UNIVERSIDADE DE LISBOA
FACULDADE DE CIÊNCIAS
DEPARTAMENTO DE BIOLOGIA VEGETAL

MODELLING EVOLUTIONARY DYNAMICS OF BACTERIAL INFECTIONS UNDER TREATMENT

Joana Alexandra Santos Teixeira

MESTRADO EM BIOINFORMÁTICA E BIOLOGIA COMPUTACIONAL
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Dissertação orientada por:
Dra. Erida Gjini e Prof. Dra. Sara Newbery Raposo Magalhães

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Resumo

Infeções bacterianas que apresentam resistência a um ou mais antibióticos não são um problema recente na prática da Medicina. Uma prova disso é Alexander Fleming, autor dos primeiros registos relacionados com antibióticos, que foi também dos primeiros a observar situações de resistência. No entanto, ao longo dos últimos anos, esta problemática tem-se agravado e tornou-se uma prioridade global no que diz respeito à Saúde Pública. A gravidade é de tal nível que esta situação já é reconhecida como uma ameaça colossal ao núcleo da Medicina Moderna pela Organização Mundial da Saúde, tendo esta instituição também já lançado uma lista de agentes bacterianos para os quais é mais urgente o desenvolvimento de novas terapêuticas. Para além disto, esta temática apresenta impactos de carácter económico que têm aumentando, o que tornam ainda mais urgente o controlo e, num nível mais avançado, a prevenção da resistência a antibióticos. Vários campos de pesquisa, como a Epidemiologia Evolutiva e Clínica ou a Modelação Matemática de Infeções e Transmissão de Doenças têm focalizado grande parte das suas pesquisas nesta questão.

Dado o aumento significativo do número de agentes bacterianos resistentes a um ou mais medicamentos antimicrobianos e a incapacidade da indústria química de criar novos antibióticos a um ritmo que faça frente a esta onda de resistência, a comunidade científica tem-se dedicado a este assunto de diferentes formas. Para além de já existirem, por exemplo, bases de dados onde é possível identificar a que substâncias é resistente cada bactéria, é frequente encontrar, na literatura científica, vários modelos biomatemáticos associados ao tema. Na maioria deles, o principal objetivo é identificar, entre os diversos potenciais tipos de tratamento antibacteriano, aqueles que minimizam tanto quanto possível a seleção de resistência a medicamentos, sem

comprometer a saúde do paciente. Serão os tratamentos mais agressivos os ideais para alcançar este propósito? Ou a resposta implicará alterar todo a paradigma associado ao uso dos antibióticos, tornando a sua administração flexível e em função da resposta do paciente, em tempo real?

Apesar de todo o progresso significativo alcançado nos últimos anos, continua a ser uma tarefa árdua travar o surgimento de resistência a novas terapias antimicrobianas. Para além disso, lidar com agentes bacterianos que já apresentam resistência continua a tratar-se de uma tarefa ingrata ao nível da prática clínica. Neste momento, tudo aponta para que a chave deste enigma implique explorar e conhecer os diferentes mecanismos de controlo durante as infeções bacterianas e ainda as dinâmicas evolutivas nos diferentes cenários de doença.

Nesta dissertação, abordamos essa questão desenvolvendo vários modelos matemáticos e explorando-os através de diferentes ferramentas computacionais, desde análise numérica até séries de simulações. Para isso, estabelecemos inicialmente três cenários biológicos que descrevem o estado da infeção bacteriana. Um primeiro, denominado colonização, para a situação em que, apesar do hospedeiro estar infetado por bactérias, não há estimulação de resposta imunitária e a infeção mantém-se sob controlo por ação da densidade equilibrada máxima. Um outro, apresentado como persistência em que, por sua vez, a existência de bactérias, em valores mais elevados, implica uma consequente resposta imunitária. Este cenário pode ser associado na prática clínica a uma infeção estacionária crónica. Um último é definido como eliminação, momento a partir do qual o hospedeiro está livre da infeção, panorama comum após uma infeção aguda.

Primeiramente, recorremos a modelos determinísticos. As grandes novidades, quando comparados com os modelos já propostos na literatura, surgem na modelação logística do crescimento bacteriano e ainda na utilização de uma equação única para descrever toda a resposta imunitária. Estes são utilizados para analisar as condições de

equilíbrio que permitem a passagem de um cenário de infeção para outro, entre colonização, persistência e eliminação. Estes resultados são repetidos para infeções bacterianas sem e com tratamento. A administração de agentes antimicrobianos é modelada, nesta dissertação, recorrendo a diferentes abordagens, que em última análise, são comparadas entre si. Os resultados apontam para que a modelação clássica e mais simples, que implica uma dose constante ao longo do período de tratamento, é representativa do processo. Contudo, tanto a farmacodinâmica das drogas como a sua eficiência podem ser modeladas de outras maneiras, o que poderá influenciar os resultados e trazer novos conhecimentos para a área.

Este tipo de modelo permite um estudo assintótico, descrito acima, mas também uma análise das dinâmicas transientes. Nesse campo, foram comparadas infeções bacterianas crónicas e agudas. No primeiro caso, foi observado que o início da administração do antibiótico em diferentes dias, que correspondem a diferentes combinações de bactérias sensíveis e resistentes, vai resultar em diferentes desenlaces para o hospedeiro. No caso de se tratar de uma infeção aguda e considerando os valores dos parâmetros usados na dissertação, o hospedeiro é capaz de eliminar todas as subpopulações bacterianas, sem recorrer a qualquer tratamento, apenas por ação do seu sistema imunitário. As consequências do uso de antibióticos podem, neste caso, ser dúbias: o tratamento tanto pode resultar na seleção de bactérias resistentes, fazendo com que a infeção piore e acabe por progredir para um caso crónico, como pode acelerar o processo de cura, reduzindo os efeitos prejudicais para o hospedeiro.

Os últimos resultados da dissertação surgem associados às dinâmicas evolutivas das infeções bacterianas com tratamento. Neste campo, são estudadas em particular infeções bacterianas agudas cujo tratamento é iniciado antes do sistema imunitário estar a funcionar no seu pleno. O modelo matemático híbrido apresentado aqui tem uma componente na qual a estocasticidade é imposta no surgimento de

novas estirpes bacterianas e uma outra componente determinística, associada ao crescimento bacteriano. Cada estirpe bacteriana é caracterizada por dois traços fenotípicos: o custo na taxa de crescimento exponencial intrínseca e a suscetibilidade aos antibióticos. Este modelo é usado como uma ferramenta exploratória para simular e estudar a seleção de resistência. É também através dele que se estuda o impacto de diferentes tipos de tratamento, variando a sua dose e duração e que nesta dissertação surgem em cinco grupos diferenciados: tratamento com dose baixa e duração baixa; tratamento com dose alta e duração baixa; tratamento com dose média e duração média; tratamento com dose baixa e duração alta; e ainda tratamento com dose alta e duração alta. Os resultados preliminares mostram que a ideia geral de que tratamentos agressivos (doses e durações mais altas) resultam numa maior probabilidade de cura acoplada a uma diminuição da seleção não pode ser comprovada. Por sua vez, doses baixas ou curtas durações geram mais oportunidades para uma maior evolução, e estão, portanto, associadas a cenários de maior resistência. No geral, as simulações fazem crer que se o tratamento se iniciar no momento adequado, com uma dose moderada e considerando que o hospedeiro é competente a nível imunitário, é estimulada uma interação sinérgica entre hospedeiro, infeção e tratamento. Neste caso, a probabilidade de eliminação torna-se mais elevada. Um dos maiores desafios que advém da elaboração desta dissertação prende-se com a capacidade de associar ao modelo observações experimentais de sistemas particulares compreendidos pelo hospedeiro e pela população bacteriana, onde uma visão mais geral e realista das dinâmicas de tratamento e evolução da infeção possam ser integradas.

Em linhas gerais, esta investigação assenta na modelação matemática de infeções bacterianas e comprova, de novo, o poder avassalador desta ferramenta quando associada a análises numéricas e simulações computacionais. Todo o trabalho levado a cabo durante esta dissertação permite-nos afirmar que, no campo da resistência a antibióticos, es-

tamos agora mais perto do objetivo último: o seu controlo e a sua prevenção.

Palavras Chave: Infeções bacterianas, Modelos matemáticos, Resistência a antibióticos, Dinâmicas de tratamentos, Evolução

Abstract

Antimicrobial resistance in bacterial infections is not new. However, in the last years, it has become a global public health priority, already recognized as a colossal threat to the core of modern medicine by World Health Organization. In view of the urgency of its management and, at a more advanced level, its prevention, several research fields, such as Evolutionary Epidemiology, focus their work in this major problem.

Given the dramatic increase in the number of bacterial agents resistant to one or more antimicrobial drugs, it is frequent to find, in the scientific literature, biomathematical models whose main goal is to identify, among the diverse potential treatment regimes, those that minimize selection for drug resistance while seeking for general quantitative principles of infection clearance.

Despite this progress - and because several gaps are found when the scope of this problem is being determined - it still remains a difficult task to stop the emergence of resistance to new antimicrobial therapeutics and to deal with already resistant bacterial pathogens.

In this study we visit this question by developing several mathematical models of infection under treatment and exploring them computationally. Specifically, deterministic models are used to analyze the equilibria conditions which allow to move from one infection scenario to another, among colonization, persistence and clearance. These findings, in the absence and in the presence of treatment, are conjugated with evolutionary dynamics. Evolution is modeled through a series of stochastic events, giving rise to bacterial strains with different growth and antibiotic resistance phenotypes. The hybrid model, in which stochasticity is imposed in the emergence of new bacterial strains and

followed by deterministic growth, is used as an exploratory tool to simulate and study resistance selection and treatment outcomes. Our preliminary findings show that high cost, high resistant mutations are not directly favored by aggressive treatments. Sub-inhibitory doses or short durations generate more opportunity for further evolution.

Finally, we discuss future directions for improving the mathematical models and assess their realism; and also propose a series of extensions worth exploring with this framework.

Keywords: Bacterial infections, Mathematical models, Antibiotic resistance, Treatment dynamics, 2-trait Evolution

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The laws of nature are but the mathematical thoughts of God.

Euclid

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Chapter 1

Introduction

1.1 Motivation

The earliest records on antibiotic drugs date back to 1929 and are authored by Alexander Fleming, who notticed that *Penicillium notatum* produced a substance with antibacterial effects (Bush, 2010). Penicillin only started to be used for therapeutic purposes in the early 1940s (Fleming *et al.*, 1946). Since then, the use of these drugs has contributed to a significant decrease of illness and deaths due to infections (Cohen, 1994). But from the beginning of this era of discovery, the optimism was being questioned. Fleming was aware that not all microbes were sensitive to this drug (Fleming *et al.*, 1946). In 1942, particular cases of resistance were already being described in scientific articles (Rammelkamp & Maxon, 1942). Antimicrobial resistance became a reality to almost every new antimicrobial substance, after the beginning of its use in the clinical practice, predominantly in hospital environment (Macfarlane *et al.*, 1960; McGowan Jr, 1983; Peacock *et al.*, 1980; Webb *et al.*, 2005). As time passes by, there are more resistant organism, more geographically spread, and several of them not respond to many substances, instead of just one (Levy, 1998; Levy & Marshall, 2004). Beyond the adverse influence on the public health, this problem has had a big economic impact in the last decades (Holmberg *et al.*, 1987; Rubin *et al.*, 1999).

Each day, antimicrobial resistance of infectious agents increases dramatically worldwide (Organization *et al.*, 2014, 2015). The situation is so critical that

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World Health Organization already published a priority list of resistant bacteria for which new antibiotics should be developed faster (Organization *et al.*, 2017). More and more, human and monetary resources are being promptly applied in this field of investigation, in order to control this issue (Roca *et al.*, 2015; Theuretzbacher *et al.*, 2017). There is even a comprehensive antibiotic resistance database, in which knowledge about antibiotic resistance genes is concentrated (McArthur *et al.*, 2013). It is expected that, in a near future, new infections will not be treatable and the prophylactic strategies working nowadays will fail. So far, there are no other pratical and effective alternatives to antibiotics, despite all the efforts from the scientific community (Allen *et al.*, 2014). To fight this global threath is urgent and imperative (Perron *et al.*, 2015).

Concerning antimicrobial resistance, this question can be adressed in two distinct perspectives. First of all, resistance to the majority of antimicrobial drugs in use already exists (Lipsitch *et al.*, 2000). If that is the case, there is the need to manage resistance, in an individual and populational levels (Purohit *et al.*, 2017). A resistant bacterial subpopulation, present in an infection, is sufficient to threat the success of the treatment and compromise the host health. Focusing on the community, many people suffer from resistant bacteria acquired in hospital envinronment, after they are already infected (Lipsitch *et al.*, 2000; McGowan Jr, 1983).

On the other hand, resistance may emerge by *de novo* mutations (Davies & Davies, 2010; Munita & Arias, 2016). In that particular case, a better understanding of how different types or strategies of treatment affect selection and spread of drug resistance may allow to expand the life span of the drugs (Geli *et al.*, 2012). Resistance emergence is even influenced by how these drugs are consumed in the community (Bell *et al.*, 2014). If an antibiotic is effective for more time, the probability of resistance evolution decreases and the consequences for the host are less likely to be adverse. Evolutionary epidemiologists have spent a lot of time focused on this. A direct effect is in the hospital procedures and, consequently, on hospital antimicrobial swetwarship programs (Allerberger *et al.*, 2016; Hamilton *et al.*, 2015).

Nowadays, it is possible to find, in the literature, many mathematical models whose main goal is to identify, among the diverse potential treatment regimes,

those that minimize selection for drug resistance while not compromising patient health (Spicknall *et al.*, 2013). Different therapeutic strategies were already presented by many authors, corroborated by empirical and theoretical evidences. Mathematical models are used to study not only bacterial infections but also other diseases or other types of therapeutics (Schirm *et al.*, 2013). Biomathematical modelling is, without any doubt, a simplification of the study case and its outcomes are always approximations of the reality. However, its success comes from the possibility to start with a complex biological system, summarize the available knowledge about it and end it up with a formal representation. Additionally, through a set of parameters and variables, it is possible to access the dynamics of the system and to distinguished which components play a bigger role in each. Summarily, a more realistic model implies a higher number of variables and parameters. Because some of them are approximations, the more authentic the model, the higher the error associated to it. At the end, mathematical modelling implies a good harmony between how close it is to reality to be representative of it and how it is not too detailed in order not to have a higher error than desired. The key word in this process is balance.

A big part of the scientific community advocates to use an antimicrobial treatment as agressive as possible to deal with bacterial infections (Ankomah & Levin, 2014). A high dose of drug, tolerable by the host, was thought to be enough to kill the host and, at the same time, to reduce the rate of *de novo* mutations and its evolution. However, many cases have been described in which this strategy did not work (Day & Read, 2016). For low mutation rates pathogens, it is very questionable if this option succeeds since higher doses seem to favor selection of resistant pathogens, specially if the resistance already exists. Contrarily to what happens with community acquired infections, such as TB (Pienaar *et al.*, 2015), if the pathogen is able to mutate at a high rate, as it happens with HIV, this treatment strategy may work (Read *et al.*, 2011). Based on this, new alternatives started to be studied (Goulart *et al.*, 2013; Jassim & Limoges, 2014). That is when one might consider the option of prescribing a more moderate treatment, or to discard the classical regime and opt by an adaptive one, in which treatment is flexible and follows the changes in the host health (Gjini & Brito, 2016).

Currently, all alternatives are being debated, both on experimental and theoretical levels, and this amount of results shows that we are far away from having defined general practices for treatment of bacterial infections. It is from this state-of-art that the main idea of this thesis is constructed and motivation emerges.

Several models assume for simplicity an exponential growth of bacteria in acute infections, either they are sensitive or resistant (Ganusov *et al.*, 2002; Gjini & Brito, 2016). In this thesis, resource limitation is taken into account, which means we will be dealing with a logistic growth model, a very useful approach to control bacterial growth setting a maximal carrying capacity in the absence of immunity. The main simplification from the model in Gjini & Brito (2016) is in the process of the immune system modelling. Instead of considering different types of immune cells, since they play different roles in the organisms, or compare different immunity models for the same system (Handel *et al.*, 2009), here we represent immune response by a single variable. A major step forward in this master thesis is an extensive analysis of the equilibria of the model. The output are explicit mathematical expressions which produces the necessary conditions to adjust the therapeutics, if parameters involved in the antibiotic prescription are known. To end in great, deterministic and stochastic versions of the same model are compared. Individual bacterial cells present a heterogeneous behavior, which is not usually considered when bacterial populations are modelled (Koutsoumanis & Lianou, 2013). In here, pathogens can mutate, in a pleitropic manner (Perron *et al.*, 2015), and consequently evolve, which may influence the infection dynamics. We model evolution as stochastic emergence of new bacterial sub-populations with different fitness cost and antibiotic susceptibility (Kepler & Perelson, 1995). This approach/ simplification, focusing only on phenotype, may not be too restrictive since there is a dissociation between genotype and phenotype (Hughes & Andersson, 2017). Factors, such as antibiotic administration, change the phenotypic expression of resistance mutations and this information leads, in practice, to the comparison of a larger number of various antimicrobial treatments. At the end, ideally, the model will predict the probability of resistance selection and identify the phenotypic traits of the selected strains (Oz *et al.*, 2014) and related that to a successful bacterial infection treatment.

1.2 Objectives

The main ambition adressed in this thesis is to study the dynamics of bacterial infections under different types of treatment, based on the definition of mathematical models and computational simulations.

To start this research, deterministic bacterial infection models are used, mostly, for the precise identification of the conditions with parameters combinations that allow an infection, either acute or chronic, to go from one state to another, between colonization, persistence and clearance. Initially we fix the phenotypes of two bacterial subpopulations (sensitive and resistant) that compete within a host. Besides that, bacterial dynamics study concede the opportunity to compare "the same infection", when in the presence or in the absence of antibiotics. Therefore, a better understanding of the concept of ideal treatment regime depending on the pathogen and the host (in particular its immunity) is achieved. When stochasticity is incorporated in the model, the focus shifts to answer these questions: How does evolution of a pathogen affect the dynamics of a bacterial infection under treatment? And even, how do dynamics of infection affect the evolution of the pathogen? Here, the infection will be composed by multiple heterogeneous subpopulations which compete for resources and grow under immunity and antibiotics. If we are working with the correct mathematical models, the main focal point is to better distinguish the distinct infection types and to reveal the key strategies to deal with them, focusing always on resistance control.

1.3 Contributions

As slightly revealed before, to pursue the main goals of this thesis, two mathematical modelling approaches are chosen. In a first technique, a deterministic model which aggregates three ordinary differential equations is designed. Through it, sensitive and resistant bacterial subpopulations and immunity of the host can be studied. This model, with an array of adequate parameters, generates graphic interpertable simulations and mathematical expressions of equilibria conditions. The second one needs to be planned even with more detail, due to the imposed stochasticity. This element allows the pathogen to have some variability in traits

such as its fitness cost and its antibiotic susceptibility, which is reflected in the infection process and evolutionary dynamics. The main message of all this investigation high-lights the interdependency between host immunity, pathogen characteristics and evolution and type of treatment in order to obtain a successful treatment regime for bacterial infections.

1.4 Overview

The thesis architecture is as follows. Chapter 2 focus on the dynamics of bacterial infections, without treatment. It starts to provide an overview of the mathematical modelling process. Besides that, key biological concepts are introduced, such as colonization, persistence and clearance. Study of the stability of the system brings out a set of conditions of equilibria enumerated in here. It ceases with simulations focused on the role of carrying capacity and host immunity. Chapter 3 arises with the administration of antibiotics. Asymptotic analysis of stability of the system is performed for different treatment modelling approaches: constant antibiotic dose, with pharmacodyanmics and with the effective dose. Chapter 4 is centered on the transient behavior of the system. Two dissimilar infection types are compared: a persistent infection (with variation of treatment onset) and an acute infection. Bacterial dynamics in the presence of treatment are studied in order to compare types of treatment and obtain an adequate high-light of the best treatment regime. The mathematical expressions computed in here are the start point for Chapter 5, in which the types of treatment are distinguished in a more systematic way. Besides that, stochasticity allows to mimic the pathogen evolution, using different mutation rates and generating different random evolutionary trajectories even for the same parameters. Again, acute infections are scrutinized through their bacterial dynamics and interpretation of summary measures and infection outcomes scenarios. On all produced models, numerical computations and simulations were performed using *Wolfram Mathematica 11.0* and *MATLAB R2016a*. In the final chapter, central messages are reviewed. Aside from the wealth of thesis results to the scientific community, the potential applicability in the clinical practice is discussed. The last point adressed are the future perspectives of this field, reinforcing the usefulness of interdisciplinarity.

