reduz

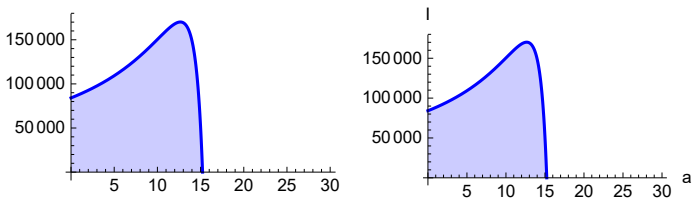
$$
(\text{beta} \mid \text{gamma} \mid K \mid m \mid Ns) \in \mathbb{R} \;\&\&\; \left((a \leq 0 \;\&\&\; (\cdots 1 \cdots)) \;||\; (a > 0 \;\&\&\; (\cdots 1 \cdots)) \right)
$$

large output | **show less** | **show more** | **show all** | **set size limit...**

```
r = 0.09;
K = 10^8;
beta = 1.2 * 10^(-7);
delta = 0.2;
gamma = 0.1;
Ns = 100;
Bext = 0.1;
v = 1 * 10^(-5);
sigma = 2;
k = 10^4;
m = 10^(-7);
c = 2;
Tmax = 100;
region2 = condExist && Max[Re[Eigenvalues[Jac5]]] < 0 && Ir /. eqlist[[6]] ;
                          má·· p··· autovalores
region1 = condExist  && Ir /. eqlist[[6]] ;
g1 = Plot[{region1, region2}, {a, 0, 30},
      gráfico
    PlotStyle → {Blue, {Red, Thick}}, Filling → Bottom];
    estilo do gráfico  azul   ve···  espesso   coloração   inferior
Show[
mostra
 g1]
```




```
region3 = condExist && Max[Re[Eigenvalues[Jac5]]] < 0 &&
                          má·· p··· autovalores
     Max[Im[Eigenvalues[Jac5]]] > 0 && Ir /. eqlist[[6]] ;
          p··· autovalores
g2 = Plot[{region1, region2, region3}, {a, 0, 30}, PlotStyle →
      gráfico                                    estilo do gráfico
     {Blue, {Red, Thick}, {Green, Thick}}, Filling → Bottom, AxesLabel -> {"a ", "I"}];
       azul   ve···  espesso   verde   espesso   coloração   inferior   legenda dos eixos          unidade
GraphicsRow[{Show[g1], Show[g2]}]
linha de gráficos  mostra      mostra
```



Coexistance Equilibrium - Stability

```
eigs6 = Eigenvalues[Jac6];
        autovalores
Reduce[eigs6[[1]] < 0 && eigs6[[2]] < 0 &&
reduz
  eigs6[[3]] < 0 && eigs6[[4]] < 0 && eigs6[[5]] < 0 && eigs6[[6]] < 0 ]
```