
Modelling the dynamics between Enteropathogenic *Escherichia coli* infections, the immune response and antibiotic therapies

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Abstract

Enteropathogenic *Escherichia coli* (EPEC) is a virulent strain of bacteria that is a leading cause of death in children with diarrhoea, especially in countries with underdeveloped healthcare. EPEC attach to host intestinal epithelial cells (IECs) and secrete proteins into them that subvert the immune response. In particular, these proteins inhibit pro-inflammatory signalling, IEC apoptosis and phagocytosis by leukocytes. It is estimated that there are approximately 800,000 fatalities worldwide per year due to EPEC. Therefore, finding effective treatments for EPEC infections is crucial to the improvement of human health across the globe. In order to find effective treatments, a detailed understanding of EPEC and its infection strategy is needed. Researchers at the Centre for Biomolecular Sciences at the University of Nottingham have recently discovered experimentally that EPEC internalise host pro-inflammatory cytokines, granting dynamic control of protein secretion and reduced susceptibility to aminoglycosides (a class of antibiotic). However, while biological studies have uncovered vital information on many of the mechanisms of EPEC's infection strategy, much is still unknown about the infection strategy.

In this thesis, we first formulate and analyse a mathematical model describing a general infection resulting from interactions between host epithelial cells and non-specific bacteria in the human gut. The model comprises a set of ODEs describing the dynamics between a bacterial infection and the innate immune response. We then extend the model to account for EPEC-specific mechanisms with analysis providing insight into the effectiveness of the various aspects of EPEC's infection strategy, allowing the effectiveness of an EPEC infection to be compared to that of a general infection. The EPEC model was then adapted into an *in silico* multi-scale hybrid model that includes mechanisms on the macro-, cell-, and sub-cellular scales, implemented deterministically and stochastically, with the aim of exploring the dynamics between EPEC and the host's immune system both spatially and temporally. Finally, the hybrid model is extended to explore the effects of bacterial biofilms on the success of the infection. In each of the EPEC models, we account for the effects of antibiotics and the recent experimental discoveries discussed above. Analysis of the models via steady state, stability, sensitivity and bifurcation analyses and numerical simulations allows us to make predictions on the effectiveness of antibacterial treatment strategies. Our models predict effective treatment strategies targeting bacterial protein synthesis and replication, the immune response, the application of antibiotic, the positioning of bacteria and the ability of bacteria to form biofilms. We hope our predictions will inform, and reduce the time and cost of, future experimental work.

Acknowledgements

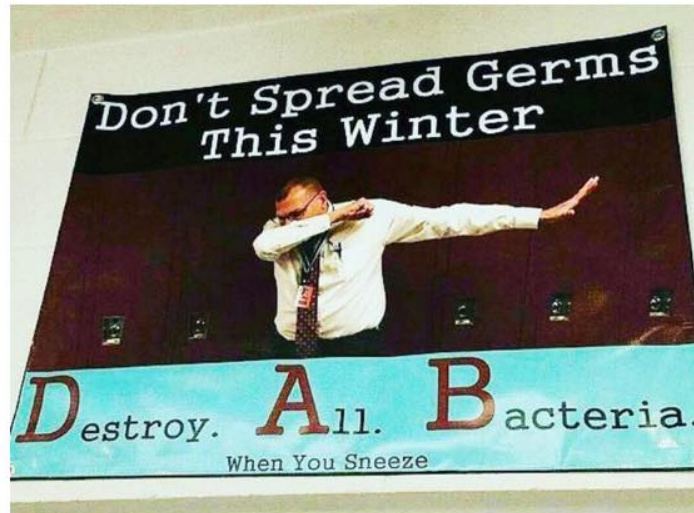
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“Well Seymour, I made it...”

Gary Chalmers



Contents

1	Introduction	8
2	Literature review	11
2.1	Overview	11
2.2	The innate immune response	11
2.2.1	Biology background of the innate immune response	11
2.2.2	Mathematical models of the innate immune response	13
2.3	Bacteria protein synthesis	15
2.3.1	Biological background of bacteria protein synthesis	15
2.3.2	Mathematical models of bacterial protein synthesis	16
2.4	Enteropathogenic Escherichia Coli	19
2.4.1	Biology of an EPEC infection	19
2.5	Antibiotics	21
2.5.1	Mechanism of antibiotics	21
2.5.2	Mathematical models of antibiotic application	23
2.6	Biofilms	25
2.6.1	Biology of biofilms	25
2.6.2	Mathematical models of biofilms	27
2.7	The Gillespie algorithm	28
2.7.1	Overview	28
2.7.2	The algorithm	29
2.8	Mathematical models of chemotaxis	29
2.9	Volume exclusion	32
2.10	Summary	34
3	A mathematical model of bacteria-immunity interaction	35
3.1	Overview	35
3.2	Model formulation	35
3.3	Steady state and stability analyses	38
3.3.1	Steady state analysis	38
3.3.2	Stability analysis	41
3.3.3	Steady state analysis summary	43
3.4	Sensitivity and bifurcation analyses with numerical simulation	43

3.4.1	Steady state sensitivity analysis	44
3.4.2	Bifurcation analysis	47
3.4.3	Numerical simulations	50
3.5	Summary	53
4	A mathematical model of an EPEC infection	56
4.1	Overview	56
4.2	Basic model formulation	57
4.3	Basic model: steady state and stability analyses	65
4.3.1	Steady state analysis	65
4.3.2	Stability analysis	68
4.3.3	Steady state analysis summary	71
4.4	Basic model: sensitivity, bifurcation and numerical simulation analyses	72
4.4.1	Overview	72
4.4.2	Steady state sensitivity analysis	73
4.4.3	Bifurcation analysis	77
4.4.4	Numerical simulations	82
4.4.5	EPEC basic model summary	88
4.5	A subcellular gene network (GRN) for bacterial protein synthesis	89
4.5.1	Introduction	89
4.5.2	Formulation of the GRN	90
4.5.3	Constant antibiotic concentration	91
4.5.4	Varying antibiotic concentration	94
4.6	The coupled EPEC model	100
4.6.1	Introduction	100
4.6.2	Formulating the model	100
4.6.3	Steady state and stability analysis	101
4.6.4	Numerical simulations	103
4.6.5	Coupled model summary	109
4.7	Summary	110
5	A hybrid model of an EPEC-specific infection	113
5.1	Introduction	113
5.2	Model formulation	114
5.2.1	Overview	114
5.2.2	Macro-scale model equations	115
5.2.3	Cell-scale dynamics	121
5.2.4	Sub-cellular scale dynamics	122
5.3	Numerical implementation	125
5.3.1	Macro-scale	125
5.4	Cell level mechanisms	134
5.4.1	Overview	134
5.4.2	Movement of bacteria	134
5.4.3	Movement of leukocytes	135

5.4.4	Phagocytosis	137
5.4.5	Volume exclusion	138
5.4.6	Recruitment and death of leukocytes	141
5.4.7	Production of pro-inflammatory mediators by leukocytes	142
5.5	Sub-cellular scale processes	142
5.6	Summary	145
6	Results of the hybrid model	147
6.1	Overview	147
6.2	Numerical experiments and parameters	147
6.3	Results in the absence of antibiotic	148
6.4	Results in the presence of antibiotic	167
6.5	Discussion	175
7	A PDE model for chemotactic leukocyte recruitment	178
7.1	Overview	178
7.2	Model formulation	178
7.3	Model analysis	180
7.3.1	Numerical simulations	180
7.3.2	Stability analysis	181
7.4	Discussion	183
8	The formation of bacterial biofilms and their effects on the infection	185
8.1	Overview	185
8.2	Model development	185
8.2.1	Biofilms and their effects	185
8.2.2	Pro-inflammatory mediator production	187
8.3	Model implementation	188
8.4	Results	191
8.5	Discussion	192
9	Conclusions and further work	195
9.1	Preamble	195
9.2	Models and results	195
9.3	Treatment strategies	198
9.4	Further work	200
A	Appendix	203
A.1	Showing that equation (3.10) only has one root with $p > 0$:	203
A.2	EPEC ODE model — Jacobian elements	205
A.3	EPEC ODE model — reducing the Jacobian matrix at the disease free state	206
A.4	A toy model of pro-inflammatory mediator production by leukocytes	209
A.4.1	Introduction	209
A.4.2	Model formulation	210
A.4.3	Summary	216

A.5 Hybrid model - showing numerical convergence for a decreasing time-step	217
A.6 Hybrid model - deriving expression for an approximation to the second derivative for a non-uniform mesh	217
A.7 Hybrid model - deriving the probability of phagocytosis	219
A.8 Hybrid model default parameter sets	222
A.9 Biofilm model baseline parameter values	227
A.10 Stability analysis of (7.2)	227

Chapter 1

Introduction

Enteropathogenic *Escherichia coli* (EPEC) is a leading cause of death in children in countries with underdeveloped healthcare and it is estimated to contribute to approximately 800,000 fatalities worldwide per year. It is therefore important that effective treatments for EPEC infections are found, but for this to be achieved, a detailed understanding of EPEC and its infection strategy must be developed. Numerous experimental studies have uncovered vital information on many of the mechanisms of EPEC's infection strategy, in particular the binding to host cells and the actions of secreted immuno-suppressing proteins, termed effector proteins. However, much is still unknown about the infection strategy; the times and rates at which the immuno-suppressing proteins are secreted are not known and the interactions between EPEC and antibiotics in the presence of the host immune system are poorly understood. This thesis focuses on the formulation and analysis of mathematical models describing the dynamics between a human host and the initial stages of an EPEC infection, intending to address the gaps of knowledge on the infection strategy, and thereby providing insight into potential treatment strategies.

Chapter 2 presents a review of the relevant biological and mathematical literature. First, we provide an overview of the innate (naturally present and not due to prior sensitization) immune response, in which we describe the inflammatory response, the leukocyte response and host cell death. We also discuss the defining features and infection strategy of EPEC and review the mechanisms of action, benefits and drawbacks of various classes of antibiotic. Sub-cellular protein synthesis is key to the survivability of cells and a particular class of antibiotic, known as aminoglycosides, disrupts bacterial protein synthesis when internalized and creates a positive feedback loop for further internalization by increasing bacterial membrane permeability. As we consider the effects of aminoglycosides on EPEC infections in detail in this work, we include a thorough description of bacterial protein synthesis. In [1], Mahdavi et al. showed that the pathogen *Neisseria meningitidis* can modulate gene expression via uptake of a type of pro-inflammatory mediator, leading to increased virulence. More recently, Easton et al. at the Centre for Biomolecular Sciences, University of Nottingham, have recently discovered that when internalized by EPEC, certain pro-inflammatory mediators reduce the sensitivity of EPEC to aminoglycosides (unpublished). A key aim of this thesis is to understand how such internalization of pro-inflammatory mediators influences EPEC population dynamics. We then discuss the mechanisms of bacterial biofilm formation and its consequences on the dynamics

and efficacy of the infection. We also present the mathematical models and methods from the literature that we used to inform our models.