Chapter 2

Bacterial Infection Dynamics Without Treatment

2.1 Mathematical Model

The general mathematical model is designed to explore the interplay between antibiotic treatment strategies and host immune response, during a partial drug-resistant bacterial infection. The major formulation is based on a previous within-host model of bacterial infection dynamics (Gjini & Brito, 2016), in which two pathogen phenotypes are identified: the sensitive bacterial subpopulation, B_S, and the resistant one, B_R. These two subpopulations can be distinguished by two essential rates: their intrinsic growth rates, r_0 (Stromberg & Antia, 2011; Tuomanen *et al.*, 1986) and r_1 (Levin *et al.*, 2000), and the killing rates by the antibiotic, δ_0 and δ_1, respectively. Two major parameters are considered in this model: the fitness cost of resistance, $c = r_0\text{-}r_1$ ($0 \leq c \leq r_0$), and, on the other hand, the fitness benefit of resistance, $a = \frac{\delta_1}{\delta_0}$ ($0 \leq a \leq 1$). In this model, the fitness benefit of resistance can be seen as the way in which bacterial resistance reduces the killing capacity of the antibiotic.

One of the main differences remains on the fact that the action of host immunity is simplified. Instead of having one equation for each type of immune cell, there is only one equation that describes the entire action of the immune system. This mathematical model is inevitably a simplification of complex interactions

between immunity, bacteria and antimicrobial drugs but the underlying assumptions do capture the major dynamics of the immune system: growth by antigen stimulation (at equal rates by any types of bacteria) and possible decline.

Another crucial assumption in the model is that the killing rate d by the action of the immune system cells (Barber *et al.*, 2003; Stromberg & Antia, 2011; Yates *et al.*, 2007), known as immune response, is equal for B_S and B_R, independently of their antimicrobial susceptibility. Another one regards the function of the stimulation of immunity, by the entire bacterial population within host, B_{TOT}. For immune stimulation by antigen, a monotonically increasing saturating function of pathogen density (Hill function, coefficient 1) is assumed, by default. In this function, the parameter k (De Boer *et al.*, 2001; Stromberg & Antia, 2011) represents the half-saturation constant for activation of the immune response, in this work, as the host immunity threshold. Other parameters are σ and h (Allan *et al.*, 2004; De Boer *et al.*, 2001; Stromberg & Antia, 2011), which represent maximum immune cell recruitment and immune system action decay rate, respectively.

To the general mathematical model described above will be added some distinct extensions, in order to study different scenarios, which are considered critical in this investigation. A detailed description of model parameters is given in Table 2.1. Aditionally, other parameters will be described later. The simulations are based on a limited set of parameter values, likely to apply to a range of diferent infections types. Another important feature of the model is that the parameters values do not reflect any particular antibiotic-species combination.

The first extension to the previous described model is to consider that both subpopulations experience a logistic growth, instead of exponential. This asumption provides another way to control the bacterial growth, beyond the control via host immune responses. This alteration requires a new parameter: the carrying capacity, C, known as the maximum population size of the species that the within-host environment can sustain indefinitely. This parameter takes into account the within-host resources, habitat, other necessities available in the environment and even the crowding effects and bacterial competition. At this point, the model is more general and C can take every value. It will be important to study the interaction between this limit for pathogen growth and the immunity stimulation threshold.

Table 2.1: Model parameters and interpretation

Symbol	Interpretation	Value	Range	Units
r_0	Sensitive bacteria growth rate	3.3	1-8	day^{-1}
r_1	Resistant bacteria growth rate	1.1	$\leq r_0$	day^{-1}
d	Pathogen killing rate by immunity	10^{-5}	10^{-5}-10^{-4}	μl/cell/day
δ_0	Killing rate of B_S by antibiotics	1	Scaled	l/mg/day
δ_1	Killing rate of B_R by antibiotics	$a\delta_0$	Scaled	l/mg/day
σ	Maximum immune response growth rate	2	1.2-3	day^{-1}
k	Host immunity threshold	10^5	10^4-10^5	cell/μl
h	Immunity action decay rate	0.35	0.1-0.8	day^{-1}
A_m	Average antibiotic concentration	1-50	0.03-128	mg/l
C	Carrying capacity	10^5	10^2-10^9	cell/μl

Within-host dynamics for a mixed infection with a drug-sensitive, B_S, and pre-existing partially resistant, B_R, bacterial strains and additionally the immune system (I) are described by the following set of ordinary differential equations:

$$\frac{dB_S}{dt} = r_0 B_S\left(1 - \frac{B_S + B_R}{C}\right) - dB_S I - \delta_0 B_S A_m \tag{2.1}$$

$$\frac{dB_R}{dt} = r_1 B_R\left(1 - \frac{B_S + B_R}{C}\right) - dB_R I - \delta_1 B_R A_m \tag{2.2}$$

$$\frac{dI}{dt} = \frac{\sigma I(B_S + B_R)}{k + B_S + B_R} - hI \tag{2.3}$$

The initial conditions of the model are $B_S(0) = 10$, $B_R(0) = 2$ and $I(0) = 200$, which satisfies $B(0) \ll k$ and $I(0) \ll \frac{r_0}{d}$. To be able to consider the pathogen's discrete nature, an extinction threshold is assumed, when pathogen density of either bacterial subpopulation falls below a critical level $B_{ext} = 10^{-1} cell/\mu$.

A special case of this model is $A_m = 0$, which means that part of both equations (Equation 2.1 and Equation 2.2) are not considered in the analysis. Biologically, the infection is not being treated. This particular case of the equations system is essential because the main goal, in this chapter, is to study the dynamics of bacterial infections that are not going under any antimicrobial treatment.

2.2 Equilibria for the case $A_m = 0$

From the analysis of a mathematical model, several points of equilibria can arise, by solving a system of equations:

$$\frac{dB_S}{dt} = 0 \tag{2.4}$$
$$\frac{dB_R}{dt} = 0 \tag{2.5}$$
$$\frac{dI}{dt} = 0 \tag{2.6}$$

Each of them can be interpreted biologically, corresponding to a known infection fixed scenario, summarized in this dissertation as colonization, persistence or clerance. By the term **colonization** we refer to the situation in which the pathogen is present in the host system, and there is no immune response to fight it. By the term **persistence**, we mean that the presence of the pathogen in the host system stimulates an immune response, that persists at equilibrium. Clinically, it stands for a chronic infection. **Clearance** represents the scenario in which the host is free of pathogen and the immune response, at that time point, is null. This picture can arise as a direct outcome of an acute infection.

2.2.1 Fixed Points

The fixed points of the mathematical model of bacterial dynamics without any antimicrobial treatment are enumerated and described below:

- Colonization by B_S at C and no B_R, by B_R at C and no B_S and by B_S and B_R where $B_R + B_S = C$:

$$\begin{bmatrix} B_S^* = & C \\ B_R^* = & 0 \\ I^* = & 0 \end{bmatrix}, \begin{bmatrix} B_S^* = & 0 \\ B_R^* = & C \\ I^* = & 0 \end{bmatrix} \textit{ and } \begin{bmatrix} B_R^* = & C - B_S^* \\ I^* = & 0 \end{bmatrix}$$

- Persistence of B_S with some immunity and no B_R, and persistence of B_R with some immunity and no B_S:

$$\begin{bmatrix} B_S^* = & \frac{hk}{\sigma - h} \\ B_R^* = & 0 \\ I^* = & \frac{r_0}{d}\left(1 - \frac{B_S^*}{C}\right) \end{bmatrix} and \begin{bmatrix} B_S^* = & 0 \\ B_R^* = & \frac{hk}{\sigma - h} \\ I^* = & \frac{r_1}{d}\left(1 - \frac{B_R^*}{C}\right) \end{bmatrix}$$

- Clearance of the infection:

$$\begin{bmatrix} B_S^* = & 0 \\ B_R^* = & 0 \\ I^* = & 0 \end{bmatrix}$$

All these fixed points can be compared to each other, concerning the values of B_S, B_R and I (Figure 2.1). It is possible to check that, in the absence of immune response, at equilibrium, there is no simultaneous persistance of both bacterial subpopulations. However, their coexistence without immune response is possible.

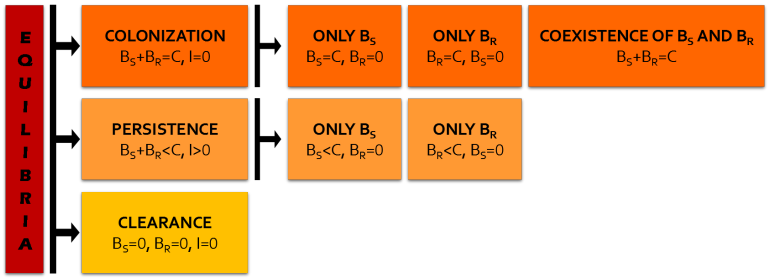

Figure 2.1: **Summary of Equilibria of the Mathematical Model.**

2.2.2 Conditions for Stability

Next, we check if the equilibria have the robustness against perturbations of the bacterial subpopulation sizes and the immune response levels around the steady state. Linear stability analysis requires studying the properties of the Jacobian matrix, evaluated at (B_S^*, B_R^*, I^*) and allow to determine the stability, identifying the critical parameter values. The real part of all eigenvalues of the Jacobian Matrix must be negative for the equilbria to be stable.

2. BACTERIAL INFECTION DYNAMICS WITHOUT TREATMENT

- **Stability of Colonization**

When only one of the bacterial subpopulations is responsible for the colonization of the host (equilibrium $(C, 0, 0)$ and $(0, C, 0)$), the eigenvalues are:

$$\lambda_1 = 0$$

$$\lambda_2 = -r$$

$$\lambda_3 = -h + \frac{C\sigma}{C+k}$$

. where $r = r_0$ or $r = r_1$ for colonization by B_S or by B_R, respectively. Regarding bacterial subpopulations coexistence (equilibrium $(B_S^*, C - B_S^*, 0)$), λ_1 and λ_3 are the same. Differences are only found in the second eigenvalue:

$$\lambda_2 = \frac{(r_1 - r_0)}{C} B_s^* - r_1.$$

For all the above cases, the first eigenvalue, λ_1, being zero, gives one neutrally stable direction for free variation between B_R and B_S, always ensuring that $B_S^* + B_R^* = C$. The second eigenvalue is always negative because r_0 is always positive and because $\frac{B_S^*}{C} < 1$, leading to $(r_1 - r_0)\frac{B_S^*}{C} < r_1$. For the last eigenvalue to be negative, $\lambda_3 < 0$, the condition $C < \frac{hk}{\sigma - h}$ has to be satisfied. Thus, only when the carrying capacity is low enough, relative to host immune activation parameters, that the bacterial populations will be controlled exclusively by resource limitation.

- **Stability of Persistence**

The eigenvalues of these fixed points (equilibrium $(B_S^*, 0, I^*)$ and $(0, B_R^*, I^*)$) follow this structure:

$$\lambda_1 = (r_0 - r_1)\Big(\frac{1}{C}\frac{hk}{\sigma - h} \pm 1\Big) \text{ for } B_R \text{ and } B_S \text{ persistence, respectively}$$

$$\lambda_{2,3} = -A \pm \sqrt{B},$$

where $A = \frac{hkr}{2C(\sigma - h)}$ and $B = hr[4Chk(\sigma - h)^2 + hk^2 r\sigma - 4C^2(\sigma - h)^3]$, where $r = r_0$ or $r = r_1$ depending on which equilibrium we are dealing with.

The conditions for existence of these fixed points are $\sigma > h$, to ensure $B_S^* > 0$ or $B_R^* > 0$, and $C > \frac{hk}{\sigma - h}$, to ensure $I^* > 0$.

Considering r the growth rate of the bacterial subpopulation that persists, if $B \geq 0$, λ_2 and λ_3 are real, which generates a node and the equilibrium is approached in a monotonous manner. For $B \geq 0$, requires the satisfaction of the following condition:

$$\frac{hk}{\sigma - h} < C \leq \frac{1}{2}\left(\frac{hk}{\sigma - h} + \sqrt{H}\right), \text{ where } H = \frac{hk^2(h^2 - (h + r)\sigma)}{(h - \sigma)^3}.$$

Given the parameters values, $A > 0$, which means $-A < 0$. Following that idea, $-A - \sqrt{B} < 0$, which ensures eigenvalue $\lambda_3 < 0$. $\lambda_2 = -A + \sqrt{B}$ will be negative when $B < A^2$, ensured by $C \leq \frac{1}{2}\left(\frac{hk}{\sigma - h} + \sqrt{H}\right)$.

If $B < 0$, the eigenvalues λ_2 and λ_3 are complex. This occurs for values of carrying capacity C exceeding a critical value

$$C > \frac{1}{2}\left(\frac{hk}{\sigma - h} + \sqrt{H}\right).$$

In that situation, the stability of the equilibrium can be verified just considering the real part of the eigenvalues ($Re(\lambda_{2,3})$), given by $-A < 0$. Thus whenever a focus exists, it is always stable. In these cases, persistence is approached in an oscillatory manner. As C increases further, the amplitude of the oscillations increases.

- **Stability of Clearance**

The fixed point associated with the scenario of clearance (equilibrium $(0, 0, 0)$) is the trivial one and the correspondent eigenvalues are:

$$\lambda_1 = -h$$

$$\lambda_2 = r_0$$

$$\lambda_3 = r_1.$$

This point is always unstable, given that both growth rates are always positive.

2.3 Simulations

2.3.1 The Role of Carrying Capacity

Here we study, through simulations, the role of the new parameter, C, since now it is a model containing logistic growth dynamics independently of immunity or antibiotics.

In order to get as much information as possible, the simulations can be divided into different scenarios that differ on the value of C, in order to satisfy the conditions of stability of each scenario. These four scenarios allow to compare the logistic growth model against the exponential one, making our results and investigation more general.

- **Case 1: Colonization**

Taking into account the fact that the carrying capacity, $C = 10^2$, is way lower than the immunity threshold, $k = 10^5$, which allows to satisfy the condition of stability of colonization, there is no sufficient stimulation of the immune system and the immune response decreases over time from its initial levels (Figure 2.2, Panel E). Without control by immune system, both bacterial subpopulations, individually or simultaneously, can grow up to the value near to the carrying capacity, where they remain indefinitely (Figure 2.2, Panel A). In this case, the host is colonized by the pathogen and it will suffer from a chronic infection.

- **Case 2: Persistence**

When, for example, the carrying capacity C increases to 10^5 and has the same value of the immunity threshold k, the critical condition for stability of persistence is satisfied. There is a fine incentive of the immune system's action (Figure 2.2, Panel F). Considering our default value of h, the immune response will be able to result in the clearance of the resistant bacterial subpopulation and the persistence of the sensitive one (Figure 2.2, Panel B). The values of B_S load, when persistent, are lower than C, as expected from the stability analysis abovementioned. This is

consequence of the action of immune system. It can happen to occur persistence of B_R instead of B_S, but not both simultaneously.

- **Case 3: Persistence with oscillations**

We find some special cases in which C slightly exceeds the value of the immunity threshold, k, leading to a particular scenario of persistence. Mathematically, when $C = 10^6$ the condition of stability of oscillatory persistence is satisfied. Both B_S (Figure 2.2, Panel C) and immune response (Figure 2.2, Panel G) present oscillations. Over time, there is a general damping of the oscillatory behavior. This can be seen approximately after 5 months of persistence of bacteria and it is maintained over time (Figure 2.3).

- **Case 4: Clearance**

If the value of the carrying capacity C increases further, for example 10^8, greatly exceeding the value of k, the model approaches the exponential growth scenario. It means the dynamics mimic a system in which there is no limitation of resources. If this is the case, asymptotically we will observe the extinction of both bacterial subpopulations, and the host will be free of the infection, a process known as clearance (Figure 2.2, Panel D). This will correspond to an acute infection. It happens because bacteria are able to grow until a level in which there is a continuous stimulation of the immune system (Figure 2.2, Panel H).

The clearance observed here does not correspond to the fixed point. Because an extinction threshold is considered, when the amplitude of the oscillations in the persistence scenario are high enough, B_S hits B_{ext} and clearance is imposed on the system via our numerical threshold.

Overall, it is possible to check that Case 1 mimics a Logistic Growth Model, while Case 4 is a closer scenario to Exponential Growth Model. Clearance is a more likely outcome when C increases, in comparison to k.

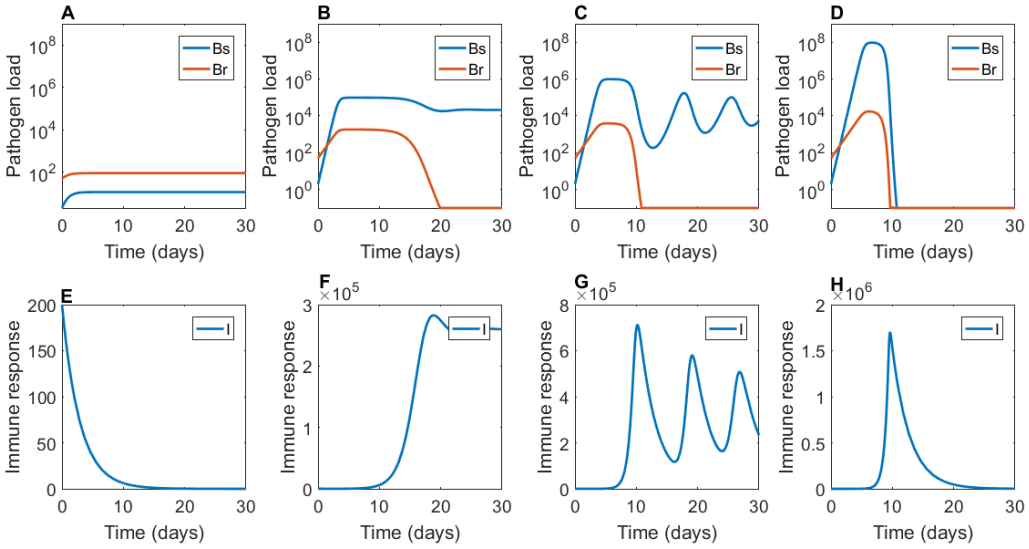

Figure 2.2: **Simulations of the dynamics of B_S, B_R and I, during 30 days.** Panels A-D for Pathogen Load and Panels E-H for Immune Response. Panels A and E $C = 10^2$; panels B and F $C = 10^5$; panels C and G $C = 10^6$; and panels D and H $C = 10^8$. Other parameters as in Table 2.1.

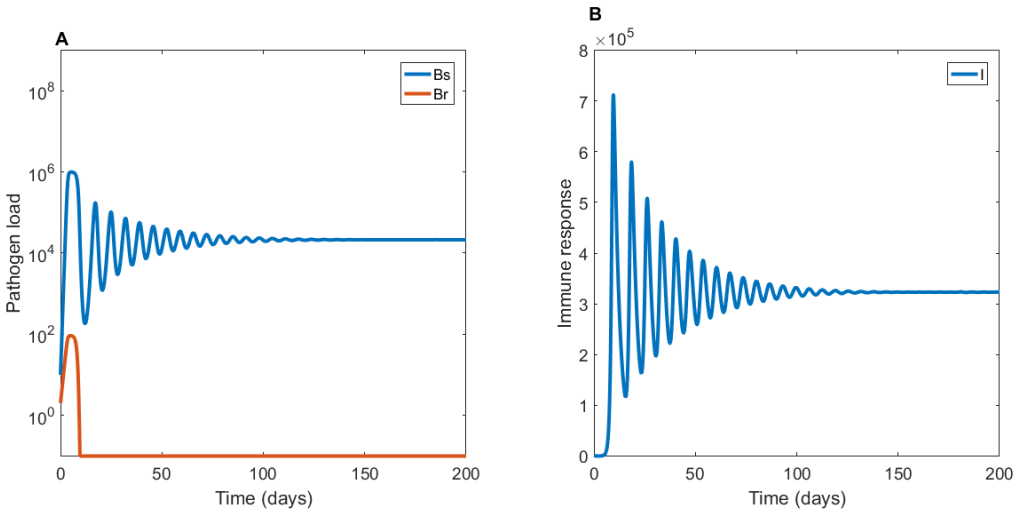

Figure 2.3: **Simulations of the dynamics of B_S, B_R and I, during 200 days.** On both panels $C = 10^6$. Other parameters as in Table 2.1.

2.3.2 The Role of Host Immunity

Although C plays a major role in the growth logistic model, immunity is also an important player on these interactions. Here we focus on persistence cases. This has been verified by the equations of the fixed points of persistence and by the stability conditions. To illustrate further the role of the immune parameters, the following simulations are focused on the outcome of the relation between σ, rate of immune activation, and C, carrying capacity. To have a wider spectrum of values, different outcomes are compared: the final pathogen load, the total pathogen burden and the maximum value obtained during the simulation.

- **Final Pathogen Load**

Final pathogen load corresponds to the value in the simulation's last time point, which in this case is $T = 30$ days.

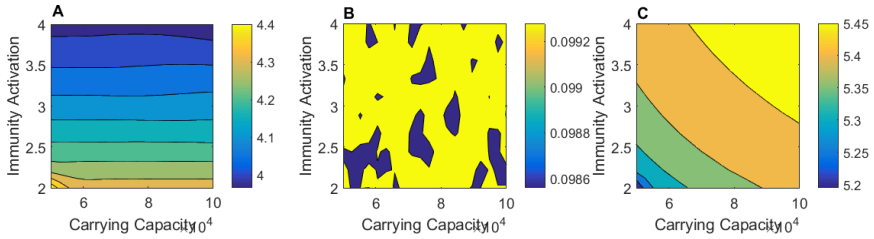

Figure 2.4: **Contour plots of Final Values of the simulations of B_S, B_R and I.** Panel A presents the final value of B_S load, B for B_R and C for immune response. σ varies from 2 to 4. C varies from 0.5x10^5 to 10^5. All values are their common log values. Other parameters as in Table 2.1.

In cases of lower σ, the levels of B_S are high, independently of C; the decrease of the burden follows the increase of σ, since the value of the bacterial loas, at equilibrium, is only dependent of immunity parameters (Figure 2.4, Panel A). Concerning B_R, the pattern differs, and this measure almost does not change varying C and σ (Figure 2.4, Panel B). Concerning immunity, if C is too low, it won't be activated (because k won't be exceeded by the bacterial population size); on the other hand, when C is higher, and more if σ is high too, the immune system is acting at its maximum capacity (Figure 2.4, Panel C).

- **Total Pathogen Burden**

We define total pathogen burden as the cumulative value of the pathogen over the interval $[0, T]$, mathematically known as $B_{TOT} = \int_0^T B(t)dt$, where $T = 30days$. B_S, B_R and I burdens are, respectively, $\int_0^T B_S(t)dt$, $\int_0^T B_R(t)dt$ and $\int_0^T I(t)dt$.

Concerning pathogen burden of B_S, it is more dependent of the immunity activation, defined by σ (Figure 2.5, Panel A). This value is maximum with the highest C and the lowest σ. Nevertheless, when the focus is on B_R, the values of the pathogen burden are dependent of other parameter combinations (Figure 2.5, Panel B). Here, it is possible to verify that the higher the σ, the lower the burden, as expected. Additionally, higher values of the pathogen tend to be related to higher C. In the last plot (Figure 2.5, Panel C), related to immunity, the higher the C and the higher the σ, the greater the immune response, because of the activation and stimulus due to bacteria presence.

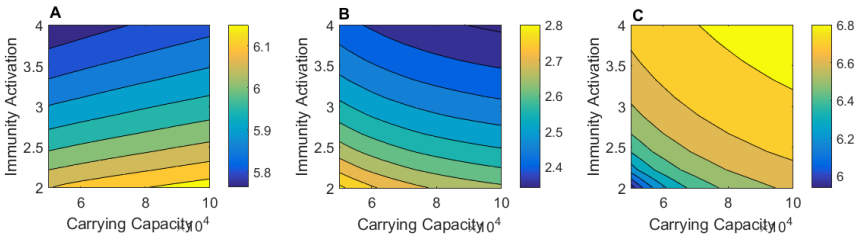

Figure 2.5: **Contour plots of total bacterial burden as a function of carrying capacity and immune activation rate.** Panel A presents the B_S burden, panel B for B_R and panel C for the immune response burden, all over 30 days. σ varies from 2 to 4. C varies from $0.5x10^5$ to 10^5. All values are their common log values. Other parameters as in Table 2.1.

- **Maximum Values**

Another summary measure of infection we can study is the maximal value of each variable in the system. Looking at each bacterial subpopulation (Figure 2.6, Panels A and B), the maximum value of bacteria during the pre determined time span of the simulation does not depend on the parameter σ. This value allows to have some clues about the transient dynamics of the system.

The higher the C, the higher the maximum value, because bacteria will grow more if there are more resources. Notice that this value is not the value at equilibrium, which is always lower (Figure 2.2, Panel B). This will typically lead to an acute infection, brought to control only via action of the immune response. This shows that the peak of the infection does not depend on the immune system activation. However, this parameter σ will interfer with the duration of the infection. Parameter σ also plays a role on the immune response (Figure 2.6, Panel C), since these values are lower if σ is lower as well. High values of both parameters will result in a more efficient stimulation of the immune response.

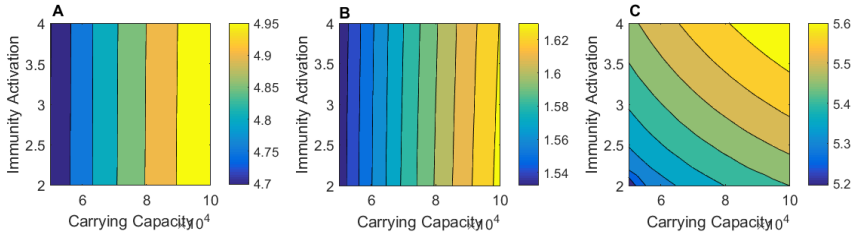

Figure 2.6: **Contour plots of Maximum Values of the simulations of B_S, B_R and I.** Panel A presents the B_S maximum value, panel B for the B_R maximum value and panel C for the immunity response maximum value, during the 30 days simulation. σ varies from 2 to 4. C varies from $0.5\text{x}10^5$ to 10^5. All values are their common log values. Other parameters as in Table 2.1.

In summary, regarding the final load, higher values of σ, and in particular higher values of C, lead to a less likely clearance. A higher immune response, at the end, depends on high values of one or both parameters. On the other hand, total pathogen burden depends on different conditions, concerning which subpopulation is the focus: the higher the C, the higher the B_S burden; however, the B_R burden essentially depends on σ, except when C is very low. The I burden increases when these two parameters increase as well. To finish, maximum values simulations allow to point some interesting facts: bacterial population peak during infection, that increases with a higher C, does not depend on σ, which affects the infection duration. A good immune response depends on high values of both parameters.

2.4 Sensitivity of the model

2.4.1 Sensitivity to intrinsic growth rates

Initially, there is the need to study how the ratio between B_R and B_S is affected by some parameters of the model or the initial conditions of the simulation.

The first study is focused on the influence of the ratio of both growth rates, $\frac{r1}{r0}$ (Figure 2.7). The closer the growth rates are, the higher the ratio $\frac{B_R}{B_S}$ is, due to the advantage of the resistant bacteria compared to sensitive bacteria. However, $\frac{B_R}{B_S}$ never exceeds 0.2 if $r_1 \leq r_0$, which biologically means that the plateau value of B_S is always much higher than the plateau value of B_R (Figure 2.2).

Another perspective to study this influence is to check the impact of the fitness cost of resistance, $c = r_0 - r_1$, on the ratio $\frac{B_R}{B_S}$ (Figure 2.8). The higher the fitness cost of resistance, the lower the ratio $\frac{B_R}{B_S}$. If the cost is higher, resistant bacteria have less chances to proliferate and the differences between the bacterial subpopulations become more significant.

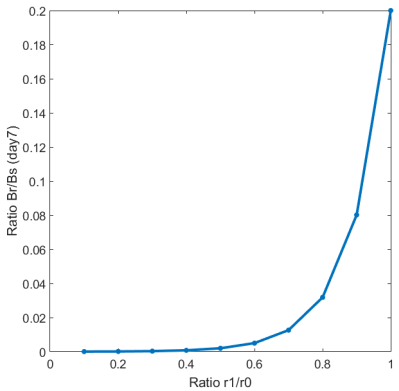

Figure 2.7: **Influence of the ratio of growth rates on the ratio of bacterial subpopulations at day 7.** With r_0 fixed to 3.3, r_1 varies from 0.33 to 3.3. Other parameters as in Table 2.1. Default ratio value, as in Table 2.1, is 0.3.

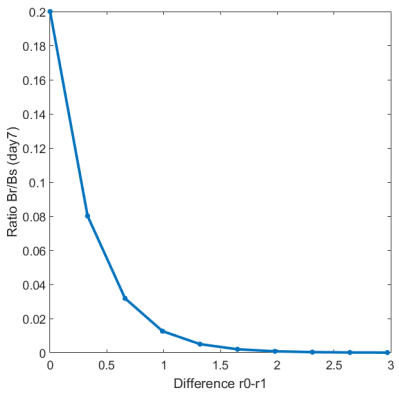

Figure 2.8: **Influence of the fitness cost of resistance on the ratio of bacterial subpopulations at day 7.** With r_0 fixed to 3.3, r_1 varies from 0.33 to 3.3. Other parameters as in Table 2.1. Default difference value, as in Table 2.1, is 1.1.

2.4.2 Sensitivity to ratio $\frac{B_R}{B_S}$ in the initial conditions

The second one is focused on the influence of the ratio $\frac{B_R}{B_S}$ on the beginning of the simulation (Figure 2.9). The main goal is to verify if different initial conditions affect the ratio of the plateau values.

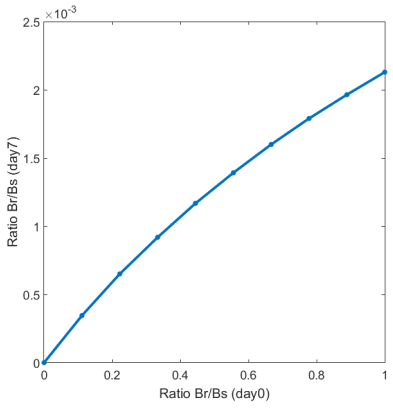

Figure 2.9: **Influence of the ratio of bacterial subpopulations in the initial conditions on the ratio of bacterial subpopulations at day 7.** The total bacterial load is mantained constant in the plot. Other parameters as in Table 2.1. Default ratio value, as in Table 2.1, is 0.2.

It is evident that an increase of the ratio at day 0 results in an increase of the same ratio at day 7. However, at day 7 the ratio $\frac{B_R}{B_S}$ is much smaller than in the beginning of the infection, which means that, over time, the two bacterial subpopulations tend to be more similiar in size.

A new question arises at this point: how does this relation between both ratios at different time points is affected by the carrying capacity of the system? In general, the higher the carrying capacity, the smaller is the ratio $\frac{B_R}{B_S}$, which means that the sizes of bacterial subpopulations are closer (Figure 2.10).This happens because the smaller the carrying capacity, the less time the bacterial subpopulations have to grow, which means initial conditions determine more strongly the dynamics. It is even possible to verify that with a higher C, the influence of the ratio of bacterial subpopulations in the initial conditions on the ratio of bacterial subpopulations at day 7 becomes less significative, proved by smaller slopes.

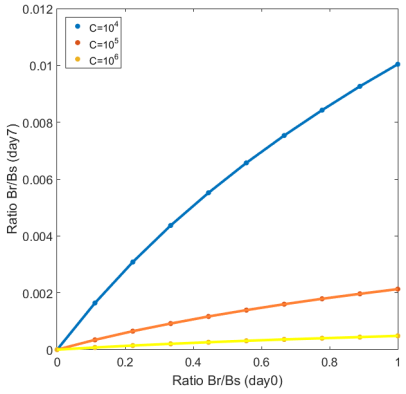

Figure 2.10: **Influence of the ratio of bacterial subpopulations in the initial conditions on the ratio of bacterial subpopulations at day 7, for different carrying capacity values.** The slope becomes smaller with a higher C, which means the influence becomes less representative ($C = 10^4$ in blue, $C = 10^5$ in orange and $C = 10^6$ in yellow). The total bacterial load is mantained constant in this plot. Other parameters as in Table 2.1.

Chapter 3

Asymptotic Analysis of Bacterial Infection Under Treatment

The next three sections will approach asymptotic dynamics of bacterial infections considering that the host is being treated with antimicrobial drugs. These sections differ on the manner how the treatment is modelled and the main goal is to verify which is the most realistic way to model antibiotic treatment.

3.1 Constant Antibiotic Dose

In this particular section, it is considered that the host receives a constant dosage of antibiotic, $A_m > 0$. Another important assumption is that the drug concentration in host body does not suffer any alterations over time. This is the most simple way to model the use of antimicrobial drug during an infection, similar to previous studies (Day & Read, 2016; Gjini & Brito, 2016).

3.1.1 Mathematical Model

Mathematically, this scenario can be modelled by the same system of equations (Equations 2.1, 2.2 and 2.3), presented in the last chapter (Chapter 2). However, the main difference is that A_m has to have a constant positive value, instead of 0. Combining the value of the average antibiotic concentration, A_m, with the value

of δ, the killing rate of bacteria by the antibiotic (δ_0 for B_S and δ_1 for B_R), it is possible to decrease the bacterial subpopulation sizes.

3.1.2 Equilibria

From the analysis of this mathematical model, several points of equilibria arise. It is possible to check that if any antimicrobial drug is administrated there is no chance to have colonization of the host by both bacterial subpopulations simultaneously, at equilibrium, as it happens in cases of infections with no treatment. Therefore, there are fewer distinct equilibrium scenarios (Figure 3.1). Nevertheless, asymptotic exclusion of one bacterial subpopulation does not mean that B_S and B_R do not coexsit transiently.

Steady states

The fixed points of the mathematical model regarding treatment, which can be compared to each other, concerning the values of B_S, B_R and I, reflect:

$$\begin{bmatrix} \lim_{t\to\infty} B_S(t) = B_S^* \\ \lim_{t\to\infty} B_R(t) = B_R^* \\ \lim_{t\to\infty} I(t) = I^* \end{bmatrix}$$

They are enumerated and described below:

- Colonization by B_S and no B_R, and colonization by B_R and no B_S:

$$\begin{bmatrix} B_S^* = & -\frac{C(A_m\delta_0 - r_0)}{r_0} \\ B_R^* = & 0 \\ I^* = & 0 \end{bmatrix} \; and \; \begin{bmatrix} B_S^* = & 0 \\ B_R^* = & -\frac{C(A_m\delta_1 - r_1)}{r_1} \\ I^* = & 0 \end{bmatrix}$$

- Persistence of B_S under some immunity and no B_R, and persistence of B_R under some immunity and no B_S:

$$\begin{bmatrix} B_S^* = & \frac{hk}{\sigma - h} \\ B_R^* = & 0 \\ I^* = & -\frac{-r_0 + A_m\delta_0}{d} + \frac{hkr_0}{Cd(h-\sigma)} \end{bmatrix} \; and \; \begin{bmatrix} B_S^* = & 0 \\ B_R^* = & \frac{hk}{\sigma - h} \\ I^* = & -\frac{-r_1 + A_m\delta_1}{d} + \frac{hkr_1}{Cd(h-\sigma)} \end{bmatrix}$$

- Clearance of the infection:

$$\begin{bmatrix} B_S^* = & 0 \\ B_R^* = & 0 \\ I^* = & 0 \end{bmatrix}$$

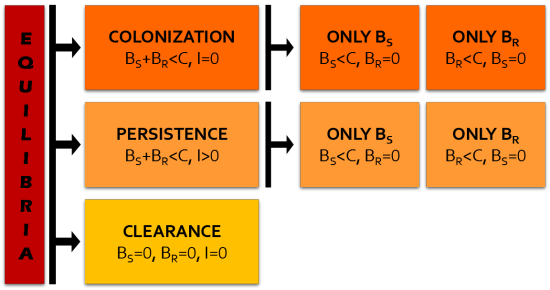

Figure 3.1: **Summary of Equilibria of the Mathematical Model, considering classical treatment.** A constant and continuous dose of antibiotic is administrated during a bacterial infection.

Conditions for Stability

- **Stability of Colonization**

The existence and stability of both cases of colonization equilibrium are comparable between them. In order for both fixed points to exist, the dose of antibiotic has to be lower than a specific value: the ratio between the intrinsic growth rate and the killing rate by antibiotic. Therefore, colonization by B_S or by B_R, respectively, is biologically realistic if

$$A_m < \frac{r_0}{\delta_0} \text{ or } A_m < \frac{r_1}{\delta_1}.$$

Concerning stability, a stable node is a fixed point that exists and it is stable. Both fixed points mentioned above are stable nodes, under similar conditions, as well. When focusing on sensitive bacteria, assuming that the condition for existence is satisfied, one of the conditions for stability is

$$r_0 > \frac{r_1 \delta_0}{\delta_1}$$

($r_0 > \frac{r_1}{a}$ if the concept of fitness benefit of resistance, a, is used). The equivalent condition for the resistant strain is

$$r_1 > \frac{r_0 \delta_1}{\delta_0}$$

($r_1 > a r_0$). Additionally, one of two other conditions needs to be satisfied to ensure stability. Both of them are responsible to constrain the growth of bacteria. This kind of control can be obtained by the immune system action (condition $h \geq \sigma$) or if the carrying capacity presents a maximum value. For the colonization by B_S or B_R, respectively:

$$C < \frac{-hkr_0}{(r_0 - A_m\delta_0)(h-\sigma)} \text{ or } C < \frac{-hkr_1}{(r_1 - A_m\delta_1)(h-\sigma)}.$$

- **Stability of Persistence**

Both steady states corresponding to persistence scenarios exist under the two same conditions, $h < \sigma$ and $C > \frac{hk}{\sigma - h}$. The lower the decay of the immune response, the higher is the minimum value of the carrying capacity. The first condition allows the maintenance of the immune system response and the second one ensures that there is enough "space" for the resistant bacteria to grow until they reach an equilibrium. There is a third condition, which establishes a maximum dose of antimicrobial drug during treatment, which differs for persistence of B_S and B_R subpopulations, respectively:

$$A_m < \frac{r_0(Ch + hk - C\sigma)}{\delta_0 C(h-\sigma)} \text{ and } A_m < \frac{r_1(Ch + hk - C\sigma)}{\delta_1 C(h-\sigma)}.$$

Concerning stability, the conditions are more complex. For both B_S and B_R persistence cases, there is, in first instance, a distinct range of values that C needs to be in. After that, it depends on the antimicrobial drug dose and the ratio between both intrinsic growth rates. Regarding B_R, the mandatory conditions are

$$r_1 > a r_0 \text{ and } \frac{hkr_1}{(r_1 - A_m\delta_1)(\sigma - h)} < C < \frac{hk(r_1 - r_0)}{(r_0 - r_1 + A_m(\delta_1 - \delta_0))(h-\sigma)}.$$

With regard to the dose, or $\frac{r_0-r_1}{\delta_0-\delta_1} \leq A_m < \frac{r_1}{\delta_1}$ or, in case the dose is lower, h is equal or lower than a determined value or it is higher and there is the need to assign σ a maximum value as well.

Sensitive bacteria present almost the same conditions for stability pattern. First of all, carrying capacity starts from the maximum value established for B_R and besides that has to satisfy the condition

$$C \leq \frac{1}{2}\left(\frac{-hkr_0}{(r_0 - A_m\delta_0)(h-\sigma)} + \sqrt{\frac{hk^2r_1^2(h^2-(h+r_1-A_m\delta_1)\sigma)}{(r_1-A_m\delta_1)^2(h-\sigma)^3}}\right).$$

And then it depends on the antibiotic dose:

$$\begin{cases} r_0 = \frac{r_1}{a}, & \text{if } A_m < \frac{r_1}{\delta_1} \\ r_0 > \frac{r_1}{a}, & \text{if } A_m < \frac{r_0}{\delta_0} \\ r_0 < \frac{r_1}{a}, & \text{if } A_m < \frac{r_0-r_1}{\delta_0-\delta_1} \end{cases}$$

For the last situation, there are additional conditions, as it happens with B_R: h is equal or lower than a determined value or, if it is higher, there is the need to assign σ a maximum value as well.

However, if C exceeds the maximum value presented in the stability conditions of B_S, and the antimicrobial drug is low enough, $A_m < \frac{r_0}{\delta_0}$ for B_S and $A_m < \frac{r_1}{\delta_1}$ for B_R, there is room for oscillations. In that case, the antibiotic dose is too low to clear the infection and a high carrying capacity allows bacteria to grow enough to activate and to be killed by the immune system. This defensive action decreases with the decrease of bacteria load, which allow them to grow again and this process perpetuates in time, generating an oscillatory behavior.

- **Stability of Clearance**

The fixed point associated with the scenario of clearance is the trivial one. It is a stable node if satisfies one of two pairs of conditions, closely related to the conditions of existence and stability of both colonization scenarios. If the antimicrobial drug dose exceeds the maximum value to maintain colonization, while satisfying the condition of stability related to the growth rate, clearance of the infection will happen.

Mathematically, clearance of the infection is a stable node if $A_m > \frac{r_0}{\delta_0}$ and $r_0 > \frac{r_1}{a}$ or $A_m > \frac{r_1}{\delta_1}$ and $r_1 \geq ar_0$.