In Chapter 3, we develop an ODE model for a general infection. This model includes the dynamics of the pathogen, the inflammatory response and the leukocyte response. Via steady state, stability, parameter sensitivity and bifurcation analyses, and numerical simulations, we identify treatment strategies that our models predict to be most effective. However, the general infection model lacks the EPEC-specific dynamics and in Chapter 4, we address this by developing two ODE models. The first model uses the general infection model as a foundation, includes host cell death and EPEC-specific immuno-suppressing effector proteins and is analyzed using the same methods that were used for the general infection model. The second model is a multi-scale model that includes a sub-cellular gene regulatory network (GRN) for bacteria protein synthesis, aminoglycosides and the novel findings from our experimental collaborators (Robin Delahay et al.). Via steady state analysis, numerical simulations and parameter sensitivity analysis of both models, we identify treatment strategies that are predicted to be most effective. We also compare the findings from the EPEC models to those from the general infection model, demonstrating the differences that the EPEC-specific mechanisms make to the success of the infection.

The ODE models developed in Chapters 3 and 4 describe population-level behaviour, necessarily neglecting both spatial variation and the stochastic effects associated with cell population growth/death and small cell populations. In Chapter 5, we address this by coupling a volume-excluding agent-based representation of EPEC, leukocyte and effector protein behaviour, with a continuum description of the inflammatory and antibiotic responses. In particular, this hybrid model includes: (1) a stochastic sub-cellular gene regulatory network for bacterial protein synthesis and replication; (2) reaction-diffusion PDEs for the production, degradation and transport of inflammatory mediators and antibiotic and (3) leukocyte recruitment to the infection site in response to inflammatory status via a chemotactically-biased random walk. This model allows for a detailed exploration into relevant treatment strategies. We then compare the stochastic hybrid model and the deterministic multi-scale ODE model, highlighting the strengths and drawbacks of both models. In Chapter 6, we present results obtained through simulation of the hybrid model, focusing on the infection size and the immune response strength over time and the percentage of infections that are eradicated. We explore the relationship between the spatial profile and the success of the infection and consider the course of the infection both in the absence and presence of antibiotic. Via numerical simulations and parameter sensitivity and phase plane analyses, we identify treatment strategies that are predicted to be most effective. For example we find that frequent, but weaker doses of an aminoglycoside treatment are more likely to lead to eradication than one strong dose. We compare the obtained results to those from the multi-scale ODE model and highlight the similarities and differences. In particular, the two models disagree on the effectiveness of targeting the effector proteins that inhibit phagocytosis.

In Chapter 7, we present a spatio-temporal PDE model for the recruitment of leukocytes, giving a detailed exploration of the immune response to an infection. In particular, we consider the production of pro-inflammatory mediators by leukocytes and the chemotactic attraction of leukocytes

up gradients in pro-inflammatory mediator concentration. We analyse the model numerically via simulations, bifurcation analysis and solving the boundary value problem (BVP) for the steady state profiles. We find for certain parameter regimes that the concentrations of pro-inflammatory mediators and leukocytes diverge to infinity, suggesting model instability. We explored the stability of the model analytically, but we were unable to explain the observed instability.

Bacteria are able to form biofilms; slimy encapsulating matrices that afford protection against many anti-bacterial treatments. None of the models we have developed so far have addressed the effects of biofilms, therefore in Chapter 8, the hybrid model is adapted to account for the mechanisms and consequences of biofilm formation. We present results demonstrating that biofilm infections are much more difficult to eradicate. We therefore predict that treatments that target biofilm formation are effective strategies.

Finally, in Chapter 9 we discuss the research undertaken in this project, summarizing the models developed and the treatment strategies found by each model to be effective. We compare the findings of the different models and discuss the reasons for the differences. We also outline a number of possible extensions to this work.

Chapter 2

Literature review

2.1 Overview

In this chapter, we present a detailed review of the biological and mathematical literature relevant to the infection strategy of Enteropathogenic *Escherichia coli* (EPEC) and the eradication of within-host EPEC infections. Motivated by the absence of current mathematical literature specific to EPEC infections, we use experimental studies of EPEC from the current literature as a basis to develop novel mathematical models that govern the dynamics of an EPEC-specific infection. We consider the stimulation and effects of the innate immune response, bacterial protein synthesis and replication, the infection strategy of EPEC, the effects of various classes of antibiotics and the formation and effects of bacterial biofilms. For each topic, we present a literature review of the relevant biological mechanisms and the mathematical models developed by other authors that govern the dynamics of the mechanisms. Finally, we give descriptions of the mathematical methods that are used in the multi-scale hybrid model (see Chapters 5 and 6); specifically, a stochastically simulated algorithm (Gillespie algorithm), models for chemotaxis and volume exclusion. There are numerous other mathematical methods that are relevant to the modelling of infection dynamics, including graphs and Boolean networks; however, details have been omitted for brevity. For detailed reviews on the application of graphs and Boolean networks to biological problems, we refer the reader to [2, 3] and [4, 5] respectively.

2.2 The innate immune response

2.2.1 Biology background of the innate immune response

The innate immune system is the host's first defence mechanism against invading pathogens, such as bacteria and viruses and comprises the cells and mechanisms that defend the host from infection, but without conferring long-lasting or protective immunity to the host [6]. One of the first defense mechanisms of the host is inflammation, the body's immunovascular response to an infection. Inflammatory mediators are released by a variety of host cells when stimulated by the presence of a pathogen or injurious stimuli and serve to activate and attract leukocytes to the site of infection [7]. Two of the main types of inflammatory mediators are cytokines and histamines. Cytokines are

cell signalling molecules that aid the immune response by (1) affecting the behavior of other inflammatory mediators, for example by stimulating up- or down-regulation, and (2) stimulating the production of leukocytes and attracting them towards sites of inflammation, infection and trauma [7, 8, 9]. Cytokines act by binding to receptors on the target cell's surface, initiating a chain of events that ultimately leads to the upregulation and/or downregulation of various genes and their transcription factors, resulting in the production of other cytokines, an increase in the number of surface receptors for other molecules or the suppression of their own effect by feedback inhibition [10, 11]. Individual cytokines can be categorised as pro- or anti-inflammatory, although many cytokines exhibit both pro-inflammatory and anti-inflammatory actions [12]. Proinflammatory cytokines are produced during cell injury and infection predominantly by activated macrophages and by other nonimmune cells, such as fibroblasts and endothelial cells [8]. The nuclear factor κ B (NF- κ B) pathway is a signalling pathway that is heavily involved in the expression of pro-inflammatory genes including those for the production of cytokines, chemokines, and adhesion molecules [13]. The pathway is triggered by microbial products and proinflammatory cytokines such as tumour necrosis factor alpha (TNF- α) and interleukin 1 (IL-1) and ultimately leads to altered gene transcription in favour of inflammation [11]. During an infection, the concentration of pro-inflammatory mediators increases [14]. Anti-inflammatory cytokines reduce inflammation by suppressing the production of pro-inflammatory cytokines [15], common cytokines that exhibit anti-inflammatory effects including transforming growth factor beta (TGF- β) and interleukin 10 (IL-10). Histamines cause blood vessels to dilate and become more permeable, allowing leukocytes to attach to chemotactant proteins on the endothelial cells of the locally dilated blood vessel and then squeeze through the endothelial cell walls. Once out of the blood vessel, they travel up chemokine (a type of cytokine) gradients via chemotaxis to the site of the pathogen or damage [7, 16]. Leukocytes are made in the bone marrow, are found throughout the body, particularly in the blood and lymphatic system, and engulf pathogens and digest them in a process termed phagocytosis [17]. Figure 2.1 shows the dynamics of a general infection schematically.

One of the intrinsic immune defense mechanisms of multi-cellular organisms is to sacrifice infected cells for the benefit of the remaining cells [18]. Cell death occurs when a cell is no longer able to carry out its function(s), two of the most common variations being apoptosis and necrosis. Cells undergoing apoptosis display chemical markers, such as phosphatidylserine, and secrete chemotactic factors that stimulate the attraction of leukocytes [19, 20], prompt phagocytosis allowing the safe disposal of the cells' waste products [21]. Apoptosis enables pathogens to be eliminated early in the infection without emitting alarm signals to further amplify the innate immune response [18, 22]. Necrosis describes nonapoptotic accidental cell death and is associated with unwarranted cell loss in human pathologies [23, 24]. Apoptotic cells shrink and are engulfed completely by phagocytes, whereas necrotic cells swell and are only partly taken up by phagocytes, leading to local inflammation through the unintentional release of pro-inflammatory mediators [24]. In the absence of phagocytosis, apoptotic bodies may lose their integrity and proceed to die via necrosis [23].

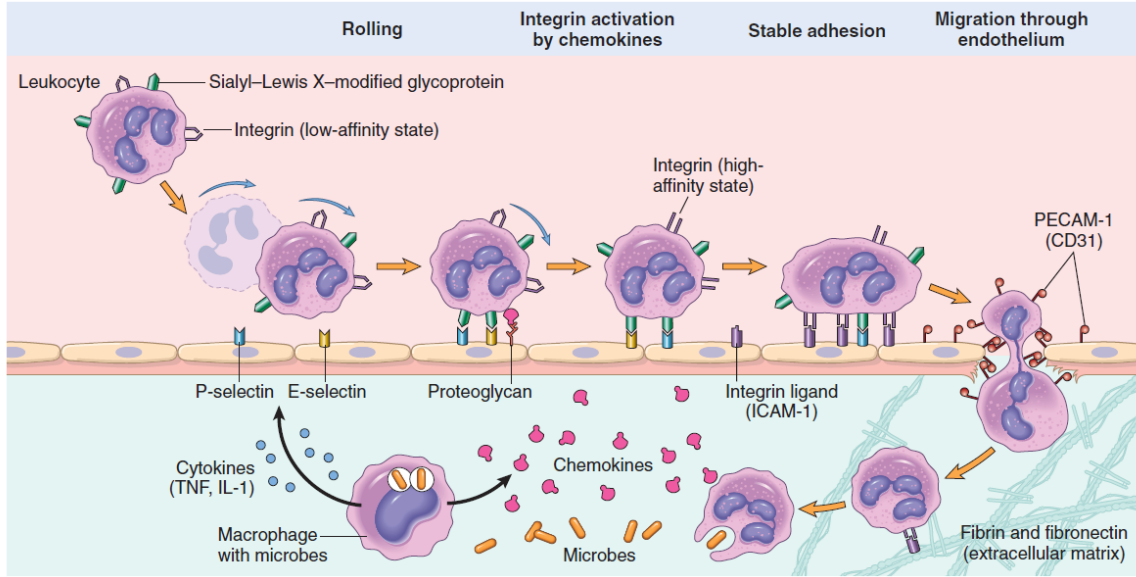


Figure 2.1: The dynamics of a general infection (taken from [7] — Figure 2-5 in book). When stimulated by the presence of a pathogen, host cells release inflammatory mediators. These mediators aid in the attraction of leukocytes to the site of infection, and in the translocation across the blood vessel layer. Once the leukocytes reach the site of infection, they can phagocytose the bacteria.