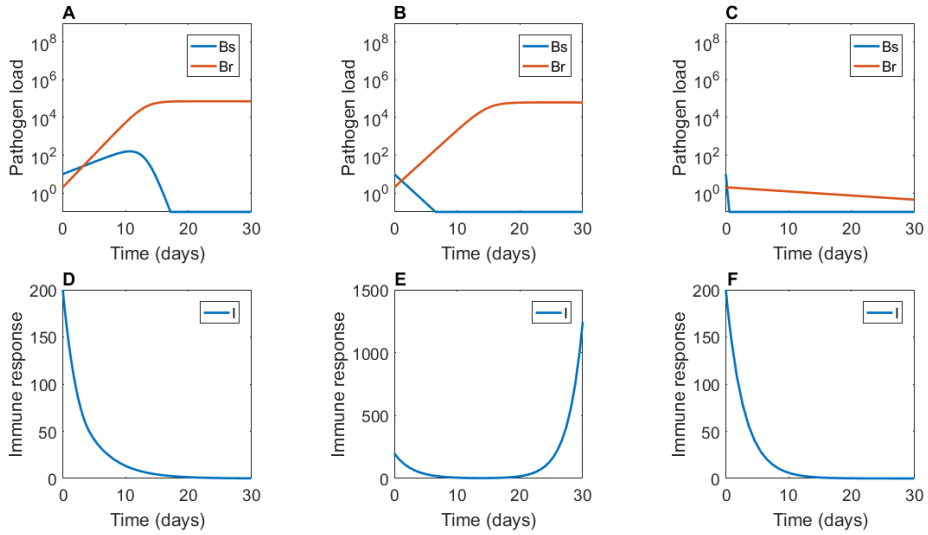

Figure 3.2: **Simulations of the dynamics of B_S, B_R and I, over 30 days, under a classical treatment.** Panels A and D represent a case of colonization of B_R, $A_m = 3$ and $\sigma = 0.3$. Panels B and E represent a case of persistence of B_R, $A_m = 4$. Panels C and F represent a case of clearance, $A_m = 11.5$. Colonization and persistence of B_S are omitted because are equivalent to B_R. Other parameters as in Table 2.1.

3.2 Antibiotic Dose with Pharmacodynamics

Despite the insights provided by the constant-dose approximation, it is known that there is an elaborated quantitative interaction between the possibly varying concentrations of the antibiotic and the growth and death rates of the target bacteria (Abdul-Aziz *et al.*, 2015; Ankomah & Levin, 2014). This role of pharmacodynamics of the drug is adressed in this section. The main goal is to verify if the increase in complexity of the model has repercussions on the results obtained.

3.2.1 Mathematical Model

Within-host dynamics for a B_S and B_R mixed infection, and additionally the immune system (I) and the antimicrobial drug concentration in the host (A), are described by the following set of ordinary differential equations:

$$\frac{dB_S}{dt} = r_0 B_S\left(1 - \frac{B_S + B_R}{C}\right) - dB_S I - \delta_0 B_S A \tag{3.1}$$

$$\frac{dB_R}{dt} = r_1 B_R\left(1 - \frac{B_S + B_R}{C}\right) - dB_R I - \delta_1 B_R A \tag{3.2}$$

$$\frac{dI}{dt} = \frac{\sigma I(B_S + B_R)}{k + B_S + B_R} - hI \tag{3.3}$$

$$\frac{dA}{dt} = 1 - \frac{A}{A_m} \tag{3.4}$$

where $B(t) = B_S(t) + B_R(t)$ is the total pathogen load at time t.

There are no additional parameters in this model, because we impose the same equilibrium concentration of the drug given by A_m, as in the constant dose model (Section 3.1) and we assume a drug inflow rate of 1 per unit of time (Equation 3.4). However, a new variable, A, changes over time to represent the alterations of the antibiotic concentration in the host, which affects the way bacteria are killed.

3.2.2 Equilibria

From this more complex mathematical model, arise the same steady states, concerning their biological interpretation and the values of the variables at equilibrium. Additionally, there is only A that, at equilibrium, is always A_m, fact that comes directly from the ordinary differential equations system.

3. ASYMPTOTIC ANALYSIS OF BACTERIAL INFECTION UNDER TREATMENT

Steady states

The fixed points of the mathematical model of bacterial dynamics with treatment considering pharmacodynamics are enumerated and described below:

- Colonization by B_S and no B_R, and colonization by B_R and no B_S:

$$\begin{bmatrix} B_S^* = & -\frac{C(A_m\delta_0 - r_0)}{r_0} \\ B_R^* = & 0 \\ I^* = & 0 \\ A^* = & A_m \end{bmatrix} \; and \; \begin{bmatrix} B_S^* = & 0 \\ B_R^* = & -\frac{C(A_m\delta_1 - r_1)}{r_1} \\ I^* = & 0 \\ A^* = & A_m \end{bmatrix}$$

- Persistence of B_S under some immunity and no B_R, and persistence of B_R under some immunity and no B_S:

$$\begin{bmatrix} B_S^* = & \frac{hk}{\sigma - h} \\ B_R^* = & 0 \\ I^* = & -\frac{-r_0 + A_m\delta_0}{d} + \frac{hkr_0}{Cd(h-\sigma)} \\ A^* = & A_m \end{bmatrix} \; and \; \begin{bmatrix} B_S^* = & 0 \\ B_R^* = & \frac{hk}{\sigma - h} \\ I^* = & -\frac{-r_1 + A_m\delta_1}{d} + \frac{hkr_1}{Cd(h-\sigma)} \\ A^* = & A_m \end{bmatrix}$$

- Clearance of the infection:

$$\begin{bmatrix} B_S^* = & 0 \\ B_R^* = & 0 \\ I^* = & 0 \\ A^* = & A_m \end{bmatrix}$$

Because all fixed points are the same, this more realistic model (in theory) does not bring any possibility to know more about the role of treatment during a bacterial infection, when compared to the initial model presented in this work.

Conditions for Stability

Given the resemblance between the fixed points of this model and the ones of the previous model, it is not a surprise that all conditions for both existence and stability are exactly the same, reason why they are not present them here again. We cannot state the same about the bacterial transient dynamics, which will surely be different.

3.3 Effectiveness of Antibiotic Dose

Another option is to assume that not all of the administrated antibiotic is able to kill bacteria. In other words, only an amount of the dose given to the host is efficient and it can be represented by $\frac{A_m}{\alpha+A_m}$. Assuming this Hill-function with coefficient 1 describes that as we increase the antibiotic dose A_m, the actual potency of the drug saturates, and the maximal effective dose is 1. The parameter α represents the drug concentration where half-maximal potency is obtained and δ becomes then the maximal killing rate of the drug per unit of time.

3.3.1 Mathematical Model

The system is composed by three equations, related to both sensitive and resistant bacterial strains (B_S and B_R, respectively) and to the immune system of the host (I):

$$\frac{dB_S}{dt} = r_0 B_S\left(1-\frac{B_S+B_R}{C}\right) - dB_S I - \delta_0 B_S\left(\frac{A_m}{\alpha+A_m}\right) \tag{3.5}$$

$$\frac{dB_R}{dt} = r_1 B_R\left(1-\frac{B_S+B_R}{C}\right) - dB_R I - \delta_1 B_R\left(\frac{A_m}{\alpha+A_m}\right) \tag{3.6}$$

$$\frac{dI}{dt} = \frac{\sigma I(B_S+B_R)}{k+B_S+B_R} - hI \tag{3.7}$$

where $B(t) = B_S(t) + B_R(t)$ is the total pathogen load at time t.

Besides all the parameters in Table 2.1, this extension has an additional parameter, α, with the same units of A_m.

3.3.2 Equilibria

As a result of the study of stability of the model, the same five steady states arised, concerning their biological interpretation (Figure 3.3). However, the values of bacteria at equilibrium in colonization scenarios and the values of the

immune response, both for cases of colonization and persistence, show some alterations, not observed before. Also in this model, there is no fixed point where there is colonization or persistence of both bacterial subpopulations B_R and B_S simultaneously, as it happens in all cases in which antibiotics are administrated.

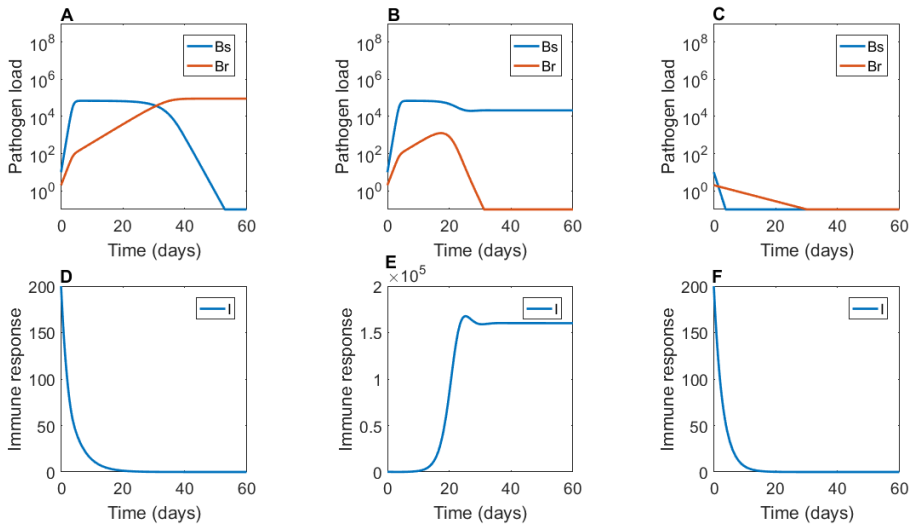

Figure 3.3: **Simulations of the dynamics of B_S, B_R and I, during 60 days, to mimic infection equilibrium scenarios considering effective dose.** Panels A and D represent a case of colonization of B_R, $\sigma = 0.3$ and $A_m = 1$. Panels B and E represent a case of persistence of B_S, $A_m = 6$. Panels C and F represent a case of clearance, $A_m = 12$. Colonization of B_S and persistence of B_R are omitted because are equivalent to the ones already shown. Other parameters as in Table 2.1.

Steady states

The fixed points of the mathematical model of bacterial dynamics considering the effectiveness of the treatment are enumerated and described below:

- Colonization by B_S and no B_R, and colonization by B_R and no B_S:

$$\begin{bmatrix} B_S^* = & -\frac{C(A_m\delta_0 - r_0(\alpha+A_m))}{r_0(\alpha+A_m)} \\ B_R^* = & 0 \\ I^* = & 0 \end{bmatrix} \text{ and } \begin{bmatrix} B_S^* = & 0 \\ B_R^* = & -\frac{C(A_m\delta_1 - r_1(\alpha+A_m))}{r_1(\alpha+A_m)} \\ I^* = & 0 \end{bmatrix}$$

- Persistence of B_S under some immunity and no B_R, and persistence of B_R under some immunity and no B_S:

$$\begin{bmatrix} B_S^* = & \frac{hk}{\sigma - h} \\ B_R^* = & 0 \\ I^* = & -\frac{-r_0(\alpha + A_m) + A_m\delta_0}{d(\alpha + A_m)} + \frac{hkr_0}{Cd(h-\sigma)} \end{bmatrix} and \begin{bmatrix} B_S^* = & 0 \\ B_R^* = & \frac{hk}{\sigma - h} \\ I^* = & -\frac{-r_1(\alpha + A_m) + A_m\delta_1}{d(\alpha + A_m)} + \frac{hkr_1}{Cd(h-\sigma)} \end{bmatrix}$$

- Clearance of the infection:

$$\begin{bmatrix} B_S^* = & 0 \\ B_R^* = & 0 \\ I^* = & 0 \end{bmatrix}$$

Conditions for Stability

Considering the alterations in the fixed points, due to the changes in the mathematical model in the first instance, it is expected that the conditions for stability suffer some changes as well.

- **Stability of Colonization**

Concerning the steady states corresponding to colonization scenarios, existence can be obtained by two distinct paths. One option is for them to grow at least at the same rate that they are killed by the antibiotics: $r_0 \geq \delta_0$ for B_S and $r_1 \geq \delta_1$ for B_R. On the other hand, that same outcome can be achieved with a maximum antimicrobial drug dose, that can be written as a minimum value of effectiveness of the drug. Concerning colonization of sensitive bacteria, there is the need to satisfy

$$A_m > \frac{r_0\alpha}{\delta_0 - r_0}$$

For the resistant subpopulation, the condition is equivalent:

$$A_m > \frac{r_1\alpha}{\delta_1 - r_1}.$$

The other conditions for stability are the same presented for the case of Constant Antibiotic Dose. The only difference is where we had r_0 or r_1, the net growth rate per capita becomes $r_0 - \frac{\delta_0 A_m}{A_m + \alpha}$ or $r_1 - \frac{\delta_1 A_m}{A_m + \alpha}$.

Comparing to the model in which is considered a constant dose (or even considering pharmacodynamics), and concerning existence, one of the conditions is equivalent: a maximum antimicrobial drug dose. However, in this last model, this condition can be substituted by other which relates growth and killing rates. Regarding stability, the behavior is similar: there is a minimum value for the intrinsic growth rate and the two same mechanisms for growth control: balance between parameters related to the immune system or a maximum carrying capacity (although this maximum value differs in both models).

- **Stability of Persistence**

The same happens concerning the stability conditions for the persistence case. The only difference is where we had r_0 or r_1, the net growth rate per capita becomes $r_0 - \frac{\delta_0 A_m}{A_m+\alpha}$ or $r_1 - \frac{\delta_1 A_m}{A_m+\alpha}$.

- **Stability of Clearance**

The fixed point associated with the scenario of clearance is the trivial one. In order to be considered a stable node, this equilibrium has to satisfy two conditions:

$$\frac{A_m}{\alpha + A_m}\delta_0 > r_0 \text{ and } \frac{A_m}{\alpha + A_m}\delta_1 > r_1.$$

Comparing to the model in which is considered a constant dose (or even considering pharmacodynamics), in this case, it does not exist a mimimum antibiotic dose. Instead, the final condition becomes:

$$\frac{A_m}{\alpha + A_m} > max\Big(\frac{r_0}{\delta_0}, \frac{r_1}{\delta_1}\Big).$$

After all these modelling approaches, the main conclusion is that the most simple model, the first one presented in the chapter, captures the major dynamics of treatment and no big advantages were found with the other extensions and that is why we opt to choose that modelling approach from now on.

Chapter 4

Bacterial Infection Dynamics Under Treatment

So far, we studied the asymptotic behavior of bacterial infections for some special cases, under antimicrobial treatment, namely in the $lim_{t\to\infty}$, which allows the identification of equilibrium states and the conditions needed to achieve them and their stability. However, it is indispensable to do an in-depth investigation of the transient dynamics of the infections. Long-term measures of bacterial subpopulations growth and death rates do not capture the population's fluctuations in the short-term. The study of transient infection dynamics provides more detailed knowledge, which can be crucial to design the best treatment strategy. The equation regarding immunity remains the same (Equation 2.3) and within-host bacterial dynamics for a mixed infection are described by the following equations:

$$\frac{dB_S}{dt} = r_0 B_S\left(1 - \frac{B_S + B_R}{C}\right) - dB_S I - \delta_0 B_S A_m \eta(t) \tag{4.1}$$

$$\frac{dB_R}{dt} = r_1 B_R\left(1 - \frac{B_S + B_R}{C}\right) - dB_R I - \delta_1 B_R A_m \eta(t) \tag{4.2}$$

where $\eta(t) = \begin{cases} 1, & \text{if } \tau_1 \leq t \leq \tau_1 + \tau_2 \\ 0, & \text{if } t < \tau_1 \text{ or } t > \tau_1 + \tau_2 \end{cases}$ represents the schedule of treatment administration.

Different treatments differ mainly in three aspects: the timing, also referred to as onset (τ_1), the dose (A_m) and the duration (τ_2). For contrasting timings, the best treatment strategy implies an equilibrium between the dose and the duration. Distinct combinations of these aspects result in divergent transient dynamics and it is possible to identify a cluster of efficient combinations for each infection scenario based on one's optimization criteria.

4.1 Persistent Infection Scenario

The first studied scenario represents a persistent infection scenario, and its parameters were already studied in detail in Chapter 2. Biologically, starting with more B_S than B_R gives them a growth advantage. Growing bacterial density within-host stimulates an immune response and, over 30 days, only B_S subpopulation persists, in the absence of antibiotic administration. We choose to focus on two interesting time points to start treatment here (Figure 4.1). The first one happens at day 7, when both bacterial subpopulations have reached a plateau and persist together (Case A, 4.1.1). The other one, temporally, happens later, at day 21. At that point, B_R has been cleared via competition with the sensitive bacteria and only B_S persists, although at a lower level (Case B, 4.1.2).

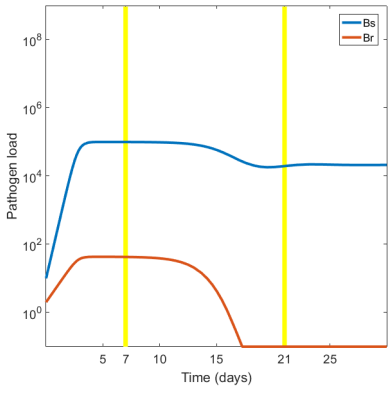

Figure 4.1: **Simulations of persistent infection dynamics of B_S and B_R, during 30 days, without treatment.** The pathogen load value is its common log value. Identification of treatment onsets: the yellow vertical lines depict case A ($\tau_1 = 7$), and case B ($\tau_1 = 21$). Other parameters as in Table 2.1.

4.1.1 Case A: Treatment onset at day 7

In this scenario, treatment onset is at day 7, in which both bacterial subpopulations persist together and the system can evolve to different scenarios, depending on the treatment strategy. Two of them are characterized by the persistence of both subpopulations: one in which $B_S > B_R$ (scenario I) and another one in which $B_R > B_S$ (scenario II). Another two possibilities are persistence of B_S and no B_R and persistence of B_R and no B_S (scenario III and IV, respectively). The last potential scenario is the total extinction, clinically known as clearance of the infection (scenario V). The dynamics with antibiotics only remain the same that without them or return to the pre-treatment level if the dose does not overcome the critical value, $A_m = \frac{r_0}{\delta_0}$, or if the duration τ_2 is not long enough (scenario III).

To study all the possibilities numerically, A_m has to vary from doses below the minimal inhitory dose for B_S ($A_m < A_m^* = \frac{r_0}{\delta_0}$) to doses above the minimal inhibitory dose for B_R ($A_m > A_m^{**} = \frac{r_1}{\delta_1}$), in the absence of immune response (Figure 4.2). An initial approach is to compare outcomes, for two specific durations that are common in the clinical practice ($\tau_2 = 7$ and $\tau_2 = 14$). We illustrate the behavior of the model for only 4 specific doses: $A_m = 1mg/l$ ($A_m < A_m^*$), $A_m = 4mg/l$ ($A_m > A_m^*$ but closer to A_m^*), $A_m = 6mg/l$ ($A_m < A_m^{**}$ but closer to A_m^{**}) and $A_m = 20mg/l$ ($A_m > A_m^{**}$) (Figure 4.2).

Shorter treatments ($\tau_2 = 7$)

- If $A_m < \frac{r_0}{\delta_0}$ ($A_m = 1mg/l$), the antibiotic treatment has almost no effects and the dynamics are similar to the ones without treatment, as expected.
- A small dose ($A_m = 4mg/l$) allows B_R subpopulation to overcome, for a short period of time, the B_S subpopulation. However, with the end of the antimicrobial administration, sensitive bacteria recover and after 30 days of simulation there is the persitence of both bacterial subpopulations.
- If the dose increases further ($A_m = 6mg/l$), only B_R can persist, with the extinction of B_R during the treatment period.
- With an even higher dose ($A_m = 20mg/l$), both bacterial populations are extinguished at the end of the treatment.

Longer treatments ($\tau_2 = 14$)

- If $A_m < \frac{r_0}{\delta_0}$ ($A_m = 1mg/l$), the antibiotic treatment has almost no effects and the dynamics are similar to the ones without treatment, as expected. This fact is due to dose and independent of the duration.
- Low doses ($A_m = 4mg/l$) combined with longer duration give a greater competitive advantage to pre-existing B_R and are more likely to transform a mixed infection into a totally resistant one.
- The same pattern happens if the dose increases ($A_m = 6mg/l$). These last two combinations are inefficient to get clearance of the infection.
- Clearance is only achieved with a much more higher dose ($A_m = 20mg/l$). In this last case, sensitive bacteria subpopulation responds and it goes extinct imediatelly with treatment onset while resistant bacteria take nearly a week to be cleared (but still during treatment).

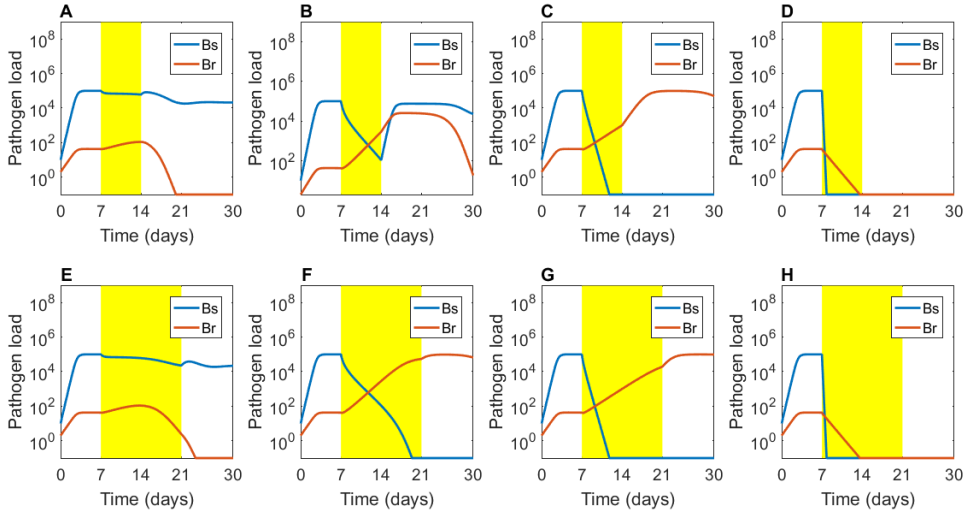

Figure 4.2: **Illustration of bacterial subpopulations model dynamics considering dose-duration interactions.** Panels A-D for shorter treatment ($\tau_2 = 7$) and Panels E-H for longer treatment ($\tau_2 = 14$). A set of different doses is displayed: $A_m = 1$ (Panels A and E), $A_m = 4$ (Panels B and F), $A_m = 6$ (Panels C and G) and $A_m = 20$ (Panels D and H). Yellow region identifies the treatment administration period. Other parameters as in Table 2.1.

In summary, different treatment regimens, differentiated by their timing, dose and duration, result in different infection outcomes, sometimes in clearance, sometimes in selection of resistance or relapses dominated by B_S. In the last instance, this is reflected by harmful consequences for the host.

Independently of the duration of the treatment, we are dealing with a change of the infection scenario only if the minimum dose required for clearance is achieved (data obtained by the asymptotic analysis). If the dose is too low, the infection will continue to persist. The host cannot get rid of the infection because the immunity response is not stimulated enough to deal with it and the dose of antibiotics is not sufficient either. However, a persistence scenario can gradually progress into clearance if A_m is higher than the critical minimum value mentioned above. In that case, because the condition is satisfied, clearance is reached in a finite time even if the duration of treatment is moderate. A free-infection host, in this case, is only possible if it is used a sufficient high dose of antibiotics, even for a short time.

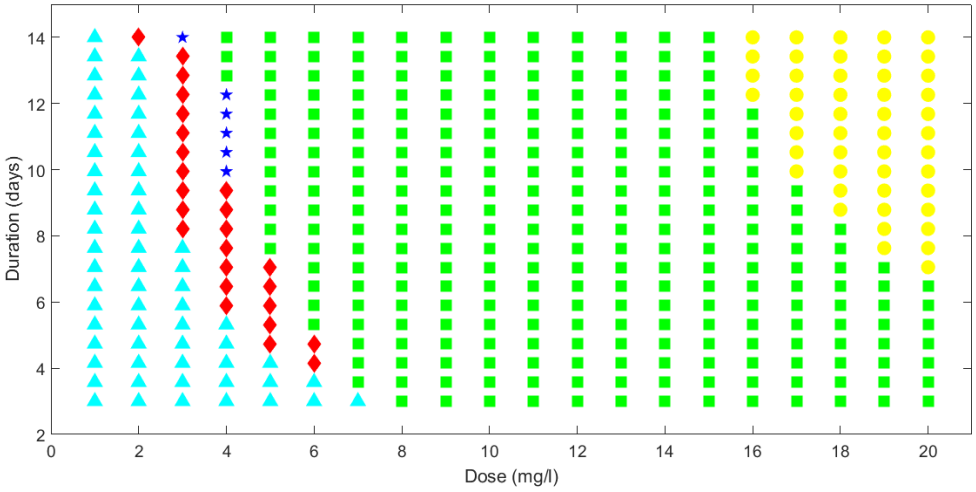

Figure 4.3: **Outcomes of treatment considering dose-duration interactions.** A_m varies from 1 to 20 mg/l and τ_2 from 3 to 14 days. Different markers for each scenario at the end of the simulation: red diamonds for Scenario I ($B_S > B_R$); blue stars for Scenario II ($B_R > B_S$); cyan triangles for Scenario III (B_S and no B_R); green squares for Scenario IV (B_R and no B_S);and yellow circles for Scenario V (clearance). Other parameters as in Table 2.1.

A more complete picture is obtained by a general study of the dose-duration interaction and the infection outcomes at the end of 30 days. For a wider range of values for duration and dose, it is possible to verify which is the final infection scenario (Figure 4.3).

It is clear, by the existence of scenarios which do not show any alterations in comparison with no treatment (Figure 4.3, cyan triangles), that there are mimimum values both for dose and duration. Significant alterations on the values of B_S and B_R only start with Scenario I (Figure 4.3, red diamonds) and Scenario II (Figure 4.3, blue stars), in which both bacterial subpopulations persist together. The majority of combinations results in Scenario III (Figure 4.3, green squares), in which resistant bacteria persist overtime. This shows that a greater part of antibiotic administration strategies tends to select B_R. In all these situations mentioned so far, over 30 days, there is still infection. Clearance (Figure 4.3, yellow circles) can only be achieved starting with a minimum dose of $A_m = 16mg/l$ and duration of 13 days. The minimal dose that achieves clearance with a 7 days treatment is dose $A_m = 20mg/l$. Only in this restricted number of cases, the host is free of the infection.

The higher the ratio $\frac{B_R}{B_S}$ at treatment onset ($\tau_1 = 7$), the smaller the yellow area will become. The green area is due to the seletion mechanism, which in this case is to free up space for B_R. Here, the free area expansion will be less restricted, which results in a higher selection for B_R. Graphically, this means that the number of green squares will be higher too. In few words, the higher the ratio $\frac{B_R}{B_S}$ at τ_1, the more likely is to select B_R and the less likely is to reach clearance at day 30.

4.1.2 Case B: Treatment onset at day 21

Considering the conditions of the second scenario, when treatment is applied at day 21, we start only with the persistence of B_S, since B_R was already extinguished. From here, it is possible to relate the subsequent decrease of sensitive pathogen load with the dose and duration of the treatment and to verify if the bacteria level falls below the threshold B_{ext}. This can be written using a inequality, in which it is assumed an exponential rate of bacterial decline, dependent of

the growth rate and treatment regime and neglecting the small role of killing by the immune system:

$$B_S^* e^{(r_0 - \delta_0 A_m)\tau_2} \leq B_{ext} \tag{4.3}$$

Asymptotic dynamics can help to estimate the value of sensitive bacterial load, when these bacteria persist alone, in the presence of immune response. Immunity plays a role only in the initial B_S levels at treatment onset, $B_S^* = \frac{hk}{\sigma - h}$. Administration of treatment is only logical when $B_S^* > B_{ext}$, i.e when the $h < \sigma < \frac{h(B_{ext}+k)}{B_{ext}}$ is satisfied.

It also gives information about the minimum dose needed to initiate bacterial decline, independently of the duration of treatment, $A_m > \frac{r_0}{\delta_0}$.

At any time point, during treatment (between τ_1 and $\tau_1 + \tau_2$), there is an immune response, $I > 0$. This means that the net growth rate of sensitive bacteria, $\frac{dB_S}{dt}$, is always lower than we assume, $\frac{dB_S}{dt} \leq (r_0 - \delta_0 A_m) B_S$. In this case, calculations are a conservative approximation, assuming the worst case scenario, in which bacteria are only killed by the antibiotics and immune system may be considered negligible during treatment.

Based on this, it is possible to calculate the dose, A_m, needed for clearance, at a particular duration τ_2 (Figure 4.4). This inequality comes directly from inequality 4.3, after having substituted the value of B_S^*:

$$A_m \geq \frac{\tau_2 r_0 - ln\left(\frac{B_{ext}(\sigma - h)}{hk}\right)}{\tau_2 \delta_0}. \tag{4.4}$$

Inequality 4.4 can be rewritten in the following way:

$$\tau_2 \geq \frac{ln\left(\frac{B_{ext}(\sigma - h)}{hk}\right)}{r_0 - A_m \delta_0}. \tag{4.5}$$

These two last inequalities 4.4 and 4.5 represent the trade-off between A_m and τ_2.

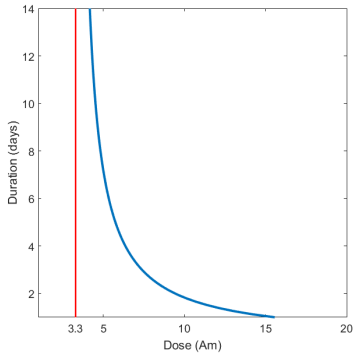

Figure 4.4: **Duration-dose interaction during treatment to get clearance of the infection.** Minimum theoretical inhibitory dose for B_S (red) and minimum dose for each duration that allow infection clearance with that particular treatment duration (blue), following from inequality 4.4 . Clearance is also obtained by any combination above the blue line. Other parameters as in Table 2.1.

One major benefit of analyzing infection clearance by a given treatment (A_m, τ_2) is the opportunity to check the sensitivity of our criterion for clearance (inequality 4.4). We study sensitivity to σ, h and k, immune response parameters which play the most crucial role in the starting bacterial load upon treatment.

If the focus is on parameter σ, related to the activation of an immune response, there is almost no sensitivity to it because all σ values used in this simulations satisfy the mentioned condition which ensures that $B_S^* > B_{ext}$ (Figure 4.5).

Considering the immune system action decay rate, h, the lower this parameter goes, the higher the dose has to be, for the same duration, to get clearance (Figure 4.6). If the immune response decays faster, this mechanism will be less efficient to fight the infection and antibiotics need to take action to compensate, in order to get clearance as well. This difference becomes less significant as the duration increases. In general lines, the interaction between dose and duration is not very sensitive to h, as observed in the numerical simulations (Figure 4.6).

This sensitivity is higher if the parameter in study is k, the host immunity threshold, since the three curves are further apart. Biologically, if the immunity plays a smaller role, treatment becomes the principal mechanism against infection. An increase of k results then in an increase of A_m (Figure 4.7).

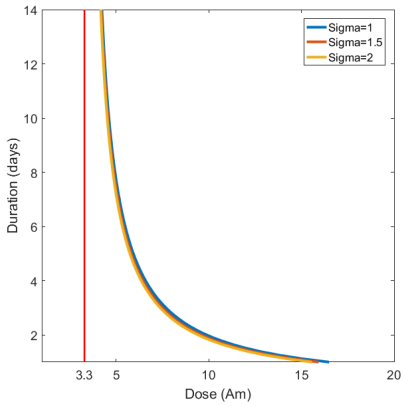

Figure 4.5: **Sensitivity of duration-dose interaction to the parameter σ.** Theoretical investigations of minimum inhibitory dose for B_S (red) and minimum dose for each duration that allow clearance of the infection changes with different values of maximum immune cell recruitment (blue for $\sigma = 1$, orange for $\sigma = 1.5$ and yellow for $\sigma = 2$). Clearance is also obtained by any duration-dose combination above the last mentioned lines. Other parameters as in Table 2.1.

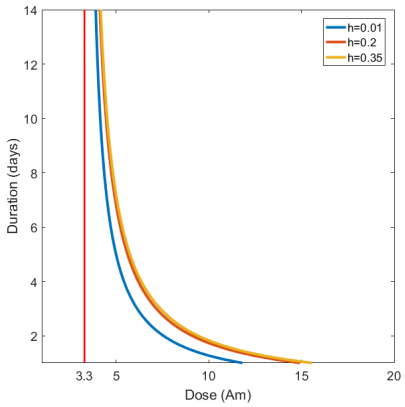

Figure 4.6: **Sensitivity of duration-dose interaction to the parameter h.** Theoretical investigations of minimum inhibitory dose for B_S (red) and minimum dose for each duration that allow clearance of the infection changes with different values of immune system action decay rate (blue for $h = 0.01$, orange for $h = 0.2$ and yellow for $h = 0.35$). Clearance is also obtained by any duration-dose combination above the last mentioned lines. Other parameters as in Table 2.1.

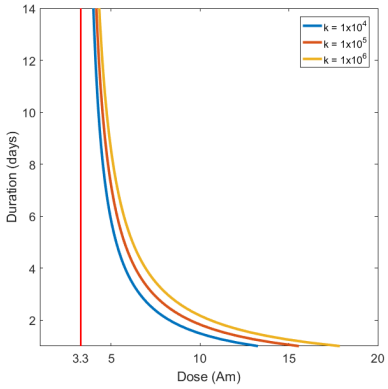

Figure 4.7: **Sensitivity of duration-dose interaction to the parameter k.** Theoretical investigations of minimum inhibitory dose for B_S (red) and minimum dose for each duration that allow clearance of the infection changes with different values of host immunity threshold. Blue line for $k = 10^4$, situation in which system responds rapidly to low bacterial loads. Orange line for $k = 10^5$ (parameter as in Table 1). Yellow line for $k = 10^6$, which mimics an immune response that acts more slowly against B_R because it needs more stimulation. Clearance is also obtained by any duration-dose combination above the last mentioned lines. Other parameters as in Table 2.1.

These three plots (Figures 4.5, 4.6, 4.7) are elucidative about the small role of immunity nonethless, noticing that not all the parameters of the immune response are important in the same way. The lower B_S is at treatment onset, which means the better the immune response has controlled bacteria, the less demand for treatment there is. This confirms the balance between both mechanisms responsible for bacterial growth control: immunity and treatment. An infection that has been brought down to a lower level by the immune response, will require less aggressive and less prolonged treatments.

For example, if the duration is 7 days, the minimum dose required to achieve clearance is $5.052 mg/l$, considering no immunity and the other parameters values by default (approximation in inequality 4.4).

But, if there is an immune response active also during treatment, the extinction of B_S takes less time (Figure 4.8), since both mechanisms of immunity and antibiotics act together.

As expected, the smaller the duration of the treatment, the higher has to be the dose of the antibiotic (Figure 4.9). A more agressive or a more moderate treatment can be chosen to get clearance, depending on the duration.

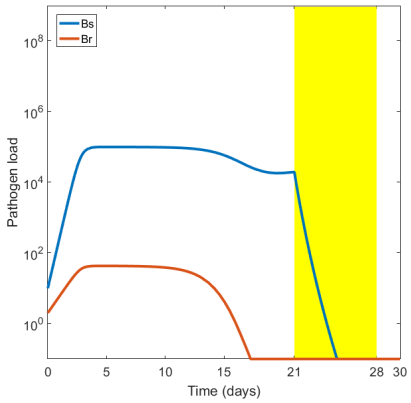

Figure 4.8: **Illustration of the clearance dynamics of B_S and B_R, over 30 days.** The pathogen load value is its common log value. Treatment starts at day 21, τ_2=7 and A_m=5.052. Yellow region identifies treatment administration period. Other parameters as in Table 2.1.

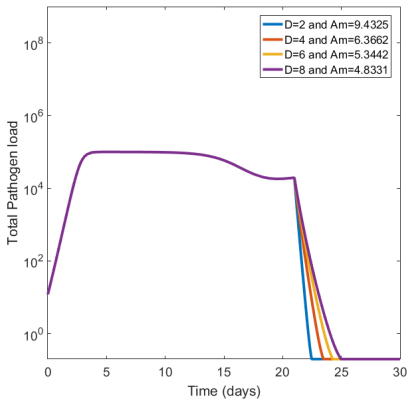

Figure 4.9: **Illustration of the clearance dynamics of the total pathogen load, with different successful treatments.** The total pathogen load value is its common log value. Treatment starts at day 21. Different combinations of duration and dose. Other parameters as in Table 2.1.

4.2 Acute Infection Scenario

The second scenario chosen to study the transient dynamics with treatment is an acute infection (Figure 4.10), characterized by higher bacterial density and higher immune response stimulated by the bacterial load. Due to that, clearance is achieved thanks to the immune system. This scenario was already studied in detail in Chapter 2. Whatever the bacterial mixture, immunity will clear the infection in this case, as it does not care whether bacteria are sensitive or resistant. The only difference lies in the net growth rate of B_{TOT}. If it is composed of mainly B_S, the rate will be higher and hence stimulate faster immunity, resulting in a shorter peak. If it is composed more of B_R, it will grow slowly, stimulate more slowly the immune response and result in a lower more extended peak.

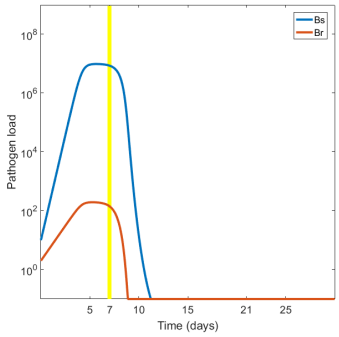

Figure 4.10: **Illustration of acute infection dynamics of B_S and B_R, over 30 days.** Approximate bacterial peak at dat 7, when treatment starts. $C = 10^7$. Other parameters as in Table 2.1.

What happen if, in this situation, antibiotics are administrated to the host? One way to understand those effects is to do an analysis of the A_m-τ_2 interaction and how it is reflected on the final infection scenario, over 30 days (Figure 4.11). Different outcomes can be identified: persistence of B_S and no B_R (Scenario III), selection and persistence of B_R (Scenario IV) and clearance (Scenario V). In this case, arises a distinct mechanism of B_R selection. Instead of the generation of free space for B_R, antibiotics do not play a role and selection is due to immune response decay. If it decays less (Figure 4.11, Panels B and C), clearance becomes a more common outcome. To apply treatment in this scenario creates a worst

outcome, since without them the infection would be always cleared. The only possible advantage is, in the cases that clearance is achieved as well, the host is infected for a smaller period of time, sufering less damage due to it.

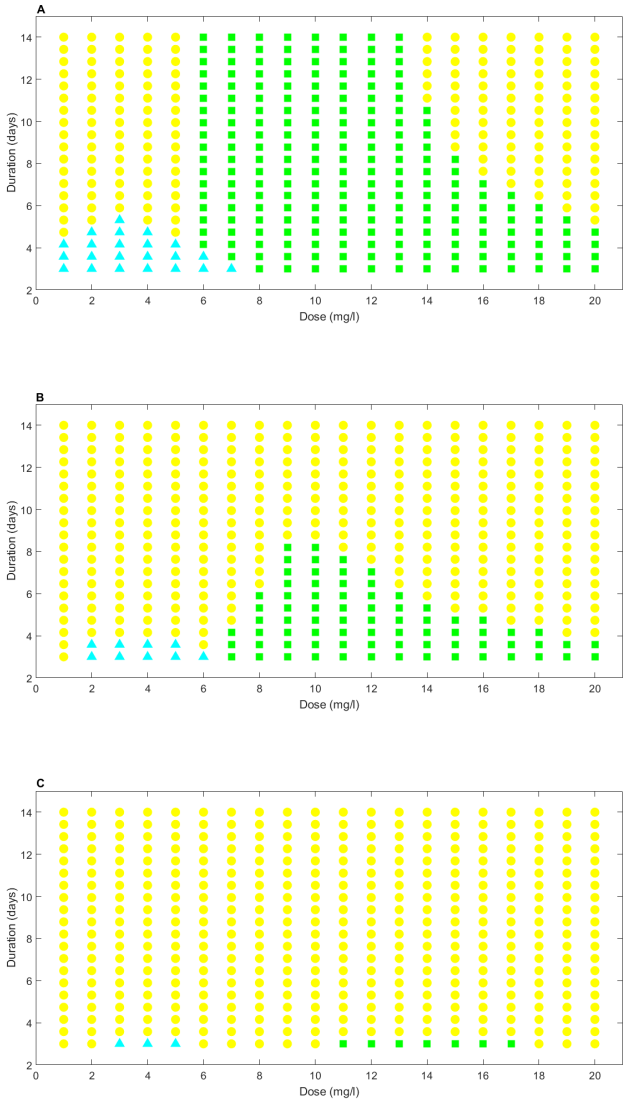

Figure 4.11: **Outcomes of treatment considering A_m-τ_2 interactions in an acute infection for different immune responses.** A_m varies from 1 to 20 mg/l and τ_2 from 3 to 14 days. Markers represent different scenarios at T=30 days: cyan triangles for Scenario III (B_S and no B_R); green squares for Scenario IV (B_R and no B_S); and yellow circles for Scenario V (clearance). $C = 10^7$. Panel A $h = 0.35$; B $h = 0.3$; C $h = 0.25$. Other parameters as in Table 2.1.

Chapter 5

Exploring Evolution of Bacterial Traits During Infection

Until now, we have studied competition between sensitive and resistant subpopulations with fixed growth phenotypes (r_0, r_1) and antibiotic susceptibility a. Now we will study the possibility of different random combinations of phenotypes and their dynamics during treatment. The main focus in this chapter is to understand how pathogen's evolution affects the bacterial infection dynamics under treatment and the other way around. Starting with an infection with only sensitive bacteria we will model how *de novo* resistance evolution will happen and how the infection can progress gradually into a resistant one. During this investigation, we will have to define and model what types of resistant strains are generated and selected by different treatments.