2.2.2 Mathematical models of the innate immune response

Baker [11] developed an activator-inhibitor model for inflammatory mediator interactions, focusing on pro-inflammatory (p) and anti-inflammatory (a) cytokines. The model accommodates background and stimulated production of pro-inflammatory cytokines, inhibited production by anti-inflammatory cytokines and cytokine degradation. The model takes the form:

$$\begin{aligned}\frac{dp}{dt} &= \left(P_{bp} + P_{pp} \frac{p^2}{\phi_1^2 + p^2} \right) \left(\frac{\phi_2^2}{\phi_2^2 + a^2} \right) - d_p p, \\ \frac{da}{dt} &= A_{pp} \left(\frac{p^2}{\phi_3^2 + p^2} \right) - d_a a.\end{aligned}$$

Hill functions have been used for the activation/inhibition terms to give switch-like behaviour in the stimulated production of pro- and anti-inflammatory cytokines. This model allows for many different types of dynamics, including bistability, hysteresis and long term steady and oscillatory behaviour. One observation from this model is that in some of the cases where bistability is admitted, increasing the concentration of pro-inflammatory cytokines leads to the eradication of chronic inflammation, since the phase plane trajectories are pushed out of the basin of attraction for the diseased (chronic inflammation) state. This is interesting as it implies that chronic inflammation can be abolished by further inflaming the environment. This model is used as a foundation for the interactions between inflammatory mediators in our models.

Jarrett et al. [25] model the dynamics between inflammation and bacteria, with the aim of reflecting particular experiments on mice infected with *Staphylococcus aureus* that led to clinically relevant vaccine strategies for multi-resistant *S. aureus*, the vaccines using target proteins specific to *S. aureus* as antigens. The model considered four components: pro- (P) and anti-inflammatory

(A) responses, inflammation/damage (I) and the bacterial infection (B). In this model, the pro-inflammatory response depends on bacterial presence and causes the level of inflammation/damage to increase, while the anti-inflammatory response suppresses the pro-inflammatory response and repairs/removes damage. The variable B describes the strength of the infection rather than the population size and it is assumed that damage benefits the infection, inhibits the anti-inflammatory response and stimulates the pro-inflammatory response. This model considers the dynamics between bacteria and the immune system while keeping inflammation and inflammatory signalling distinct and is able to accurately predict the outcome of a wide range of specific experiments and vaccinations. A sensitivity analysis highlights the immune system components that are good targets for immunotherapies. The abstraction of splitting up inflammatory responses and damage is used in our work in the modelling of host cell death and its effects on the pro-inflammatory response. A schematic of this model is given in Figure 2.2.

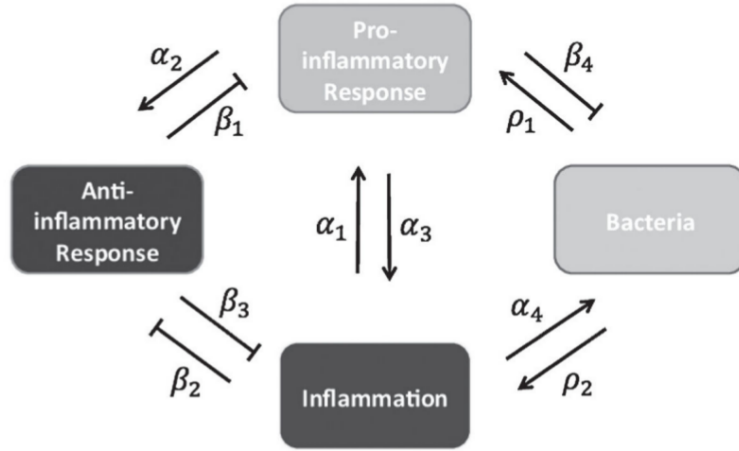


Figure 2.2: Schematic of the pathogen-immune response model presented by Jarrett et al. [25].

Rubin et al. [26] describe the interactions between bacteria and the immune response using two variables; the pathogen and activated phagocytes. Resting phagocytes are activated by the pathogen and activated phagocytes, the dependence of activation on pro-inflammatory mediator concentration modelled implicitly. The activation process is assumed to be rapid, leading to the number of resting phagocytes being in a quasi-steady state, allowing model simplification. Furthermore, in the presence of a pathogen, a rapid influx of tissue macrophages and other non-specific defenses occurs, the constituents of which are consumed by the pathogen upon encounter, thus destroying them. Anti-inflammatory mediators were then included in the model as a parameter, these mediators inhibiting the activation of phagocytes and the ability of activated phagocytes to attack pathogens. This model yields distinct parameter regions that lead to health, aseptic death¹, septic death and oscillatory behaviour and exhibits multi-stability under certain parameter regimes. Treatment interventions that are implemented by altering parameters or variables can be explored

¹Aseptic death corresponds to where the pathogen has been eliminated but with high and persistent immune activation and damage.

by considering whether or not they move trajectories from the basin of an unhealthy state to that of the healthy state. Rubin et al. further developed this model into a four variable ODE model for the innate immune response, by considering the dynamics between pathogens, activated neutrophils, damage done to the host, and anti-inflammatory mediators, the concentration of the latter now considered as a variable. The model predicts that anti-inflammation is significant in the return to health, and the avoidance of aseptic death, by allowing a larger initial infection size to be dealt with by the immune response, thus allowing the body to recover over a wider range of pathogen growth rates. Furthermore, the outcome of the infection depends significantly on the timings of interventions. The model could be made more biologically realistic by including a more detailed biological characterization of specific anti-inflammatory substances. The model could then be used to explore the therapeutic use of these substances by considering the length and intensity of intervention in a time-scale-dependent manner. In the analysis of our models, we explore the existence of multi-stability, including disease-free states with persistent immune activation, and how the timings of external treatments affect treatment efficacy.

The models explored in this section indicate that the dynamics between the immune response and a pathogenic challenge can be modelled in a variety of ways and that many different behaviours, including bistability and oscillatory behaviour, can be supported. In clinical practice, many species of bacteria are shown to have a minimum infective dose [27], demonstrating the feasibility of bistability. Additionally, infections can recur, potentially repeatedly, if host defences are insufficiently strong or the strain is sufficiently virulent [28, 29], leading to oscillatory behaviour. There is a plethora of other mathematical models for the immune response in the literature; however, the models explored here are exemplary of the mathematical techniques that can be used to model the immune response.

2.3 Bacteria protein synthesis

2.3.1 Biological background of bacteria protein synthesis

Messenger RNA (mRNA) are RNA molecules that convey genetic information from DNA to ribosomes. Proteins are synthesized by individual bacteria via the transcription of DNA into mRNA and the translation of the resulting mRNA by ribosomes. In DNA transcription, RNA polymerase (RNAP), together with one or more transcription factors, binds to the promoter region of one of the DNA strands. The RNAP creates a transcription bubble, which separates the two strands of the DNA helix forming an RNA-DNA helix, by breaking the hydrogen bonds between complementary DNA nucleotides. The RNAP then moves along the DNA strand, adding complementary RNA nucleotides to the mRNA strand being created, resulting in a strand that is a complementary copy of the DNA strand. Hydrogen bonds of the RNA-DNA helix break, freeing the newly synthesized mRNA strand, allowing the DNA to reform a helix [30, 31]. The mRNA strand is then translated by ribosomes; the mRNA is decoded to produce a specific amino acid chain (polypeptide) that later folds into an active protein. Each triplet of nucleotides (a codon) corresponds to either a specific amino acid, or a start/stop signal. Some codons code for the same amino acid; for example, the amino acid serine is encoded by 6 different codons. Transfer RNA (tRNA) are molecules that carry the amino acids to be used for the polypeptide to ribosomes. Each tRNA molecule carries one spe-

cific amino acid and certain types of tRNA are able to carry more than one specific type of amino acid. Ribosomes facilitate decoding by inducing the binding of complementary tRNA anticodon sequences to mRNA codons (see Figure 2.3). The amino acid is then added to the peptidyl site of the ribosome and the ribosome then moves (translocates) to the next mRNA codon to continue the process, creating an amino acid chain. The ribosome releases the polypeptide once a stop codon is read [31, 32]. Ribosomes also have mechanisms for proof-reading the peptide chain before and after amino acids are added [33, 34], vastly improving the efficiency of protein synthesis.

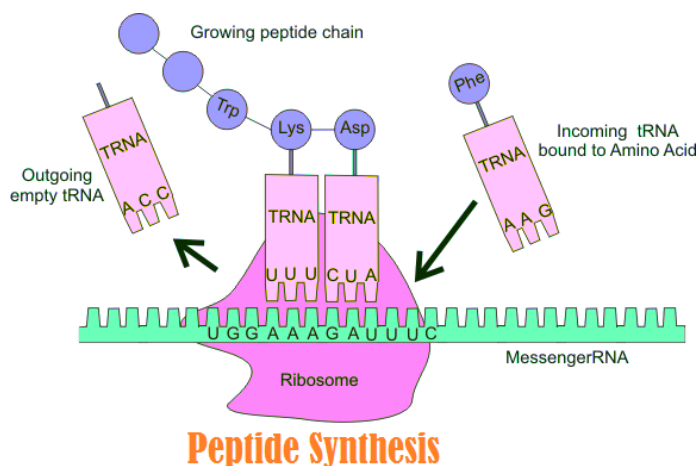


Figure 2.3: Caricature of the mechanism of action of ribosomes. Image taken from Chemistry Notes Info (<https://chemistrynotesinfo.blogspot.com/2016/10/peptide-synthesis.html>).