5.1 Mathematical Model

The mathematical model for infection dynamics described in the beginning of this thesis is a deterministic one (Chapter 2). In this chapter, we add evolutionary dynamics and stochastic emergence of new mutants. This hybrid model (Kepler & Perelson, 1995), divided into a stochastic component focused on the emergence of new resistant mutants and a deterministic component related to the subsequent bacterial growth, is defined by the following set of ordinary differential equations:

$$\frac{dB_S}{dt} = r_0\left(1-\frac{B}{C}\right)B_S - dB_S I - \delta_0 B_S A_m \eta(t) \tag{5.1}$$

$$\frac{dB_R^i}{dt} = r_0(1-c_i)\left(1-\frac{B}{C}\right)B_R^i - dB_R^i I - a_i\delta_0 B_R^i \eta(t) A_m \tag{5.2}$$

$$\frac{dI}{dt} = \sigma I\left(\frac{B}{k+B}\right) - hI \tag{5.3}$$

where $B = B_S + \sum_{i=1}^{n(t)} B_R^i$ and $\eta(t) = \begin{cases} 1, & \text{if } \tau_1 \leq t \leq \tau_1 + \tau_2 \\ 0, & \text{if } t < \tau_1 \text{ or } t > \tau_1 + \tau_2 \end{cases}$.

The initial conditions of the mathematical model are $B_S(0) = 10$, $B_R(0) = 0$ and $I(0) = 200$. At the time of emergence of each strain, $B_R^i(0) = 10\ \forall i$. Additionally, $n(t)$ is the number of existing resistant mutants at time t.

Starting with sensitive bacteria, in consecutive steps we have the emergence of the first new strain, initialized with index 1 and the other strains indexed 2, ... until n. The emergence of each new resistant strain is based on the frequencies of existing strains in that time point, giving rise to the pool of possible "parents". Each new mutant is randomly assigned a parent sub-population. The parent is sampled multinomially from the frequencies of all strains in a population, $\frac{B_R^i(t)}{B(t)}$.

The arrival time of each new strain is exponentially distributed. P is the probability of no next mutant generation. For all indexed mutants, when this probability (Equation 5.4) hits a random threshold, the emergence of the next mutant occurs and it changes by $\frac{dP}{dt} = -mPB$, considering m the spontaneous resistance mutation rate per cell per unit of time. Considering $P(0) = 1$, this probability is given by:

$$P(t) = P(0)e^{-m\int_0^t B(s)ds} \tag{5.4}$$

Each strain is defined by two traits: the fitness cost of resistance, c_i, and antibiotic susceptibility, a_i, which defines the resistance of the strain to the treatment. The 2-d phenotype of each new mutant, indexed i, is randomly drawn from a normal distribution with mean vector μ and an input covariance matrix Σ:

$$\begin{pmatrix} c_{new} \\ a_{new} \end{pmatrix} \sim \mathcal{N}(\mu, \Sigma)\,.$$

Traits of the new strain are based on the parent's traits, which determine μ, and are thus more likely to be close to the most frequent subpopulation.

If a certain sub-population has a density below the extinction threshold, B_{ext}, it will be considered extinct and its density set to 0. All these processes take space at the same time that treatment is applied. Treatment's onset happens at day known as τ_1 and it goes on for a duration of τ_2 days.

5.1.1 Pathogen's trait space

In this model, evolution is implemented at a phenotypic level. Spontaneous mutations are simulated by the discrete alterations in the phenotypic space. Each bacterial strain, either sensitive or resistant, as described before, can be characterized by two phenotypic traits, which can vary between 0 and 1, inclusive. Fitness cost, here represented by $c_i = 1 - \frac{r_i}{r_0}$, is described as a relative decreased competitive ability of a drug-resistant mutant without treatment (Andersson, 2006). Notice that this definition of c_i is slightly different from the presented in Chapter 2. Pleiotropy occurs when the same genetic mutation affects multiple traits. This is the underlying assumption in our evolution model: one same mutation event is associated to simultaneous changes in 2 phenotypes (c_i, a_i). This parameter, c_i, is imposed on the intrinsic growth of each B_R^i subpopulation. On the other hand, susceptibility to antibiotics measures how bacteria respond to treatment, by reducing, proportionally, the killing rate by antibiotics relative to the wild-type sensitive bacteria. A wild type bacteria is the one which presents a null fitness cost and a total susceptibility to the drugs. Any mutational event which changes one or both of these traits is sufficient to consider the emergence of a new resistant bacterial strain in the within-host population.

The possible pathogen trait space can be conceptualized considering different assumptions, on the correlation between the magnitude of resistance and the associated fitness cost, explicited in the covariance matrix Σ. This matrix evidences the mutation step size and how the other trait is affected.

A first approach is to consider a trade-off assumption, for example, linear, in which higher resistance mutations are associated to higher cost (Figure 5.1, Panel A). Here, the two traits are related one to another. To obtain the covariance matrix based on this trade-off model, we do the following. The phenotypic trait c_i is randomly generated, between 0 and 1. Antibiotic susceptibility value is calculated by $a_i = 1 - \beta c_i + error$. The error follows a normal distribution $\mathcal{N}(0, \sigma^2)$. In this chapter, parameters are $\sigma^2 = 0.1$ and $\beta = 0.5$. One covariance matrix, since c_i is ramdomly generated, is $\Sigma = \begin{pmatrix} 0.0828 & -0.0441 \\ -0.0441 & 0.03520 \end{pmatrix}$.

An alternative approach assumes no correlation between the two traits (Figure 5.1, Panel B). Here, a random covariance matrix is $\Sigma = \begin{pmatrix} 0.0926 & 0.0087 \\ 0.0087 & 0.0968 \end{pmatrix}$.

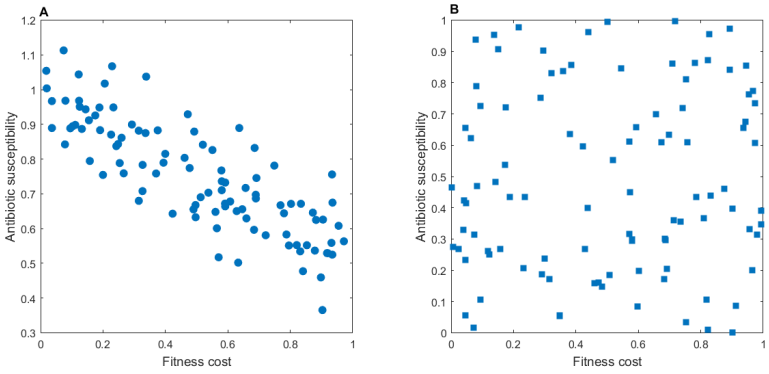

Figure 5.1: **Mutant's available trait space.** Constrained fitness cost and antibiotic susceptibiblity definition ($\sigma^2 = 0.1$, $\beta = 0.5$) (Panel A) and unconstrained random definition (Panel B).

5.1.2 Types of Treatment

The emergence and spread of antimicrobial resistance is influenced, among other factors, by different treatment strategies (Figure 5.2). In this model, we study only classical treatment with fixed dose-duration regime.

Two particular parameters are very important for the treatment design: dose of antimicrobial drugs, A_m, which we vary from 2 to 20 mg/l, and duration, which we vary from 3 to 14 *days*. Here, the killing rate by the antibiotics, δ_0, is simply set to 1 mg/l/day and the influence of treatment on how resistant bacteria respond to treatment is responsability of the parameter a_i.

We grouped different combinations of dose and duration into five types of treatment defined in this investigation. For each type of infection will be assigned a number, used to refer to that treatment strategy from now on. They are:

1. Low Dose and Low Duration treatment
2. High Dose and Low Duration treatment
3. Medium Dose and Medium Duration treatment
4. Low Dose and High Duration treatment
5. High Dose and High Duration treatment

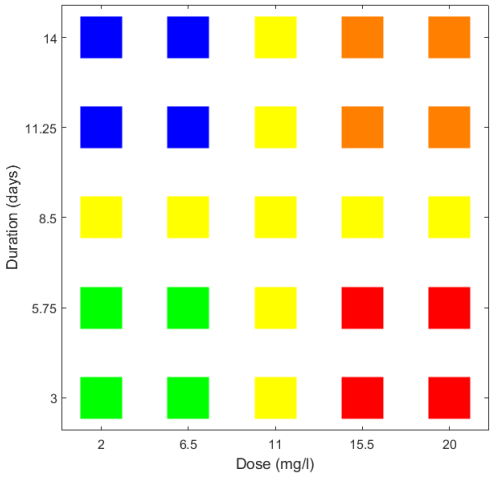

Figure 5.2: **Five broad types of treatment, defined by the combination (A_m,τ_2).** (A_m,τ_2) pairs (2,3), (2,5.75), (6.5,3) and (6.5,5.75) refer to treatment 1 (green markers). (A_m,τ_2) pairs (15.5,3), (15.5,5.75), (20,3) and (20,5.75) refer to treatment 2 (red markers). (A_m,τ_2) pairs (11,3), (11,5.75), (2,8.5), (6.5,8.5), (11,8.5), (15.5,8.5), (20,8.5), (11,11.25) and (11,14) refer to treatment 3 (yellow markers). (A_m,τ_2) pairs (2,11.25), (2,14), (6.5,11.25) and (6.5,14) refer to treatment 4 (blue markers). (A_m,τ_2) pairs (15.5,11.25), (15.5,14), (20,11.25) and (20,14) refer to treatment 5 (orange markers).

5.1.3 Simulations: Stochastic Realizations

Simulations can focus on different aspects: constraints on the evolution pathogen's trait space, range of mutation rates of the pathogen, treatment strategy, varying dose, duration and day of onset and type of infection (acute or chronic).

The conditions to generate each type of infection do not depend just on C, the carrying capacity. But, if all the other parameters are fixed, the variation of C can be used to shift from an acute infection (Figure 5.3, Panel A) to a chronic one (Figure 5.3, Panel B). The first type is described by a high peak limited by the immune response, associated to a $C = 10^7$. In contrast, a lower carrying capacity, e.g. $C = 10^5$, gives rise to a chronic infection, with lower pathogen load determined by limited resources and moderate immune stimulation.

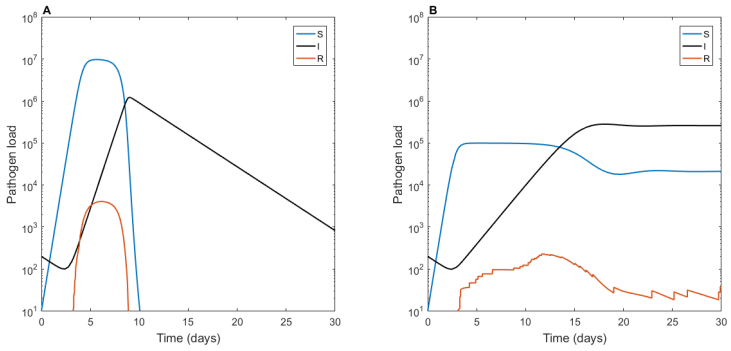

Figure 5.3: **Simulation of bacterial dynamics with evolution in the absence of treatment, during 30 days.** Blue line represents the B_S and the orange B_R. Black line corresponds to immune response of the host. The pathogen load value is its common log value. $C = 10^7$ in Panel A and $C = 10^5$ in Panel B. $m = 1\text{x}10^{-7}$. Other parameters as in Table 2.1.

In each simulation, new strains will emerge with the possibility to reach a maximal number, N, restricted for computational purposes. Strains are ordered by the time of emergence, before the simulation ends at day 30. Measures presented from now on, with few exceptions mentioned when needed, imply the summary of the results of 100 stochastic realizations, for the same parameter values. Parameter m can take three different values in this model: $0.5\text{x}10^{-7}$, $0.75\text{x}10^{-7}$ or $1\text{x}10^{-7}$.

5.2 Acute infection with treatment onset at day 4

The mathematical model described in the last section is used, in this chapter, to study a self-limiting acute bacterial infection, with treatment onset on the fourth day of infection ($\tau_1 = 4$) (Figure 5.3, Panel A).

The preference for day 4, in particular, does not relate to the particular day itself, but to a time window in which pathogen load did not reach the peak yet. Hence, B_{TOT} is still in the growth phase. In this time point, sensitive bacterial subpopulation load is around 10^6, which is less than the carrying capacity. For this reason, the immune response is still expanding and it is insufficient to control the infection. Therefore, the administration of antimicrobials will give the opportunity to have a second layer of control on the bacterial growth and treatment will have space to play a major effect.

5.2.1 Bacterial Dynamics

A good indicator to guide the study of evolutionary dynamics in an acute infection is, in the beginning, to check the changes in bacterial load and immune response over time, for different types of treatment. In these simulations, extremes of grouped treatments, concerning dose and duration, are selected. Results are compared for three mutation rates and two pathogen trait spaces. Independently of all these conditions, the qualitative results are similar for all of them.

Focusing on a paticular example, all infections, during or after treatment, are cleared (Figure 5.4, Panels A-C,E). The only exception is when lowest dose ($A_m = 2mg/l$) and highest duration ($\tau_2 = 14days$) characterize the treatment (Figure 5.4, Panel D). In that case, bacterial load will decrease significantly during treatment. However, when it ends, the value is sligthly above the extinction threshold which is enough for both B_S and B_R to be able to grow again. That is the treatment that allows the infection to relapse, which is a very bad scenario for the host. Here, low doses are more effective if given over shorter duration to minimize interference with immune activation, which was already described in previous studies (Gjini & Brito, 2016).

Another message that can be read in this figure is related to the immunity activation and following response. The real values of immunity in the presence

of antibiotics can be compared to the expected immunity in the absence of it. It is possible to verify that this two values are not always coincident because of the treatment interference. In some cases, both bacterial growth control mechanisms work together and deliver a postive outcome. However, there are other situations in which treatment application decreases the activation of immunity and, at the end of the antimcirobial administration, the conjunct action of both mechanisms is not enough to clear the infection.

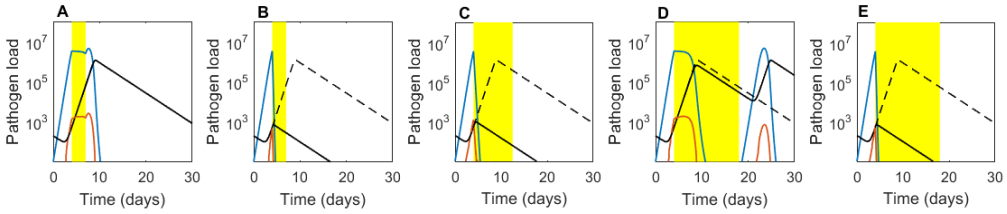

Figure 5.4: **Illustration of bacterial dynamics under treatment for 5 combinations dose-duration interactions.** Blue line for B_S and orange for B_R. Black solid line corresponds to immune response of the host, with treatment. Black dashed line corresponds to the expected immune response of the host, in the absence of treatment. The pathogen load value is its common log value. Each panel represents a type of treatment, with a particular (A_m, τ_2) combination. Panel A for treatment 1, in particular combination (2,3). Panel B for treatment 2, in particular combination (20,3). Panel C for treatment 3, in particular combination (11,8.5). Panel D for treatment 4, in particular combination (2,14). Panel E for treatment 5, in particular combination (20,14). Constrained trait space with $m = 0.75\text{x}10^{-7}$. Yellow region identifies the treatment administration period. Other parameters as in Table 2.1.

These results can be used to predict the sucess of an antimicrobial treatment, a relevant mark in the clinical practice. If both values of immunity discussed before coincide, both mechanisms will be enough to clear the infection, even if that only happens some days after treatment cessation. In contrast, very low levels of bacterial load, at the end of treatment, do not ensure that infection will be cleared (if those values are above B_{ext}). The main message is that the determination of the efficacy of treatment implies a good perception of the relationship between bacterial load and immune response values, independently of m.

5.2.2 Infection Outcome Scenarios

So far, we illustrated only particular cases (Figure 5.4). But more accurate results are obtained when the entire range of treatments is taken into account. One process to accomplish that is to check what is the most likely infection outcome scenario, at the end of the simulation. A bacterial infection, after a certain number of days, can be in four states: both B_S and B_R coexist (scenario I); only one of the bacterial subpopulations is present in the host organism (scenario II for B_S, and scenario III for B_R); or both of them have values below the extinction threshold and the infection is cleared (scenario IV). Knowing these probabibilities for each (A_m,τ_2) combination allows a more conscient choice of treatment strategy (Figure 5.5).

For each parameter combination, we simulated 100 independent stochastic realizations. Afterwards, we computed the proportion of stochastic realizations that ended up, at day 30, in each scenario $[p_1, p_2, p_3, p_4]$, where $p_1+p_2+p_3+p_4 = 1$. In Figure 5.5, we plot these probabilities, knowing that the $max(p_1, p_2, p_3, p_4)$ is the most likely outcome scenario for each treatment.

Independently of the pathogen trait space or the pathogen mutation rate, there are only two (A_m, τ_2) combinations in which the probability of clearance of the infection is not 1: (6.5, 3) and (11, 3). These combinations belong to treatment 1 and 3, associated to intermediate doses and low durations.

In the constrained trait space (Figure 5.5, Panels A-C), the outcome scenarios found in these two combinations are the same (scenario I and II), but they differ in the proportions. Even in those cases in which scenario II is the most likely, because mutational events can occur, to have sensitive bacteria persistence is enough to expand to coexistence, and switch to scenario I. With higher doses, the proportions between these two scenarios are closer, but again, moving from one setting to another is easy because of pathogen evolution.

If the evolution of the pathogen is unconstrained (Figure 5.5, Panels D-F), a similar pattern is found for (A_m, τ_2) combination (6.5, 3). However, in the case with a higher dose (11, 3), for the two highest mutation rates, scenario I becomes more likely than scenario II, but the proportions are similar between them.

All these results do not seem to coincide with the evidence obtained through the study of bacterial dynamics, for which only one stochastic realization was run. However, it cannot be ignored the fact that calculations of these probabilities were done with data of day 30, at the end of the simulation, with 100 realizations.

To have a cleared infection at that time point, does not imply that the treatment was efficient. There may even have been a relapse of bacterial load that cannot be detected, using this measure, as it happens with treatment 4 (Figure 5.4, Panel D). On the other hand, for treatments 1 and 3, were not detect relapses in one stochastic realization (Figure 5.4, Panels A,C), but evidences show that they can occur with high probability.

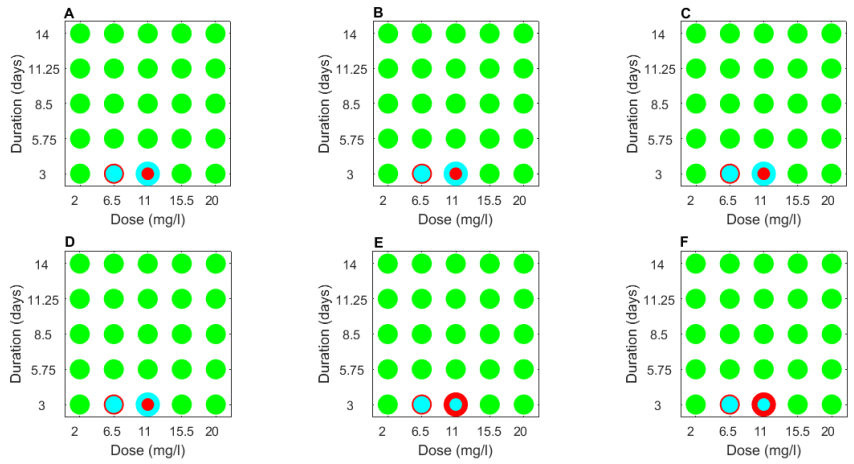

Figure 5.5: **Most likely outcome scenario of a self limiting acute infection at day 30, under treatment.** Panels A-C for constrained and Panels D-F for unconstrained evolutionary dynamics. Panels A,D with $m = 0.5\text{x}10^{-7}$, Panels B,E with $m = 0.75\text{x}10^{-7}$ and Panels C,F with $m = 1\text{x}10^{-7}$. Red stands for scenario I, blue for scenario II, yellow for scenario III, and green for scenario IV. Single color marker means that the probability of that outcome is 1. Bi-color marker implies that probability of the most likely outcome is below 1. For combinations (6.5, 3) and (11, 3) the proportions $[p_1, p_2, p_3, p_4]$ are, respectively: [0.01, 0.99, 0, 0] and [0.56, 0.44, 0, 0] (Panel A); [0.09, 0.91, 0, 0] and [0.58, 0.42, 0, 0] (Panel B). [0.05, 0.95, 0, 0] and [0.54, 0.46, 0, 0] (Panel C). [0.05, 0.95, 0, 0] and [0.57, 0.43, 0, 0] (Panel D). [0.05, 0.95, 0, 0] and [0.46, 0.54, 0, 0] (Panel E); [0.07, 0.93, 0, 0] and [0.48, 0.52, 0, 0] (Panel F). Other parameters as in Table 2.1.

5.2.3 Cumulative Summary Measures

Since the observation of the scenario at the end of the simulation is not representative of the history of the infection, some other summary measures are calculated to clarify this process (Figure 5.6).

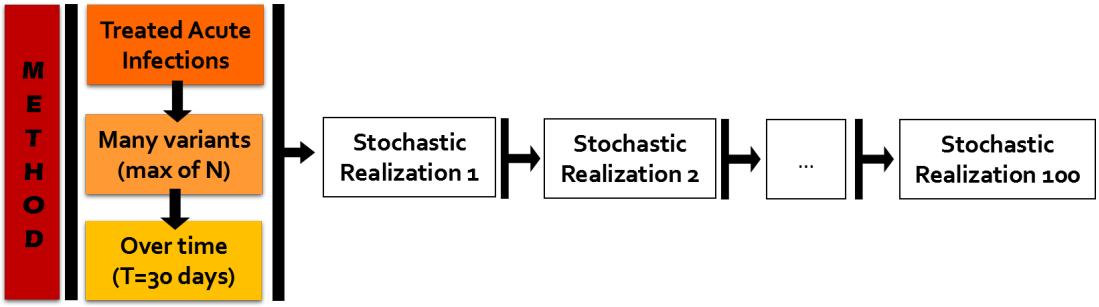

Figure 5.6: **Methods of generating Summary Measures.** During the course of an acute bacterial infection under treatment, there is the emergence of many variants over time. Each simulation with fixed parameters, known as a stochastic realization, lasts 30 days. Stochastic realizations are repeated 100 times and the outputs are saved to be used later, in the generation of summary measures.

The cumulative summary measures are: number of emerged variants by a certain time and the burdens of B_S and total resistance over a certain period from the beginning of the infection until time T, respectively obtained by:

$$B_S\_Burden = \int_0^T B_S(t)dt \tag{5.5}$$

and

$$B_R\_Burden = \sum_{i=1}^{n(t)} \int_0^T B_R^i(t)dt. \tag{5.6}$$

All of them are obatined in each stochastic realization and the mean of each measure is calculated over the 100 repetitions. These particular measures are not interpreted considering the different types of treatment mentioned before.

Considering the results are similar for the three mutation rates we studied and both pathogen trait spaces used in these simulations, we decided to display all

these summary measures for the constrained evolution case with the intermediate mutation rate 0.75x10^{-7} (Figure 5.7).

Concerning the number of resistant emerged variants, the higher values are found for low doses, independently of the duration, and for medium doses if the duration is short (Figure 5.7, Panel A). These are the types of treatment in which relapses were identified, and it is logical that the existence of a second peak of bacterial load implies a generation of new variants, increasing this number. We can see exactly the same qualitative pattern when we look at the burden of B_S over the entire simulation, which is also explained by the relapses of sensitive bacterial subpopulation due to less aggressive treatments (Figure 5.7, Panel B). Concerning the burden of B_R over the entire simulation, we see that it is orders of magnitude lower than the burden of B_S (Figure 5.7, Panel C). Competititon favors B_S overall even if transiently B_R may have been dominant.

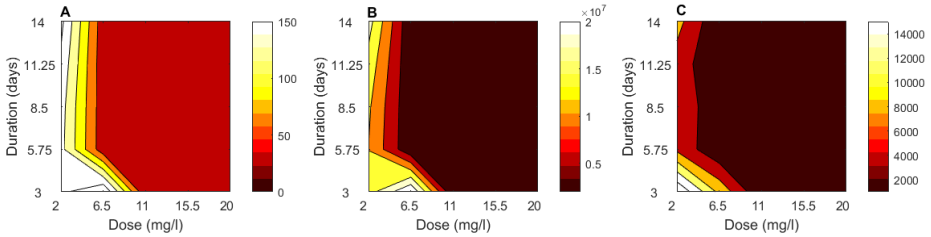

Figure 5.7: **Cumulative summary measures of a self limiting acute infection until day 30, under treatment considering dose-duration interactions.** Number of emerged resistant bacterial strains during the simulation (Panel A). Cumulative burden of sensitive bacterial subpopulation during the simulation (Panel B). Cumulative burden of resistant bacterial subpopulation during the simulation (Panel C). Constrained evolutionary dynamics. $m = 0.75\text{x}10^{-7}$. Other parameters as in Table 2.1.

Another option is to look at the mean value and standard deviation of these cumulative summary measures over the 30 days of simulation, averaging overall types of treatments applied, but as a function of mutation rate (Figure 5.8).

We see variations in mutation rate only affect the resistant strains, since there are no differences in the B_S burden for the three values of mutation rate (Figure 5.8, Panel B). On the other hand, the higher the mutation rate, the higher

the mean number of resistant emerged variants and the mean burden of B_R (Figure 5.8, Panels A,C). If bacteria can mutate at higher rate, it is predictable to have more variants, and as whole they can grow more and reach a higher bacterial load. Stochaticity imposed by the model explains the variation in the standard deviation values, more significant in the B_R burden. The constrast found between B_S and B_R burdens is due to the rarity of mutational events in a bacterial infection landscape.

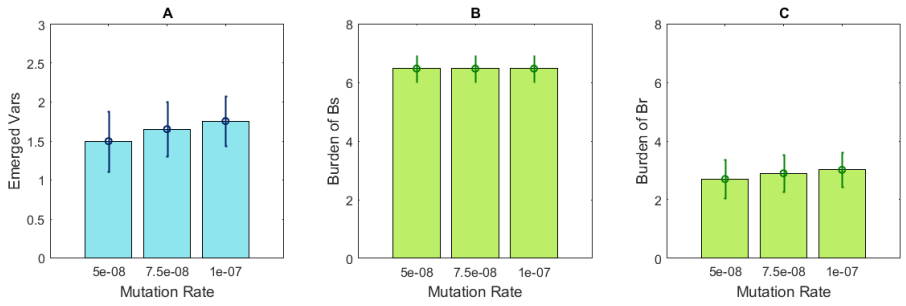

Figure 5.8: **Cumulative summary measures of a self limiting acute infection until day 30, under treatment with mutation rate variation.** Number of emerged resistant bacterial strains during the simulation (Panel A). Cumulative burden of B_S during the simulation (Panel B). Cumulative burden of B_R during the simulation (Panel C). All plots are in logarithmic scale. Error bars indicate standard deviation and circles represent the mean value. Constrained evolutionary dynamics. Other parameters as in Table 2.1.

5.2.4 Resistance Burden and Number of Emerged Variants

But is the increase of the total resistance burden related to the increase in the number of resistant emerged variants? One way to determine it is to plot the number of emerged variants against the respective B_R burden for each infection realization across all dose-duration scenarios and to verify if there is a clear pattern between them (Figure 5.9).

Independently of the pathogen trait space or the pathogen mutation rate, we see a clear pattern and it can be divided into two parts. Firstly, a vertical cluster with a smaller number of emerged variants, in which the burden of B_R can

increase until 10^5, which can be related to a primary infection peak. And secondly, a horizontal cluster, with a greater range of resistant emerged variants, in which the burden of B_R does not increase as much, representing a bacterial relapse. The small increase in the B_R burden from one cluster to another is due to the differences in magnitude between the first and second infection peaks. In a second peak, many new variants may emerge, but because space is already occupied and immune control is increasing, they cannot grow considerably. Mutation rate shifts the minimum number of emerged variants to the right, independently of the evolutionary dynamics.

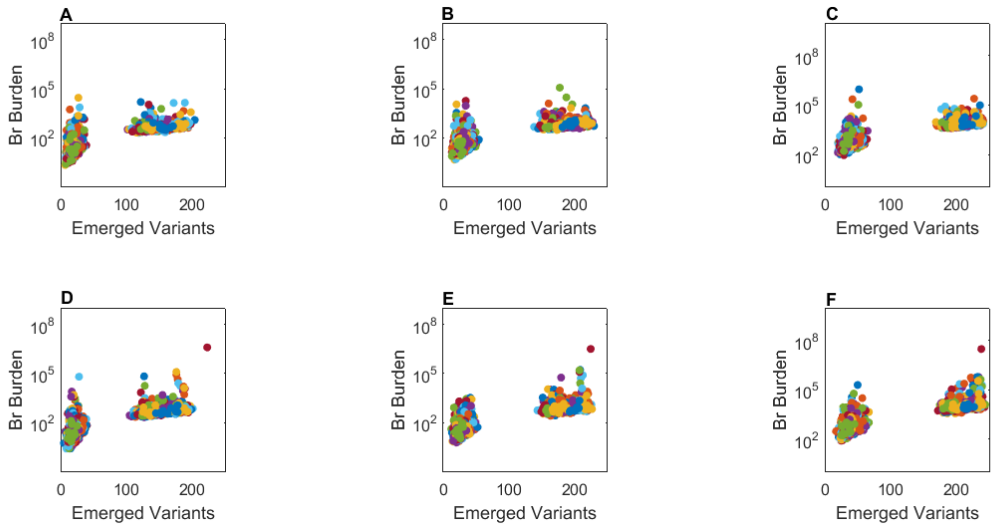

Figure 5.9: **Relationship between the number of emerged variants and the cumulative burden of B_R, in a self limiting acute infection under treatment.** Panels A-C for constrained evolutionary dynamics and Panels D-F for unconstrained evolutionary dynamics. Panels A,D with $m = 0.5\text{x}10^{-7}$, Panels B,E with $m = 0.75\text{x}10^{-7}$ and Panels C,F with $m = 1\text{x}10^{-7}$. Other parameters as in Table 2.1.

5.2.5 What strains emerge during treatment?

Up to now, it is accurate to state that pressure for selection is a reality in acute bacterial infections under treatment. But which strains of the resistant bacterial subpopulation are selected, in the 2-d phenotypic space? To answer this, we

use other summary measures, which in this case are related to two traits of the pathogen: the fitness cost, c_i, and the antibiotic susceptibility, a_i. In each stochastic realization (Figure 5.6), summary measures related to the two traits are obtained across all dose-duration scenarios or for each of the five types of treatment mentioned in this chapter.

First of all, we need to know the frequency of each strain i at time t:

$$f_i(t) = \frac{B_R^i(t)}{B(t)}. \tag{5.7}$$

Then, we compute the mean trait dynamics summing over all emerged variants:

$$\bar{c}(t) = \sum_{i=1}^{n(t)} f_i(t) c_i \tag{5.8}$$

$$\bar{a}(t) = \sum_{i=1}^{n(t)} f_i(t) a_i. \tag{5.9}$$

Another layer arises when we calculate the mean cost or susceptibility of the entire population over the entire simulation ($T = 30 days$) for one run:

$$\bar{c}_T = \frac{1}{T} \int_0^T \bar{c}(t) dt \tag{5.10}$$

$$\bar{a}_T = \frac{1}{T} \int_0^T \bar{a}(t) dt. \tag{5.11}$$

The last mean that is calculated regards the 100 stochatic realizations which were run, and these are the values used as summary measures about the emerged strains from now on:

$$E[\bar{c}_T] = \frac{1}{100} \sum_{k=1}^{100} \bar{c}_T^{(k)} \tag{5.12}$$

$$E[\bar{a}_T] = \frac{1}{100} \sum_{k=1}^{100} \bar{a}_t^{(k)}. \tag{5.13}$$

A first approach exploits the potentialities of a graphical techique called contour plots. Variation of each trait, considering the treatment dose-duration combinations, was done for both pathogen evolutionary dynamics (Figures 5.10 and 5.11), by the mean over all stochastic realizations (Equations 5.12 and 5.13). A modest alteration was performed: instead of plotting the antibiotic susceptibility, we illustrate resistance. This trick is related to the visual advantages of having both measures varying in similar ranges.

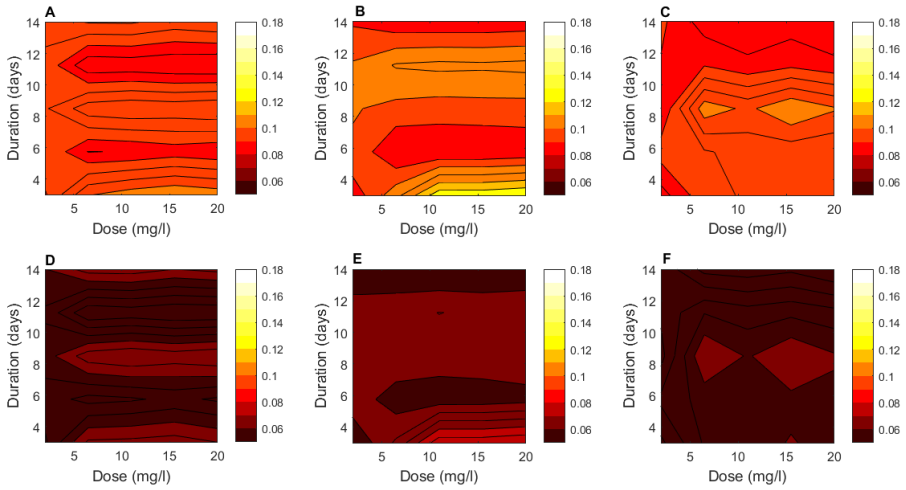

Figure 5.10: **Mean fitness cost and mean resistance of bacterial populations over 30 days and over all stochastic realizations of a self limiting acute infection under treatment for constrained evolutionary dynamics.** Fitness cost on top row (Panels A-C) and resistance at bottom row (Panels D-F). Left column (Panels A,D) with $m = 0.5\text{x}10^{-7}$, medium column (Panels B,E) with $m = 0.75\text{x}10^{-7}$ and the right one (Panels C,F) with $m = 1\text{x}10^{-7}$. Other parameters as in Table 2.1.

In the constrained pathogen trait space, both fitness cost and antibiotic resistance values do not vary considerably with the alteration of the treatment (Figure 5.10) and no clear pattern can be found to study the impact of mutation rate variation.

A similar behavior is found in the unconstrained pathogen trait space (Figure 5.11). The fitness cost, independently of the mutation rate, is lower than when constrained evolutionary dynamics are considered. Fitness cost, although only slightly, decreases with an increase of duration of treatment. However, the opposite effect happens concerning resistance: this trait, in mean, is much higher is this case, when compared to the unconstrained one. Here, resistance maximum values are associated with shorter durations, if mutation rate increases.

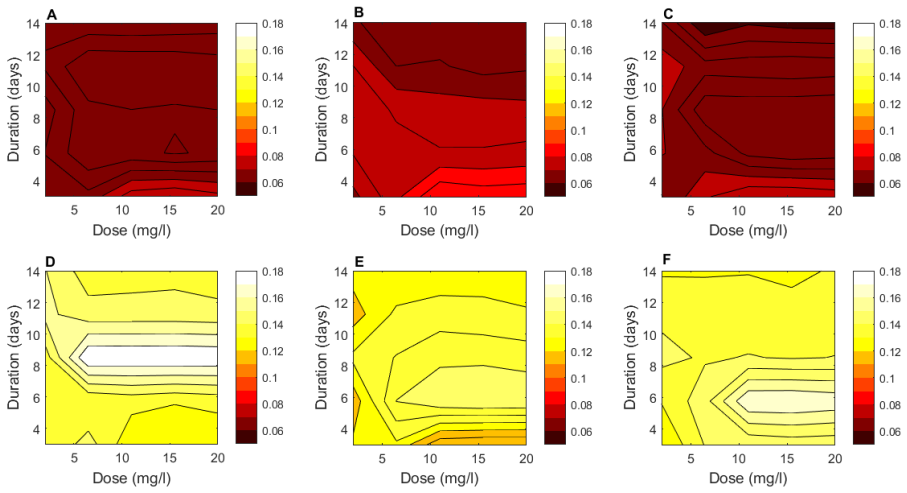

Figure 5.11: **Mean fitness cost and mean resistance of bacterial populations over 30 days and over all stochastic realizations of a self limiting acute infection under treatment for unconstrained evolutionary dynamics.** Fitness cost on top row (Panels A-C) and resistance at bottom row (Panels D-F). Left column (Panels A,D) with $m = 0.5\text{x}10^{-7}$, medium column (Panels B,E) with $m = 0.75\text{x}10^{-7}$ and the right one (Panels C,F) with $m = 1\text{x}10^{-7}$. Other parameters as in Table 2.1.

In general lines, which do not discard the need to look deeper into this subject, the strains that emerge in an unconstrained trait space, are worst for the host, since the higher resistance to antibiotics is coupled to a lower fitness cost.

So far, we looked at the mean traits over infection separately. Next, we plot the 2-d trait evolution for each scenario to answer several questions. What is the relationship between these two pathogen phenotypic traits? And does this relationship depend on the pathogen evolutionary dynamics?

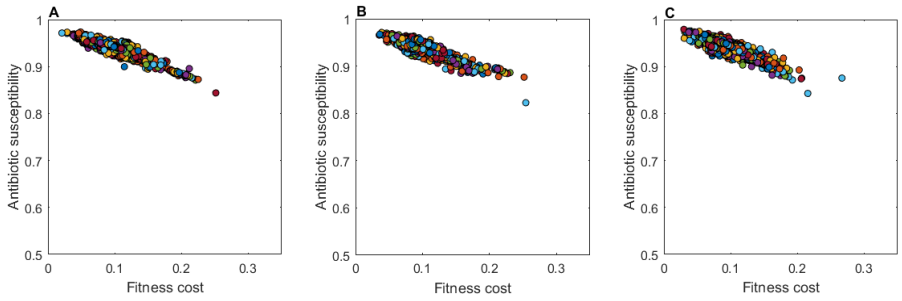

Figure 5.12: **Evolution of mean fitness cost vs. mean antibiotic susceptibility of an infection over 30 days for a constrained trait space.** Each dot represents the mean of all strains over time of 1 stochastic realizations of the simulation (Equations 5.10 and 5.11), independently of the dose-duration combination. Panel A with $m = 0.5\text{x}10^{-7}$, Panel B with $m = 0.75\text{x}10^{-7}$ and Panel C with $m = 1\text{x}10^{-7}$. Other parameters as in Table 2.1.

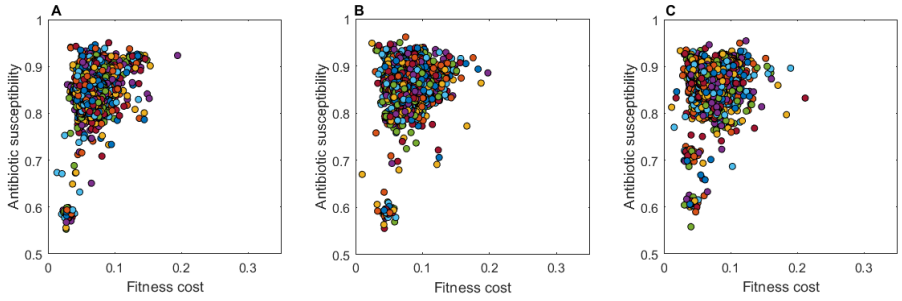

Figure 5.13: **Evolution of mean fitness cost vs. mean antibiotic susceptibility of an infection over 30 days for an unconstrained trait space.** Each dot represents the mean of all strains over time of 1 stochastic realizations of the simulation, independently of the dose-duration combination (Equations 5.10 and 5.11). Panel A with $m = 0.5\text{x}10^{-7}$, Panel B with $m = 0.75\text{x}10^{-7}$ and Panel C with $m = 1\text{x}10^{-7}$. Other parameters as in Table 2.1.

When we look at the constrained pathogen trait space, a defined pattern is common to all mutation rates: a decrease in the antibiotic susceptibility results in an increase of the fitness cost of the pathogen (Figure 5.12). The slope of this straight lines arising after treatment dynamics is similar to the slope imposed in the constrained pathogen trait landscape (Figure 5.1, Panel A), for the chosen parameter values. A small but relevant detail is the higher dispersion in evolved traits, associated to the highest mutation rates, despite the constraints. The faster new strains are generated, the further from the sensitive wild type the evolution during treatment.

The results become more interesting when pathogen trait space is unconstrained (Figure 5.13). In this case, there is more divergence between all stochastic realizations. Here, a decrease in the susceptibility (which is equivalent to say that resistance is increasing) does not necessarily imply a higher fitness cost. Some of the infections move horizontally, which means that they contain strains which suffer from a higher cost, on average, but are still very susceptible to the antimicrobial administration. The fitness cost does not overcome the value of 0.2, in the majority of cases, which means that bacterial growth overall is similar to the case of constrained evolution (Figure 5.12). On the other hand, some other treated infections progress vertically, towards strains with very high resistance. Their antibiotic susceptibility is reduced by almost 50% and they present a small cost. This type of trait combination is unique, i.e not observed in Figure 5.12. Between these two extreme cases, all the other samples are found, with a more pronounced dispersion if bacteria mutate at a higher rate.

The scattering of the stochastic realizations of this simulation can be described in another perspective. There is a big cluster, whose characteristics are closer to the sensitive wild type bacteria. A second and much smaller cluster can be found in the lower left corner of the trait landscape, which is related to the resistance selection. An increase in the mutation rate seems to facilitate the transition from the first cluster to second one, which means that the infection becomes more resistant to treatment, making the situation more complicated for the host.