2.3.2 Mathematical models of bacterial protein synthesis

De Jong and Geiselmann [35] present a simple gene regulatory network (GRN) model for two genes, in which each gene encodes a regulatory protein that inhibits the expression of the other gene. This model provides a simplistic representation of GRN-controlled protein synthesis and the model is given by the following ODEs:

$$\begin{aligned}\dot{x}_a &= r_a \frac{\theta_b^n}{\theta_b^n + x_b^n} - d_a x_a, \\ \dot{x}_b &= r_b \frac{\theta_a^m}{\theta_a^m + x_a^m} - d_b x_b,\end{aligned}$$

where x_a and x_b are the concentrations of proteins a and b respectively. The negative feedback of protein expression is represented by a decreasing Hill function of the other protein with half maximal expression θ_b, θ_a and Hill coefficient n, m for proteins a, b respectively. When $m, n \rightarrow \infty$, the model displays switch behaviour, with x_a production ‘on’ when $x_b < \theta_b$ and x_b production ‘on’ when $x_a < \theta_a$ (and vice versa). The system has either (1) two stable steady states, one in which x_a dominates and one in which x_b dominates, separated by an unstable state or (2) one (stable) steady state in which one of x_a or x_b dominates depending on parameter values. The regulatory nature of the model prevents unbounded growth of either type of protein. In the case of linear protein expression, then there are no stable steady states — all trajectories except those along the stable manifold of the saddle point blow up. However, the analysis of otherwise intractable local dynamics

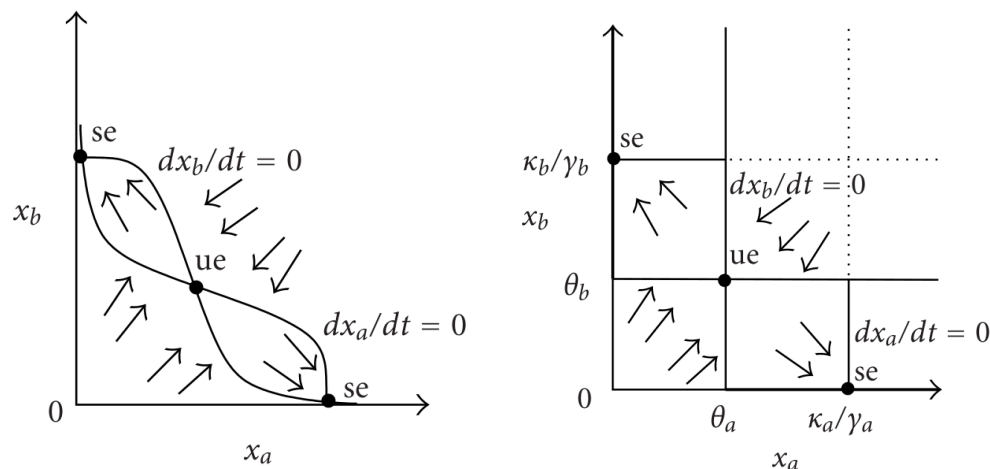


Figure 2.4: Example phase planes of a GRN presented by De Jong et al. using Hill function growth terms (left) and Heaviside growth terms (right) [35]. The latter model is piecewise linear and ‘se’ and ‘ue’ denote stable and unstable equilibrium points respectively. Both plots are taken from [35].

can be made tractable by approximating complicated functions with linearised equivalents. Heaviside functions can also be used instead of Hill functions, making the model piecewise linear and bounded. Figure 2.4 shows example phase planes for this gene regulatory network model with Hill functions and Heaviside functions. This work demonstrates the suitability of various functions in the modelling of GRNs and we use the ODE structure of the presented GRN as a foundation in the development of the GRNs in our models.

Gorban et al. [36] present models describing the dynamics of protein transcription, translation and degradation. They initially model the dynamics using four variables: mRNA with their translation initiation site not occupied and occupied by a ribosome (M, F respectively), the number of ribosomes actively synthesizing proteins (R), and the number of proteins (P). A schematic of this basic model is given in Figure 2.5. mRNA are produced at a constant rate and bind with free ribosomes, which translate the mRNA into proteins. The distinction between occupied mRNA strands, unoccupied mRNA strands and active ribosomes allows the binding of multiple ribosomes to a single mRNA strand to be included in the model. Furthermore, the linearity of the model allows exact solutions to be found algebraically. They then build upon this model by defining mRNA as either new-born or mature and splitting ribosomes into functional subunits, emphasising that the models they have developed can accommodate specific mechanisms and phenomena, making them useful foundation models. However, the models presented are not necessarily proven valid (or invalid) by experimental data. Instead, the authors suggest a hierarchy of models of increasing complexity and emphasize that each model might be useful in particular contexts. An extensive comparison of the model results to relevant experimental data would provide useful information regarding their validity. Our GRN models for bacterial protein synthesis and replication are based on their basic model.

Finally, a more general model for mRNA-protein gene networks is presented by Edwards et al. [37] and takes the form:

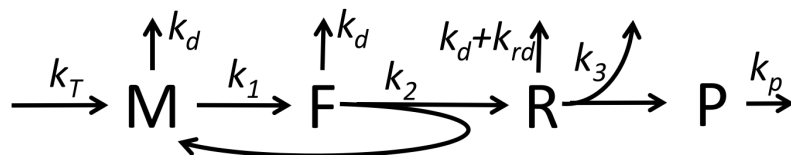


Figure 2.5: Schematic of a protein synthesis model presented by Gorban et al. [36].

$$\begin{aligned}\dot{x}_i &= F_i(\mathbf{Z}) - \beta_i x_i, \\ \dot{y}_i &= \kappa_i x_i - \alpha_i y_i, \quad i = 1, \dots, n,\end{aligned}$$

where x_i and y_i are the concentrations of the mRNA and the protein product of gene i respectively. $\mathbf{Z}$ is a vector of functions governing the production of mRNA by each protein. The i th element of $\mathbf{Z}$ given by:

$$Z_i = \frac{y_i^{\frac{1}{q}}}{\theta_i^{\frac{1}{q}} + y_i^{\frac{1}{q}}}, \quad i = 1, \dots, n, \quad 0 < q \leq 1.$$

mRNA production by each protein is controlled by an increasing Hill function in the concentration of the respective protein. $F_i(\mathbf{Z})$ governs the global production of x_i , where F_i is a function with respect to each Z_i . The model is bounded since all components of $\mathbf{Z}$ are bounded. The authors demonstrate the ability of this general model to accommodate a variety of features appropriate to describe specific GRN dynamics, such as when protein translation is rapid compared to mRNA transcription and vice versa, highlighting the effectiveness of the model as a foundation model. Rapid translation can be modelled by:

$$\begin{aligned}\dot{x}_i &= F_i(\mathbf{Z}) - \beta_i x_i, \quad i = 1, \dots, n, \\ \epsilon \dot{y}_i &= (\kappa_i x_i - \alpha_i y_i),\end{aligned}$$

where $0 < \epsilon \ll 1$. We use this ODE model for the synthesis of several distinct types of proteins in a GRN in the development of our GRN models; however, for simplicity we consider a single timescale for all mechanisms.

Many species of bacteria form spores to increase resilience and survivability in prolonged harsh conditions, such as nutrient deficiency and extreme temperatures. Jabbari et al. [38] developed an ODE model of the sporulation-initiation GRN in a *Bacillus subtilis* cell, accounting for the protein PhrA; a quorum-sensing signal that leads to sporulation activation. Population size, DNA damage, competence² and nutrient availability are the chosen signals that can induce sporulation. The model correctly reproduces nearly all predicted bacterial phenotypes, the predictions based on combinations of the above signals that the authors believe are, or are not, desirable for sporula-

²Competence is the ability of a cell to alter its genetics by taking up DNA from adjacent cells or its environment, allowing the cell to adapt genetically to the environment.

tion. They show that PhrA also acts as a timing device, delaying sporulation until conditions are appropriate. The model is detailed and provides a base on which additional signals, such as pH and temperature, can be added to further investigate the conditions that lead to sporulation in *B. subtilis*. Furthermore, the model can be adapted to fit the sporulation-initiation GRNs of other bacteria, such as *Clostridium difficile*.

2.4 Enteropathogenic Escherichia Coli

2.4.1 Biology of an EPEC infection

EPEC are a species of bacteria that target the host's intestinal epithelial cells (IECs). They are described as attaching and effacing bacteria as they bind to IECs and efface their microvilli-covered surface using bundle-forming pili and other adhesins [39, 40, 41]. Via a type III secretion system (T3SS), EPEC secrete a variety of effector proteins into their host IEC, the majority of these proteins contributing to the subversion of the immune response [42]. The core T3SS apparatus is needle shaped and forms a translocon (channel) and a pore in the IEC's membrane [43], allowing the injection of effector proteins into the IEC.

The first protein to be injected is translocated intimin receptor (Tir), intimin being a bacterial membrane protein. Tir anchors itself to the host cell's cytoskeleton via α -actinin [44, 45]. Bacteria are defined as being intimately attached to an IEC when the T3SS can efficiently inject effector proteins into the IEC and the binding of Tir and intimin results in an intimate attachment to the IEC [46, 47]. Tir is then phosphorylated, leading to the local surface of the IEC being pushed up into a pedestal, upon which the bacterium sits more stably [45, 47]. This process also allows the bacterium to withstand the buffeting of the stream of digested materials that flows through the intestinal tract [44]. Figure 2.6 shows a schematic of the attachment of EPEC to host IECs.

Once the bacterium and IEC are intimately attached, the bacterium can efficiently inject other effector proteins that perform a wide range of functions to enable the bacteria to evade the immune response [49]. Key functions include the inhibition of:

- host inflammatory signalling pathways, such as the NF- κ B pathway,
- apoptosis of host IECs,
- phagocytosis of the bacteria.

The inhibition of inflammatory signalling leads to fewer leukocytes being attracted to the site of infection, thereby allowing the infection more time to grow before detection. Inhibition of IEC apoptosis is beneficial to EPEC for two reasons. Firstly, it allows adherence to IECs that would otherwise undergo apoptosis due to bacteria-induced damage, allowing bacteria more time to inject further effector proteins into IECs [50]. Secondly, host cell apoptosis stimulates the attraction of leukocytes and phagocytosis via the display of chemo-attractive markers, therefore preventing apoptosis reduces the efficacy of the leukocyte response in infection eradication. Conversely, the effector protein EspF induces host cell apoptosis [51, 39] and is seemingly antagonistic to the infection strategy. However, it has been shown that EPEC's effector proteins are injected sequentially.