5.2.6 What strains emerge for each type of treatment?

Based on this, the question that arises is related to the origin of these clusters. Is there any relationship between the distribution of the mean traits and the type of treatment? And what is the impact of pathogen mutation rate variation? The answer comes from the partition of these scatter plots considering the five described types of treatment and the three distinct mutation rates.

In the constrained trait space, there are no differences in the relationship between pathogen traits when different types of treatment are compared, for which reason the figure is not shown here. However, discrepancies are found in the unconstrained case (Figure 5.14).

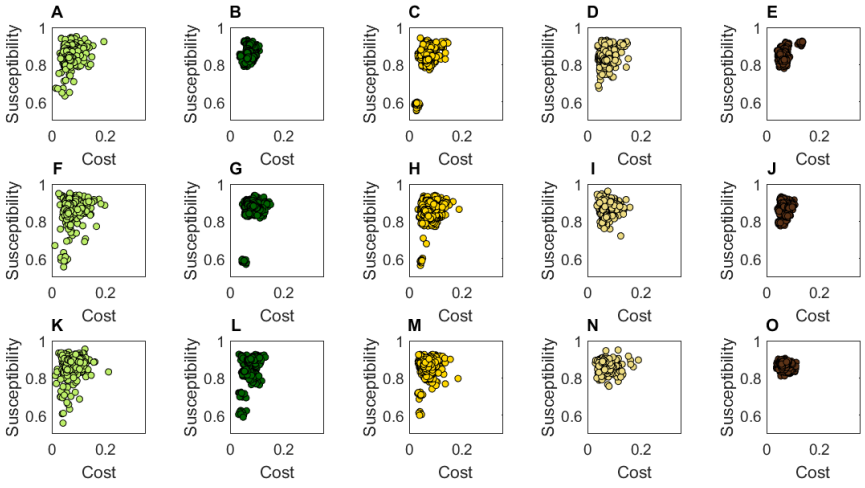

Figure 5.14: **Relationship between mean fitness cost and mean antibiotic susceptibility over 30 days in an acute infection under treatment.** Study of the impact of m and type of treatment. Panels A-E with $m = 0.5\text{x}10^{-7}$; Panels F-J with $m = 0.75\text{x}10^{-7}$; and Panels K-O with $m = 1\text{x}10^{-7}$. Panels A,F,K with treatment 1; Panels B,G,L with treatment 2; Panels C,H,M with treatment 3; Panels D,I,N with treatment 4; and Panels E,J,O with treatment 5. Unconstrained evolutionary dynamics. Other parameters as in Table 2.1.

When the dose is sufficiently high (treatment 2, High Dose and Low Duration, and treatment 5, High dose and High Duration), the infection is controlled

quicker by the conjunct action of both mechanisms (Figure 5.14, Panels B,E). Therefore, the main traits over each infection are more similar to the sensitive wild type bacteria. A high dispersion of infections, with a clear movement for the location of the second cluster mentioned above, is seen for treatments in which clearance is not the most likely outcome at the end of the simulation and relapses occur (treatment 1, Low Dose and Low Duration, and treatment 3, Medium dose and Medium Duration) (Figure 5.14, Panels A,C). Relapses can also happen, but for a shorter period of time, with a treatment 4, Low Dose and High Duration, and that explains the intermedium level of dispersion (Figure 5.14, Panel D). In general lines, more predictable evolution is found in more aggressive treatments. More resistant infections are related to treatments previously recognized for generating second peaks of bacterial load.

Next, we summarize quantitavely results by type of treatment focusing only on the case of mutation rate of $0.75\text{x}10^{-7}$. We use two different abstract measures to describe evolution in the 2-d trait space: Divergence Distance and Divergence Angle. Together, these measures allow to have a notion of how distant is each resistant emerged strain from the original sensitive one.

Divergence distance is the mathematical Euclidian distance, between the mean pathogen traits over a treated infection ($\bar{c}_T$, $\bar{a}_T$), and the traits of sensitive wild type bacteria,(c, a)=(0,1) . The greater this distance, the further the evolution of the bacterial population over infection from the original sensitive strain. For each infection, divergence distance is given by:

$$d = \sqrt{(\bar{c}_T - 0)^2 + (\bar{a}_T - 1)^2} \tag{5.14}$$

Divergence angle is measured between the y axis and the line which connects the infection scatter point and the sensitive strain and it goes from 0 to 90 degrees. The smaller the angle, the more vertical are the evolutionary dynamics in the two traits landscape, which means the more resistant bacteria and the smaller the cost. For each infection, divergence angle is given by:

$$\alpha = \arctan\left(\frac{\bar{c}_T}{1 - \bar{a}_T}\right) \tag{5.15}$$

Concerning fitness cost, the highest mean over all stochastic realizations is associated to treatment 3. The intermedium values are similar and lowest one can be found in the more aggressive type: treatment 5. Standard deviation values vary more between types of treatment than the mean itself. However, all these values are very similar, which allow to state that, for these parameter values we explored, on average, different types of treatment do not have an extensive impact on the evolved mean fitness cost of resistant bacteria over a 30 days simulation of an infection (Figure 5.15, Panel A).

Focusing on the other pathogen trait, the antibiotic susceptibility, the differences in the mean value are almost imperceptible and all of them are sligtly above 0.8. Even the variation in standard deviation among all types of treament can be considered neglegible (Figure 5.15, Panel B).

The mean divergence distance is very similar when the duration is low or medium, independently of τ_2. The correspondent standard deviations are significant, in particular for treatment 3. In addition to similarity, these values are higher since they correspond to the relapsed infections, in which resistant strains are less related to B_S. Higher durations impede resistant emerged variants to evolve and become more distant to the initial spot, associated with B_S. Quantitavely, both means and standard deviations are lower (Figure 5.15, Panel C).

Much more variation, specially in the mean values, are found for the divergence angles. Treatments 1 and 3 present the higher angles. Despite the formation of the second cluster, in mean, for these cases, the angle shows a favoring of the increase in the fitness cost, when compared to the decrease in the antibiotic susceptibility. For the other types of treatment, the mean and standard deviations are similar. However, on average, the divergence angle do not vary more than 10 degrees between all antimicrobial regimes (Figure 5.15, Panel D).

Once again, these same measures can be seen by other viewpoint, which can concede a new interpretation. The study of the frequencies distribution of mean divergence distances and angles allows to verify which are the most frequent values and if there is a identifiable particular pattern (Figure 5.16).

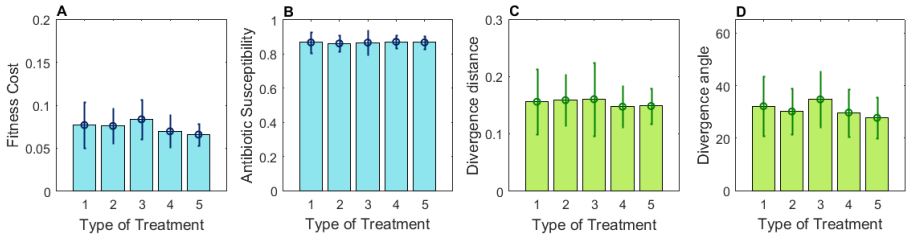

Figure 5.15: **Mean summary measures of a self limiting acute infection until day 30, with treatment administration considering distinct types of treatment.** Mean fitness cost of B_R (Panel A). Mean antibiotic susceptibility of B_R (Panel B). Mean divergence distance of B_R when compared to B_S (Panel C). Mean divergence angle of B_R when compared to B_S (Panel D). Error bars indicate standard deviation and circles represent the mean value. All bars plot the mean over 100 stochastic realizations. $m = 0.75\text{x}10^{-7}$. Unconstrained evolutionary dynamics. Other parameters as in Table 2.1.

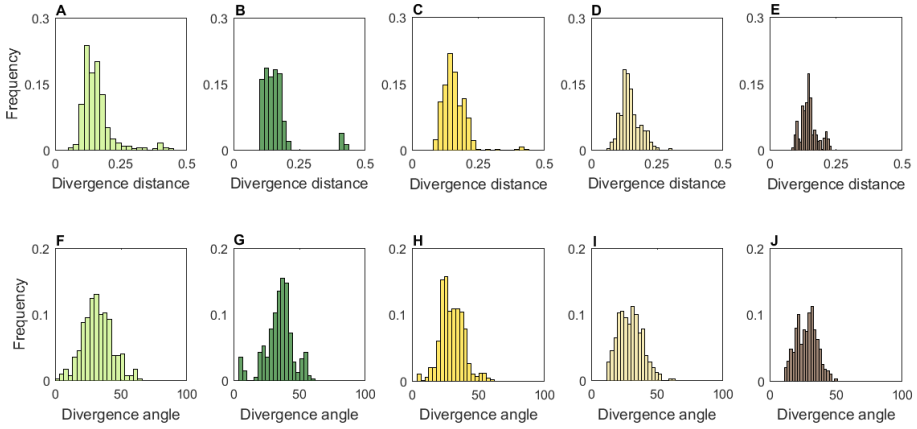

Figure 5.16: **Impact of the type of treatment on the frequencies distribution of mean divergence distance and divergence angle of B_R when compared to B_S.** Panels A,F with treatment 1; Panels B,G with treatment 2; Panels C,H with treatment 3; Panels D,I with treatment 4; and Panels E,J with treatment 5. All bars plot the mean over 100 stochastic realizations of the simulation, crossing overall dose-duration interactions. $m = 0.75\text{x}10^{-7}$. Unconstrained evolutionary dynamics. Other parameters as in Table 2.1.

Concerning divergence distance, the distribution is similar between all regimes. The greatest remoteness of this arises in the treatment 4 case (Figure 5.16, Panel B), in which a first cluster seems more rectangular and there is a second smaller peak. The same qualitative consistency arises in the case of divergence angle frequencies distribution (Figure 5.16, Panels F-J). With a margin of error, a relation between divergence distance and divergence angle can even be established.

However, the fact that we do not find big difference in these evolutionary dynamics for different treatments for these parameter combinations, does not mean that if we increase m or change host immunity parameters, we will not find significant differences. This remains to be investigated in the future.

5.2.7 Evolution features by infection windows

Because the focus, in this chapter, is on the resistance selection in self limiting acute infections under treatment, another important feature to pay attention is the comparison between what happens before, during and after treatment is applied.

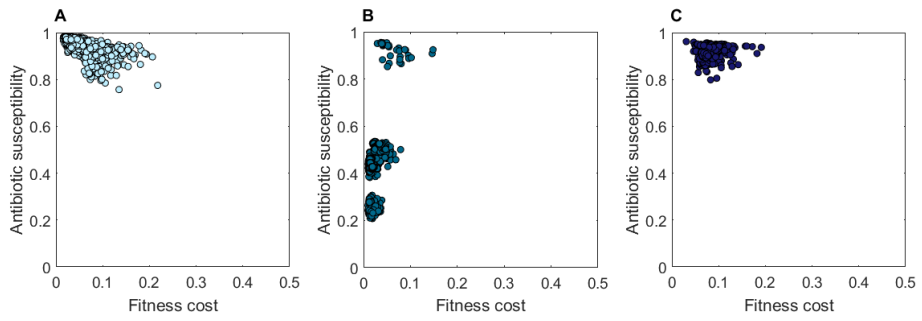

Figure 5.17: **Impact of timing on the relationship between mean fitness cost and mean antibiotic susceptibility during the 30 days simulation in a self limiting acute infection under treatment.** Each dot represents one infection, considering timing: Panel A with mean during the period before treatment is applied (from day 0 to day τ_1); Panel B with mean during treatment administration (between day τ_1 and day $\tau_1 + \tau_2$); and Panel C with mean during period after treatment cessation (from day $\tau_1 + \tau_2$ until day 30). $m = 0.75\text{x}10^{-7}$. Unconstrained evolutionary dynamics. Other parameters as in Table 2.1.

On a first approach, the relation between fitness cost and antibiotic susceptibility is verified in the three time windows. Before treatment is applied (Figure 5.17, Panel A), the first mutants emerge from B_S, initially present in an infection. Even those who emerge after it have parents whose traits are similar to those found in the beginning of the infection. During the antimicrobial administration (Figure 5.17, Panel B), mutants have more time to get away from the initial conditions and, in mean, they do it in a direction where they become more and more resistant, without almost any cost. During treatment, resistant strains with no cost are selected. The majority of infections are cleared during treatment. For those that are only cleared after treatment cessation, their B_R subpopulation have to be composed by mutants which cannot be very resistant. In addition to those mutants, there are new emerged from the secondary peaks, also mentioned as relapses. Because of this, the mean fitness cost and antibiotic susceptibility of the bacterial mutants, after treatment, return to the values associated with sensitive bacteria (Figure 5.17, Panel C). Often, the second peak is also dominated by B_S.

So far, this scattering process allowed to distinguish between the three time windows. But is it possible to find a connection between the time windows during simulations and type of treatment?

Even though there are quantitative differences, qualitatively the mean fitness cost and antibiotic susceptibility vary in the same way before and after treatment, regardless of the type of treatment (Figure 5.18). Low or medium doses favor a greater divergence between mutants, associated to relapses episodes. During treatment, the expected behavior is found for treatments with low or medium duration, disregarding the dose. Neverthless, high durations favor the loss of bacterial mutants similar to the sensitive. Strains selection is more influenced by the time window than the type of treatment itself.

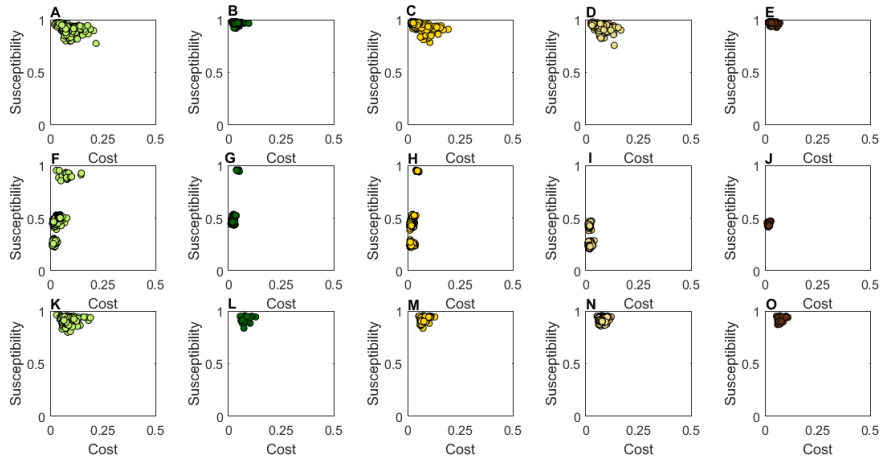

Figure 5.18: **Study of the impact of timing and type of treatment on the relationship between mean fitness cost and mean antibiotic susceptibility over 30 days in a self limiting acute infection under treatment.** Panels A-E for before treatment period; Panels F-J for during treatment period; and Panels K-O with after treatment period. Panels A,F,K with treatment 1; Panels B,G,L with treatment 2; Panels C,H,M with treatment 3; Panels D,I,N with treatment 4; and Panels E,J,O with treatment 5. $m = 0.75\text{x}10^{-7}$. Unconstrained evolutionary dynamics. Other parameters as in Table 2.1.

Chapter 6

Conclusions

The study of the dynamics of bacterial infections under different types of treatment, based on the definition of mathematical models and computational simulations is not easy. We have generated several important new findings that will be resumed and discussed now. From these, we can sketch a possible pathway to follow with regard to control of antimicrobial resistance.

With respect to infection bacterial dynamics, when no treatment is applied, studied in Chapter 2, the use of only one equation to describe the host immune system has proved to be an advantageous choice. All major dynamics are captured and, in addition, the study of the mathematical model becomes simpler and computationally lighter. The extension associated with the logistic model revealed also new phenomena and feedbacks between density-dependent resource limitation and immune control. Carrying capacity and host immune system work together in this model to control bacterial growth and can be considered more realistic for investigating different biological scenarios. One example is that lower carrying capacity limits bacterial load to lower levels, reducing the action of the host immunity. It is safe to state that all fixed points of this system correspond to biological scenarios, identified in this thesis as colonization, persistence and clearance. The absence of immune response, associated to the colonization scenario, allows the coexistence of sensitive and resistant bacteria. However, if immunity is activated, the possibility of coexistence disappears but there is an opportunity for oscillations of the resistant bacterial subpopulation. All conditions that allow to move from one state to another were identified in detail, work that had not been

developed yet, and that have a great theoretical revelance. Nonetheless, because they depend on parameters related to the bacterial agent and the immune system of the host, they have low applicability in clinical practice.

But antimicrobial treatment is almost mandatory during a bacterial infection episode and it is introduced in Chapter 3. The way this treatment is added to the mathematical model can differ. Firstly, it was studied a classical type of treatment, outlined by a constant dose administration during a certain period. The main assumption, in this case, is that drug concentration does not suffer any changes in host body over time. The equilibria analysis reveals that the fixed point related to the coexistence of both bacterial subpopulation is lost. It is even possible to state that it does not reappear, no matter how the treatment is modelled. Modelling bacterial infections under treatment highlights the key feedbacks between mechanisms of control, which, in the last instance, results in the asymptotic impossibility of coexistence due to selection.

Besides the asymptotic analysis of these models, we studied also infection history over time. To discover it implies the study of its transient behavior, found in Chapter 4. If we are dealing with a persistence infection, different timings may occur to initiate the treatment, in the clinical practice, depending for example on the host symptoms. If treatment starts when infection is mixed and contains both B_S and B_R, doses above B_R superinhibitory dose are more likely to clear the infection, with an adequate duration of treatment. Another finding is that lower doses can also produce clearance, however this is coupled to a minimum duration. By increasing the duration of treatment, serious consequences may result for the host, in addition to increasing the chance of resistance selection. When an infection starts, immunity is stimulated. So, it can happen that only sensitive bacteria are present when treatment onset happens. When that is the case, a mathematical expression was obtained to relate the dose and the duration of treatment needed to get clearance of the infection, which is not very sensitive to immunity parameters. But there is also a possibility that the host suffers from an acute infection, which would be cleared only by the action of the immune system. But what happens if this infection starts to be treated at the stage where the bacterial load is at the peak? In that case, treatment will have a higher interference on the immunity levels and combinations of dose and duration

which are able to get clearance are more affected by immunity parameters. For immune responses depending on bacterial load, interaction with antimicrobial drugs can be antagonistic. In this type of infection, treatment can result either ways: the host may continue to be infected, which is a worst outcome than it would be without treatment; or clearance is obtained anyway, but because the host is infected for a shorter period, suffers less harm due to it.

In conclusion, our preliminary results on deterministically modelling bacterial infections has proven a useful tool to study their dynamics in the presence or absence of treatment.

Chapter 5, the last part of this thesis dedicated to the evolutionary dynamics, brings stochasticity to the mathematical model. Emergence of new bacterial strains is very constrained by the approach selected during this thesis to generate the changes in the phenotypic traits, specially the first mutation step. All the results discussed from now on are very dependent on the evolutionary dynamics used to design the pathogen's trait space, which creates the opportunity to do it differently, in the future, and to understand the impact of this design on evolution and resistance selection. In this chapter, evolutionary dynamics of a self-limiting acute infections are studied in detail, using 100 stochastic realizations, with fixed parameters. Treatments, which differ on dose and duration, are grouped into five types. A primary finding related to treatment is that its success can be predict by the conjunct analysis of the bacterial load and the host immune response, which is not very affect by pathogen mutation rate. The general idea that aggressive treatments favor more resistant but with high cost mutations needs further investigation in the future. Lower or moderate doses, reinforced by short durations, create opportunities for the bacterial subpopulations to mutate and to overcome the barriers imposed by treatment. These types of treatment are, in fact, those that can be associated to second peaks of bacterial load, in which the most resistant strains, with very low fitness cost, are found. The simulations allow to state that, if treatment is administrated on the adequate timing, moderate treatments applied in an immune competent host stimulate a synergistic interplay and, with a high probabibility, clearance of the infection. To compare a treated bacterial infection against a non treated one would allow a deeper investigation. Another interesting point of research would be to define a measure to calculate the time

needed for clearance. Immunity was kept fixed during all simulations in this chapter, so its role on the evolutionary dynamics cannot be examined. However, that investigation would be very interesting to be carried out in the near future, since it is known that bacterial dynamics differ between hospitalized patients and healthier hosts. The development of the computational tools associated with this chapter was very demanding. Now that they are finished and ready to use, in addition to all the work already developed, they can be used for further investigation for different parameter values representing different host-pathogen-antibiotic combinations.

Mathematical modelling and computational simulations are, undoubtedly, tools of incalculable power to provide insights into antibioitic-immunity-pathogen dynamics. The main goal, which was to explore the interplay of different mechanisms of control during infection dynamics and to explore the evolutionary dynamics that can take place in different scenarios, was successfully reached. All the work and insights developed throughout this dissertation makes us one step closer to the ultimate goal: to have the real power to deal with resistance to antibiotics, from its prevention to its management.

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