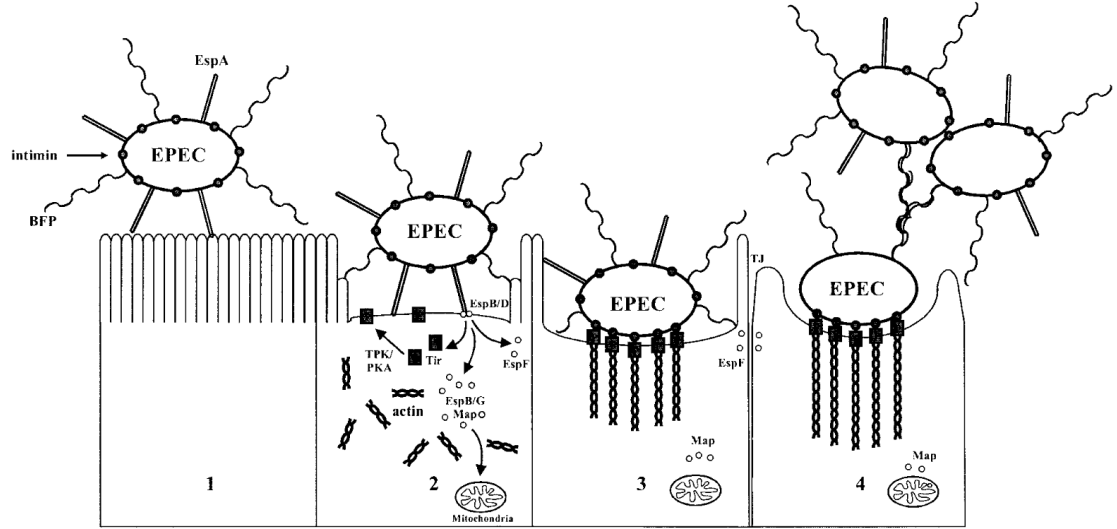


Figure 2.6: Schematic showing the attachment of EPEC to host IECs (taken from [48]). (1) An EPEC cell loosely binds to an IEC. (2) Using bundle forming pili (BFP) and other adhesins, the EPEC cell effaces the microvilli-covered surface of the IEC. Tir is secreted into the IEC and anchors itself to its cytoskeleton via α -actinin. The binding of Tir to intimin (on the surface of the bacterium) enables the EPEC cell to bind more securely to the IEC. (3) Tir is phosphorylated, leading to the local surface of the IEC being pushed up into a pedestal, upon which EPEC cell sits more stably. (4) Once the EPEC cell is firmly attached, it begins to replicate, forming a colony.

For example the effector protein EspZ, which inhibits apoptosis [49], has been shown to be injected before EspF [52] allowing EspZ to act first and prevent IEC death. It was proposed by Roxas et al. [51] that early EspZ delivery delays EspF-induced host cell death until after the bacterial colony is established, providing ample time for growth. This is advantageous to EPEC, since the alternative necrotic death (due to host cell damage caused by bacteria) would trigger a more dramatic inflammatory response [49]. The inhibition of phagocytosis results in a weaker challenge on the infection, increasing the ability of EPEC to survive the leukocyte response.

There is an ecological community of symbiotic bacteria that reside in the human gut, known collectively as the gut microbiota. The microbiota and host aid each other in numerous ways; for example, the microbiota can digest polysaccharides (such as starch from plants) that are not digestible by the host and use them for growth [53]. The microbiota then releases products during fermentation that the host can use, an example being butyrate, which is a metabolite that contributes to the prevention of colorectal cancer and the maintenance of gut health [53, 54]. The gut bacteria compete against pathogenic bacteria for resources and space and can secrete molecules that inhibit pathogens or detoxify compounds that harm the host [55]. However, normally commensal or symbiotic bacteria (including *E. coli*) can acquire genes for virulence via genetic mutation or from other bacteria via horizontal gene transfer, resulting in them becoming pathogenic to the host [56, 57].

Mahdavi et al. [1] show that the pathogen *Neisseria meningitidis* can modulate gene expression via uptake of host pro-inflammatory cytokines, leading to increased virulence. It has recently been dis-

covered by Easton et al. at the Centre for Biomolecular Sciences (CBS), University of Nottingham, that EPEC also internalize host cytokines; in particular, the two dominantly pro-inflammatory mediators TNF- α and IFN- γ (unpublished). The mechanism for this is not currently known, but it is thought that the bacteria bind the cytokines to their DNA, leading to altered gene expression in a way that is beneficial to the bacteria, given the current inflammatory conditions of the host. We hypothesize that internalized pro-inflammatory mediators alter the secretion rate of each type of effector protein. As an example, if EPEC detects that the background concentration of pro-inflammatory mediators is low (due to the low rates of internalization), then it produces more effector proteins that act to maintain the inhibition of inflammatory signalling pathways, allowing the infection more time for growth before detection by the immune response.

2.5 Antibiotics

2.5.1 Mechanism of antibiotics

Antibiotics are substances that are used to treat bacterial infections. There are many different classes of antibiotic, each with their own mechanism(s) of action. For example, penicillins and cephalosporins target the bacterial cell wall, quinolones and sulfonamides target nucleic acid synthesis, and macrolides and tetracyclines target protein synthesis [58, 59]. Antibiotics can be either bacteriostatic (prevent replication of bacteria) or bactericidal (kill bacteria) [60]. Antibiotics have been shown to have detrimental effects on the gut microbiota by unintentionally killing commensal bacteria, the induced disequilibrium facilitating intestinal colonization by virulent pathogens such as *Clostridium difficile* [61, 62].

In clinical practice, duration of therapy, dosage, and route of administration depend on site, type and severity of infection and response. Common methods of administration include oral delivery (tablets), topical delivery (creams and ointments), intravenous delivery and injections, the latter usually reserved for more serious infections [63]. Many antibiotics are administered as a course of treatments. For example, a 500 mg dose every 6 hours of the antibiotic phenoxymethylpenicillin is recommended by the National Institute for Health and Care Excellence (NICE) for the treatment of several infections, including tonsillitis, otitis media and cellulitis [64]. Some antibiotic treatments are delivered in a single concentrated dose. For example a single 1g dose of the antibiotic azithromycin is recommended by NICE for the treatment of chlamydia and gonorrhoea [65]. Many types of antibiotics, including vancomycin, ceftriaxone, and cefazolin, are delivered intravenously to treat a wide variety of infections, including skin, soft tissue, bone and joint infections. Treatments can be delivered in a clinical setting, but are often self-administered (via outpatient parenteral antimicrobial therapy) [66, 67, 68]. Self-administration offers considerable financial savings, although facilities may be needed to provide initial doses, vascular access, patient training on self-administration, pharmacy services, and medical supervision [67].

The biological half-life of a substance is the time it takes for half of the substance to be removed (via biological processes). Figure 2.7 gives the half-lives of several types of antibiotic. The minimum inhibitory concentration (MIC) of a drug is the lowest concentration at which the growth of bacteria is prevented. In order for an antibiotic therapy to be effective, the MIC of the antibiotic must be

maintained over the course of the treatment [69], therefore antibiotics with short half-lives must be administered more frequently. In contrast, azithromycin has a half-life of 68 hours, allowing the administration of a single dose to be effective for treating chlamydia [70].

Aminoglycosides are a type of bactericidal antibiotic that inhibit ribosome function, and therefore protein synthesis, in certain bacteria [71] (see section 2.3.1). The two main mechanisms of action are: (1) blocking ribosomes, preventing them from initialising translation; and (2) impairing the ability of ribosomes to proof-read proteins, potentially leading to the production of incorrect proteins or the premature termination of translation [71, 72]. The incorporation of incorrect proteins in the bacterial cell membrane has the extra benefit of making the bacterial membrane more permeable, allowing more antibiotic to enter the cell and further inhibit ribosomal activity [71, 73]. While aminoglycosides are potent antibacterials, they have been shown to be nephrotoxic (toxic to the kidneys) and ototoxic (toxic to the ears), resulting in hearing loss, vestibular impairment or renal failure in some patients [72, 74].

Aminoglycosides are typically administered in weight-based doses 1-3 times daily [75, 76]. For examples, NICE recommends that (1) amikacin be injected at a dose of 7.5 mg/kg twice daily, increased to three times daily, for up to 10 days [77] and (2) intravenous infusion of tobramycin be administered, over 20–60 minutes, at a dose of 1mg/kg three times daily for 5–8 days [78]. Aminoglycosides are not typically delivered in single doses or via continuous intravenous infusion [75].

Many strains of bacteria have developed resistance mechanisms to aminoglycoside action, two of the main mechanisms being (1) decreased uptake or accumulation of the drug (such as via efflux pumps) and (2) enzymes that modify the antibiotic to inactivate it (such as acetyltransferases) [73, 79, 80]. Plasmid exchange between bacteria facilitates the acquisition of a drug-resistance phenotype [73]; however, experimental research suggests that harbouring resistance genes comes at a fitness cost [81, 82]. Maximizing the benefits achieved with aminoglycosides, and other antibiotic classes that promote resistance, involves a trade-off between curing infections and avoiding the spread of resistance. The evolution of resistance can be slowed down by only using aminoglycosides to treat

Drug	Half-life, h
Acyclovir ^d	2–3.5
Amphotericin B	24–360
Liposomal amphotericin B	24–360
Amphotericin B lipid complex	24–360
Ampicillin	1
Ampicillin-sulbactam	1
Caspofungin	>48
Cefazolin	1–2
Cefepime	2
Cefoperazone	1.5–25
Ceftazidime	1.4–2
Ceftriaxone	5.4–10.9
Cefuroxime	1–2
Chloramphenicol	1.5–4
Clindamycin	2–3
Daptomycin	8.1
Doxycycline ^e	22–24
Erythromycin lactobionate	1.5–2
Ertapenem	4
Ganciclovir	2.5–3.6
Gentamicin	2–3
Imipenem-cilastatin	0.8–1.3
Linezolid	4.5
Meropenem	1.5
Nafcillin	0.5–1.5
Oxacillin	0.3–0.8
Penicillin G ^f	0.4–0.9
Quinupristin-dalfopristin	3; 1
TMP-SMZ ^d	8–11; 10–13
Tobramycin	2–3
Vancomycin	4–6

Figure 2.7: The half-lives of several types of antibiotics (taken from [67]).

severe infections [83].

Figure 2.8 shows time courses of the population of an EPEC cell culture as the concentrations of aminoglycoside and $\text{TNF-}\alpha$ are varied. This experimental data was obtained by Easton et al. at CBS, University of Nottingham (unpublished). By comparing the blue graphs across all panels of Figure 2.8, we see that in the presence of aminoglycoside but not $\text{TNF-}\alpha$, the infection size is reduced. By comparing the black and orange graphs across all panels, we see that for high concentrations of $\text{TNF-}\alpha$, the reduction in infection size due to aminoglycosides is smaller. This suggests a $\text{TNF-}\alpha$ -induced reduction in the susceptibility of EPEC to aminoglycosides. While the internalization of pro-inflammatory cytokines is thought to reduce the sensitivity of EPEC to aminoglycosides, the mechanism for this is currently unknown. We hypothesize that cytokines are able to deactivate aminoglycosides by binding to them.

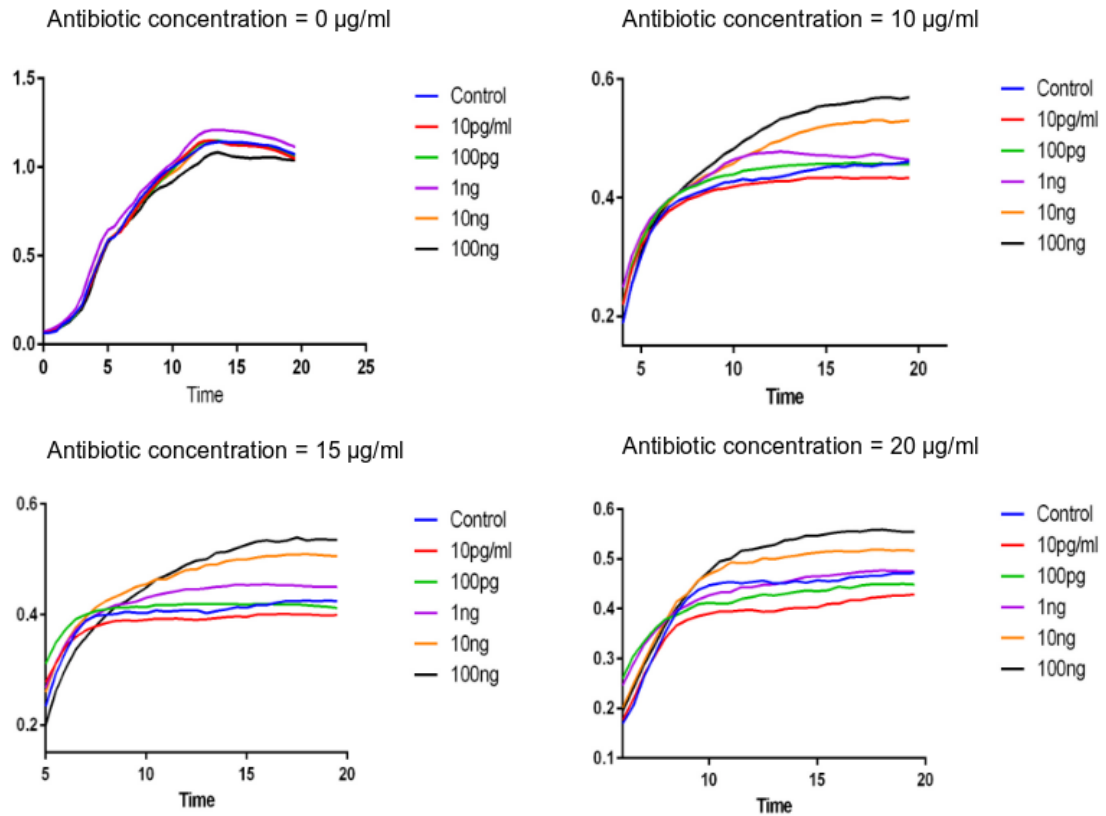


Figure 2.8: Time courses of the population of an EPEC cell culture ($\times 10^9$ cells per ml culture) as the concentrations of aminoglycoside (titles) and the pro-inflammatory cytokine $\text{TNF-}\alpha$ (legends) are varied. Time is measured in hours. Experimental data obtained by Easton et al. at CBS, University of Nottingham (unpublished).

2.5.2 Mathematical models of antibiotic application

D'Agata et al. [84] present two models to investigate the impact of different antibiotic regimens on the emergence of antimicrobial-resistant bacteria. First, they present a base model for the dynamics

between an infection, the immune response and an antibiotic treatment using only one variable: the population of bacteria. It is assumed that the activated phagocyte population has already reached equilibrium and that the phagocyte response has a bounded maximum effect on the infection. The death rate of bacteria due to antibiotic is assumed to be linear in the number of bacteria, with the antibiotic concentration remaining constant for the duration of treatment. They then develop this model by separating bacteria into those that are sensitive and those that are resistant to the antibiotic. Resistant bacteria confer their resistance to sensitive bacteria via their plasmids through horizontal gene transfer, but resistant bacteria can revert back to being sensitive due to plasmid instability or lack of antibiotic pressure — it is assumed that in the absence of antibiotic pressure, the plasmids carrying antimicrobial resistance genes are lost to minimize fitness costs that result in slower replication. Antibiotics kill bacteria at a rate that is linear in the number of bacteria; however, the rate is much slower for resistant bacteria. These models were used to show that the timing of an antibiotic treatment has a significant effect on the outcome of the treatment; earlier treatments are more successful at eradicating infections than later treatments of the same duration. They also show that the interruption of a treatment can have detrimental effects, potentially leading to treatment failure, particularly if the interruption is early in the course of the treatment. These observations indicate the presence of a basin of attraction for the diseased state which can be entered for a sufficiently large dose of bacteria and exited for a sufficiently long treatment. The authors then develop this model into one involving multi-drug resistant bacteria and multiple antibiotics, allowing the efficacy of combination treatments on complicated infections to be explored. The model could be made more biologically realistic by considering the immune response, and therefore a dynamic population of phagocytes. Doing this may shed light on synergies between the immune response and antibiotic treatments in the eradication of both susceptible and resistant bacteria. We use the ODE structure for bacteria-antibiotic dynamics presented in this paper as a basis in the development of our antibiotic-dependent models.

Ternent et al. [85] present a model that considers the effectiveness of combining antibiotics with anti-virulence drugs, Figure 2.9 shows a schematic of the model. Two bacterial sub-populations are considered: antibiotic-resistant and -susceptible. Resistant bacteria are able to confer their resistance to susceptible bacteria via conjugation (the transfer of genetic material between bacterial cells by direct cell-to-cell contact). The effect of the antibiotic on susceptible bacteria is much stronger than that on resistant bacteria. However, resistant bacteria grow at a slower rate due to the fitness costs of maintaining antibiotic resistance. The immune response affects both sub-populations equally and the anti-virulence drug is modelled as boosting the effectiveness of the immune response. Several drug dosing styles were considered, including no treatment, a single dose, periodic doses and a constant concentration, for treatments consisting of either antibiotic, anti-virulence drug or both together. The model shows that using antibiotic alone promotes the spread of resistance and using anti-virulence drugs alone is not strong enough to completely clear the infection. The authors found that the anti-virulence drug has a significant impact on the resistant sub-population due to their reduced replication rate. A subsequent antibiotic treatment will then clear the remaining susceptible bacteria and therefore eradicate the infection. They conclude that combining an anti-virulence drug with a subsequent antibiotic is not only a more effective treatment strategy, but may also help prevent the evolution of resistance to antibiotics. The varied treatment dosing styles

investigated in this paper provides a comprehensive methodology for exploring the effectiveness of external antimicrobial therapies.

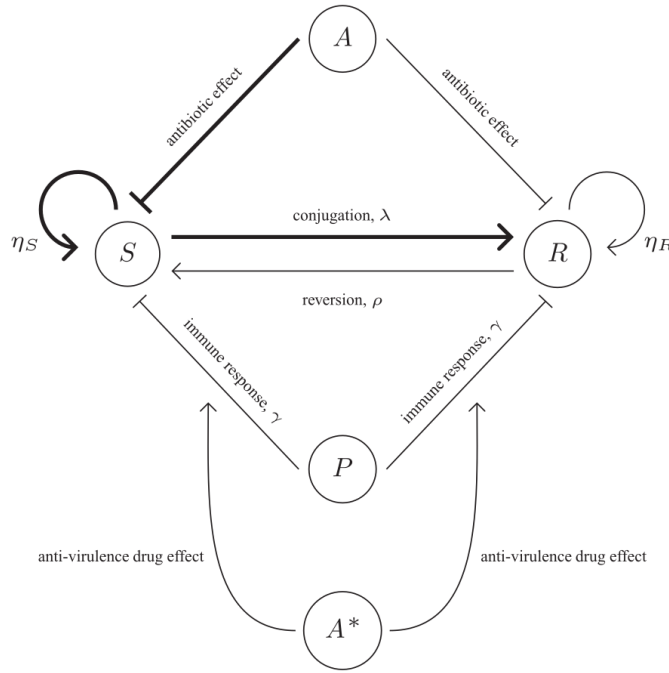


Figure 2.9: Schematic of the model presented by Ternent et al. [85]. Bacteria are split into susceptible and resistant strains. Antibiotics strongly affect susceptible strains and weakly affect resistant strains. The immune response has an equal effect on both types of strain. The anti-virulence drug is modelled as bolstering the immune response. Resistant bacteria can convey their resistance to susceptible bacteria via conjugation, but can lose it (at a much slower rate) in reversion.

Gjini et al. [86] present a model to study the interplay between pathogen-dependent host immune responses and antibiotic treatment on antibiotic-sensitive and resistant bacteria. They compare various treatment regimes, investigating how infection outcomes such as clearance time and the selection of resistant bacteria vary between regimes. They show that there is a critical strength for the immune response, above which clearance is feasible. They also show that the interaction between antibiotics and the immune response can be antagonistic when antibiotic-mediated pathogen killing decreases the intensity of the immune response; but under appropriate timing, dosage and duration, the interaction becomes synergistic, with both the antibiotic and host immunity acting in concert. This synergy also helps reduce the selection for resistance.

2.6 Biofilms

2.6.1 Biology of biofilms

Many species of bacteria can aggregate into a consortium that is embedded in an extra-cellular matrix (ECM). This ECM constitutes mainly polysaccharides, DNA and proteins, and anchors the bacteria to each other and often to an external surface [87, 88, 89]. Examples of surfaces to which biofilms attach include the teeth (plaque), industrial water systems and, of particular interest to

this research, the gut [87, 89, 90].

Biofilms are difficult for phagocytes to penetrate, making clearance of infections by the immune response much more difficult [87, 89]. Attempted clearance by the immune response often leads to frustrated phagocytosis — phagocytes are unable to fully ingest bacteria, leading to pro-inflammatory mediators and digestive enzymes spilling into the background, resulting in damage to the host and increased inflammation [7, 91, 92]. The ECM of biofilms also reduces the susceptibility of encased bacteria to antimicrobial agents, making biofilm infections much more difficult to cure [93, 94]. The phenotypic switch from a planktonic (free-swimming) lifestyle to a sessile existence in a biofilm is a highly regulated developmental process that depends on numerous environmental and genetic factors including nutrient availability and presence of antimicrobial agents [94]. It has been shown that subinhibitory concentrations of aminoglycosides can trigger biofilm formation in *P. aeruginosa* and *Escherichia coli* [95]. The physical protection and the retardation of reaction-diffusion and advection processes within biofilms leads to the reduced penetration, transport and internalization of molecules, including antimicrobials and host pro-inflammatory cytokines, by the bacteria within biofilms [93, 96].

Certain species of bacteria employ a type of organised cell-cell communication known as quorum sensing; a process that involves the production, detection, and response to extra-cellular signalling molecules called autoinducers (AIs) [97]. The accumulation of AIs enables individual bacteria to sense when the minimal number (quorum) of bacteria has been achieved for a concerted response to be initiated, one such concerted response being biofilm formation [97, 98, 99].

A small proportion of bacteria within biofilms enter a state of dormancy and metabolic inactivity [100, 101]. These cells, referred to as persister cells, have been found to have increased tolerance to antimicrobials without evidence of genetic change, implying that the increased tolerance is phenotypic [102]. It was also shown in [102] that persister cells are non-growing since they had delays in regrowth in rich medium. In many cases these cells, in addition to those with genetic resistance, contribute to the failure of antibiotic treatments to eradicate infections [100, 101, 102]. Persister cells have also been linked to an increase in the risk of the emergence of antibiotic resistance during treatment [103].

While antibiotic treatment alone is often insufficient to eradicate biofilm infections, there are many existing strategies for combating biofilm infections that when applied alongside an antibiotic treatment, often result in an improved outcome [104]. It has been shown that foreign bodies significantly down-regulate phagocytosis and the bactericidal effects of leukocytes, and provide a surface on which biofilms may form [88, 104]. Therefore if foreign bodies, such as small pieces of rock, are detected, their removal is an important prerequisite treatment step for the successful eradication of biofilm infections. The removal and replacement of indwelling devices, such as catheters and joint prostheses, reduces the risk of infection complications [104, 105, 106]. Antibiotic treatments can be made more effective by ensuring treatment is early and aggressive [99, 107], or combined with anti-virulence treatments such as quorum sensing inhibition, biofilm dispersal and increasing the susceptibility of bacteria to innate immune clearance [104, 108, 109, 110].

2.6.2 Mathematical models of biofilms

Biofilms have been modelled using a wide variety of approaches [111] and here, we review a selection of them to provide a background into this area of research.

Biofilms can be modelled using continuum approaches. Wanner and Gujer [112] develop an analytical PDE model for a multi-species biofilm, using a continuum approach and observing conservation principles. They focus on changes in biofilm thickness and the spatial distribution of microbial species and substrates in the biofilm. Rittman and McCarty [113] develop a model for steady state biofilm kinetics, coupling the flux of substrate (nutrient) into a biofilm to the mass of the biofilm. Based on kinetic and energetic constraints, this model predicts that, for a single substrate, there exists a substrate concentration below which a steady state biofilm cannot exist. Many model-simplifying assumptions were made in order to yield a workable model that can be experimentally evaluated; however, experimental verification of the reasonableness of the assumptions was not given. Providing this would allow more faith to be put into the model as a representative of biofilm kinetics. Eberl et al. [114] present a spatio-temporal continuum model for the development of spatially heterogeneous biofilm structures, describing the relationship between nutrient availability and biofilm growth. The model is able to reliably and accurately predict experimentally observed biofilm structures. If the model was to be extended to include multiple species of bacteria and nutrients, the formation and reformation of biofilms as a consequence of an antibiotic therapy could be investigated. Duddu et al. [115] present a two-dimensional continuum model for biofilm growth, the model considering fluid flow around the biofilm surface. The advection-diffusion and reaction of substrate, the variable biofilm size and biofilm erosion are assumed to be due to the interfacial shear stress at the biofilm-fluid interface. The model shows that detachment criteria which are based on the shape of the biofilm structure only, without taking local flow effects into account, are not appropriate and that biofilm cohesion strengths are also observed to be lesser near the surface and an erosion loss is therefore possible without the tearing of the biofilm.

A multi-phase system consists of substances with different chemical properties but the same phase, for example a liquid-liquid system consisting of oil droplets in water. Anguige et al. [116] developed a multi-phase PDE model of a maturing *Pseudomonas aeruginosa* biofilm, allowing for quorum sensing-controlled biofilm generation. They also account for reduced nutrient availability deep inside the biofilm, due to limited oxygen availability. Simulations of the model agree qualitatively with many experimental observations. Model results demonstrate the importance of quorum sensing for robust *P. aeruginosa* biofilm development; anti-quorum sensing therapies are shown to reduce biofilm thickness and thereby increase susceptibility to dispersal with conventional antibiotics, and the immune system. Furthermore, the model predicts that all bacteria can be eradicated by a sufficiently strong topically applied antibiotic. The model is not validated by experimental data due to its unavailability; however, if data on the kinetics of anti-quorum sensing drugs in biofilms becomes available, this model forms the basis of a mathematical investigation into the optimisation of anti-quorum sensing strategies.

An alternative approach to modelling biofilms is to employ discrete agent-based models. This approach allows numbers of cells to be tracked, rather than concentrations and densities, and enables individual cells to exhibit behaviours based on their local environment. Ben-Jacob et al. [117] present an agent-based model for the movement and aggregation of bacteria in response to gradients in nutrient concentration, and the co-operative communication between bacteria via chemotactic feedback, particularly in adverse growth conditions. In this model, random walkers represent aggregates of bacteria, rather than individual cells. They show that their predictions are in agreement with experimental results for *Bacillus subtilis* and emphasize that complex biological behaviour can be elucidated by relatively simple generic modelling in conjunction with comparison to experimental observations. Kreft et al. [118] present an agent-based model in which individual bacteria are modelled as agents, with the goal of relating the properties of cells to those of macroscopic systems such as biofilms. A colony is defined as synchronised when all cells replicate at the same time. While this model accounts for synchronous colony growth, it does not account for the chemotaxis of, or communication between, bacteria (unlike [117]). The growth of a single *Escherichia coli* cell into a colony was studied by considering substrate uptake and cell metabolism, maintenance, division and death. They found that (1) variations in the maximal rate of substrate uptake completely desynchronise the colony, (2) spatial heterogeneity desynchronises population growth by subdividing the population into parts synchronously growing at different rates and (3) variations in the critical volume at which cell division occurs have negligible effect on synchrony. Kreft et al. further developed this model into a two-dimensional multi-species model of biofilms in [119]. The authors find that, due to the high heterogeneity of substrate concentrations in the biofilm, stochasticity in initial attachment events modifies biofilm shape, the growth of single cells into clones and therefore the growth of the less abundant species. The heterogeneity of substrate concentrations also results in random variations in the growth parameters of individual bacteria having negligible effect on the long-term growth of the bacteria. The authors emphasize the importance of spreading and stochasticity, in addition to reaction-diffusion, in the emergence of complexity in biofilm communities.

We combine continuum approaches with agent-based methods in the modelling of bacterial biofilms in our work; the density of a biofilm is modelled as continuous in space and is informed by the discrete positions of the contained bacteria. We then consider the density-dependent protection that a biofilm affords the contained bacteria against phagocytosis and antimicrobial substances.

2.7 The Gillespie algorithm

2.7.1 Overview

While deterministic models are useful for considering the mean behaviour of a system, and the relationships between the variables and rates of the system, stochastic modelling additionally allows for the dynamics of individual agents to be considered, potentially having significant implications on the long-term behaviour of the system. Here, we present a high-level summary of the Gillespie algorithm; an algorithm used to simulate chemical and biochemical systems stochastically [120]. We refer the reader to [121] for an in-depth introduction to the theory and practice of the algorithm. This algorithm is used in this thesis to control bacterial protein synthesis and replication stochastically and individually, giving biologically realistic variations in the numbers of bacteria and

bacterial proteins for a given parameter regime; a feature that is not possible with deterministic well-mixed models.

2.7.2 The algorithm

For chemical reactions of the form:



one molecule of A and one molecule of B react with rate constant r to form one molecule of C . The probability that (2.1) takes place in the infinitesimally small time interval $[t, t + dt)$ is equal to $A(t)B(t)r dt$. Defining the propensity function of (2.1) as $\alpha(t) = A(t)B(t)r$, the probability that (2.1) occurs in the time interval $[t, t + dt)$ can be written as $\alpha(t)dt$. In general, the propensity function can be defined for any chemical reaction so that its product with dt gives the probability that the given reaction occurs in the time interval $[t, t + dt)$. If (2.1) occurs, we apply:

$$A(t + dt) = A(t) - 1, \quad B(t + dt) = B(t) - 1, \quad C(t + dt) = C(t) + 1.$$

In Algorithm 1, we demonstrate how the Gillespie algorithm is used to simulate the following:



We remark that while the Gillespie algorithm generates statistically correct solutions, it can be computationally expensive, and therefore slow, for large cell numbers. Approximations to the algorithm that are more efficient have been designed, for example by implementing a variable time step (tau-leaping) or time-dependent reaction rates [122, 123].

The Gillespie algorithm is widely used in the practical simulation of biochemical systems. Clote and Bayegan [124] employed the algorithm to approximate the folding kinetics of large RNA sequences. Taherkhani and Ranjbar [125] used the algorithm to simulate the oxidation of ethanol by horse liver enzymes. In modelling anaerobic digestion, Palanichamy and Palani [126] use the algorithm to predict the concentrations of glucose, acids and methane at different time intervals. Finally, von Wegner and Fink [127] simulate the local dynamics of the intracellular calcium concentration in the vicinity of a specific type of calcium channel using Gillespie's algorithm with diffusion.

2.8 Mathematical models of chemotaxis

Chemotaxis is the movement of cells in response to a chemical stimulus. The general spatio-temporal continuum model for chemotaxis has the form:

Algorithm 1 Algorithm for the Gillespie algorithm

1. Generate two random numbers R_1, R_2 uniformly distributed in $(0, 1)$.
2. Compute the propensity function of each reaction by $\alpha_1 = A(t)(A(t) - 1)r_1$ and $\alpha_2 = A(t)B(t)r_2$. Compute $\alpha^* = \alpha_1 + \alpha_2$.
3. Compute the time at which the next chemical reaction takes place as $t + \tau$ where:

$$\tau = \frac{1}{\alpha^*} \ln \frac{1}{R_1}. \quad (2.3)$$

4. Compute the number of molecules at time $t + \tau$ by

$$A(t + \tau) = \begin{cases} A(t) - 2, & \text{if } 0 \leq R_2 < (\alpha_1/\alpha^*), \\ A(t) - 1, & \text{if } (\alpha_1/\alpha^*) \leq R_2 < 1, \end{cases} \quad (2.4)$$

$$B(t + \tau) = \begin{cases} B(t), & \text{if } 0 \leq R_2 < (\alpha_1/\alpha^*), \\ B(t) - 1, & \text{if } (\alpha_1/\alpha^*) \leq R_2 < 1, \end{cases} \quad (2.5)$$

$$C(t + \tau) = \begin{cases} C(t) + 1, & \text{if } 0 \leq R_2 < (\alpha_1/\alpha^*), \\ C(t), & \text{if } (\alpha_1/\alpha^*) \leq R_2 < 1, \end{cases} \quad (2.6)$$

$$D(t + \tau) = \begin{cases} D(t), & \text{if } 0 \leq R_2 < (\alpha_1/\alpha^*), \\ D(t) + 1, & \text{if } (\alpha_1/\alpha^*) \leq R_2 < 1, \end{cases} \quad (2.7)$$

5. Repeat algorithm at time $t + \tau$.

$$\frac{\partial b}{\partial t} = \nabla \cdot \left(\mu_b(s) \nabla b \right) - \nabla \cdot \left(\chi(s) b \nabla s \right) + f(b, s), \quad (2.8a)$$

$$\frac{\partial s}{\partial t} = \nabla \cdot \left(\mu_s(s) \nabla s \right) + g(b, s), \quad (2.8b)$$

where b and s are the concentrations of the chemoattracted cells and the chemoattractant respectively, $\mu_b(s)$ and $\mu_s(s)$ are the diffusion parameters for the cells and the chemoattractant respectively, $\chi(s)$ is the chemotactic parameter and $f(b, s)$ and $g(b, s)$ are general reaction terms. There are many models in the literature, both continuous and agent-based, that employ the chemotactic attraction of cells. An extensive review of such models is provided by Tindall et al. [128]. We now present a selection of models from the literature, some of which are special cases of (2.8), as a background into the mathematical modelling of chemotaxis. In this thesis, we implement (2.8) and assume that μ_b , μ_s and χ are constants for simplicity.

Keller and Segel [129] employ (2.8) with constant diffusivities to investigate the chemotactic movement of slime moulds in response to chemical signals that stimulate aggregation (acrasins). Keller and Segel [130] then developed a phenomenological theory to explain experimentally observed travelling bands of chemotactic bacteria. In this paper, Keller and Segel model the chemotaxis of bacteria by assuming $\chi(s) = \delta(s - s_T)^{-1}$, where δ is a constant and s_T is the attractant con-

centration at which chemotaxis is activated. Equation (2.8) with this form of χ is known as the generalised Keller-Segel model. With this form of χ , the experimentally observed travelling bands of chemotactic bacteria were reproduced, the model providing an adequate description of *E. coli* migration in response to the amino acid serine [131]. However, although there is qualitative agreement between their theory and existing experimental data, the theory was based on many chemotactic assumptions, due to the lack of additional relevant experimental data; assumptions that cannot be considered justified by such agreement.

Tindall et al. [128] highlight that many subsequent continuum models, particularly early models of bacterial populations, use the Keller-Segel model as a foundation. For example, in [132], Lapidus and Schiller studied the bacterial response to various steady-state attractant gradients. They altered the chemotactic coefficient, giving it the form:

$$\chi(s) = \frac{\chi K_d}{(K_d + s)^2},$$

where K_d is the receptor-ligand binding dissociation constant. This alteration allowed them to determine values for χ and K_d and obtain model results that are in agreement with experimental results regarding the populations of motile bacteria, in particular *Salmonella typhimurium*, in fixed attractant gradients.

Hillen and Painter [133] discuss and compare ten versions of (2.8), each with a unique set of the functional forms for diffusion, chemotaxis and production, demonstrating the wide array of specific chemotaxis dynamics that can be modelled mathematically. A minimal model is presented, given by:

$$\begin{aligned} \frac{\partial b}{\partial t} &= \nabla(D\nabla b - \chi b \nabla s), \\ \frac{\partial s}{\partial t} &= \nabla^2 s + b - s, \end{aligned}$$

in which solutions exist globally only when the initial mass of b lies below a critical threshold. If the initial mass is above this threshold, solutions blow up in finite time, demonstrating the need for model modifications if global stability is required. The authors then explore adapted models in which solutions exist globally, the movement of b being dependent on mechanistic factors such as density and signal-receptor binding.

Byrne and Owen [134] present a new interpretation of the Keller-Segel model based on multiphase modelling. They describe how a population of cells moves through a fluid containing a diffusible chemical to which the cells are attracted, with the cells and fluid viewed as distinct components of a two-phase mixture. The authors specialise their model to describe the movement of slime mould cells and how the functional form of the chemical potential that drives cell locomotion influences the ability of the system to generate spatial patterns. They showed that for certain choices of the chemical potential, spatial patterning can only occur if the initial cell density lies within a well-defined

range; a finding that agrees with experimental results. Domain growth and cell proliferation have not been considered in the model, and the authors acknowledge that considering these mechanisms may yield variations in spatial patterning, such as new peaks in cell population.

McLennan et al. [135] present a model for the collective migration of neural crest cells in response to chemotactic regulatory signals. Cells undergo directed movement if they sense a sufficiently strong chemotactic gradient for a sufficient period of time and switch state to adopt leader behaviour. If leader cells sense an absence of chemotactic gradient for a sufficient period of time, then they switch back to adopting follower behaviour. A follower cell that is sufficiently close to a leader cell moves in the same direction as the leader cell, allowing the collective migration of cells in response to a chemotactic gradient.

In our agent-based models, leukocytes are modelled as cells with a defined area and surface. To model their chemotaxis, we develop a modified version of (2.8) in which ∇s is approximated across the surfaces of the leukocytes. Furthermore, the model for chemotaxis developed by McLennan et al. [135] is incorporated in our agent-based models to give pro-inflammatory mediator concentration-dependent activation of leukocyte chemotaxis.

2.9 Volume exclusion

Many individual cell-based models employ volume exclusion algorithms to ensure that cells do not overlap, making the models more physiologically realistic and forcing the expansion of aggregates that are growing. We provide here a selection of the methods used to implement volume exclusion as a background into this area of research and, where applicable, discuss how the methods inspire volume exclusion in this thesis.

The Lennard-Jones (L-J) potential, given by (2.9), is a simple mathematical model that approximates the interaction between a pair of molecules or cells [136, 137] (see Figure 2.10). The cells attract each other due to Van der Waals forces, the attraction growing stronger as the cells move closer until some critical distance is reached. Within this distance, the cells repel each other as a result of overlapping electron orbitals. The L-J potential is singular, thus ensuring that the cells cannot occupy mutual volume. Despite the functional form of the repulsive term in (2.9) having no theoretical justification, the L-J potential provides a relatively good approximation to inter-molecular dynamics, particularly for noble gas atoms [138, 139].

It is argued that r^{-12} is a poor representation of the repulsion due to overlapping electron orbitals, and that the exponential form $\exp(-r/\sigma)$ is more accurate; the exponential form is more faithful to the exponential decay of atomic wavefunctions at large distances, and hence to the overlap that is responsible for repulsion [140]. The potential with this exponential term fitted is known as the Buckingham potential (given by (2.10)) [140, 141]. Because the exponential term converges to a constant as the distance between the cells tends to zero while the r^{-6} term diverges, the Buckingham potential becomes attractive at very short distances, which may be problematic when dealing with a structure with very short interatomic distances. Figure 2.10 shows the L-J potential and

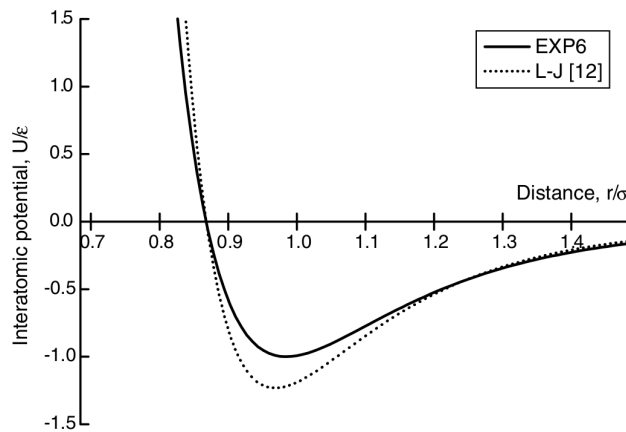


Figure 2.10: The L-J potential and the Buckingham potential plotted on the same axes for comparison (taken from [142]).

the Buckingham potential plotted on the same axes for comparison. We base the volume exclusion of bacteria in this thesis on the shapes of the L-J and Buckingham potentials; however, we use a modified version in which the potential is always defined and the range of attraction is finite for computational simplicity.

$$V_{LJ} = 4\epsilon \left[\left(\frac{\sigma}{r} \right)^{12} - \left(\frac{\sigma}{r} \right)^6 \right], \quad (2.9)$$

$$V_B = 4\epsilon \left[\exp \left(-\frac{r}{\sigma} \right) - \left(\frac{\sigma}{r} \right)^6 \right]. \quad (2.10)$$

Despite being less accurate, the L-J potential is still used extensively in computer simulations due to its computational simplicity. L-J potentials are used in [143] to implement volume exclusion in crowded biological environments, in [144] to describe the interactions between a pair of colloidal particles in a thermal bath of depletants, in [145] in an *in silico* investigation of depletion effects on the structure of and interactions between polymers and in [142] to simulate the molecular dynamics of water in contact with an iron pyrite surface.

An alternative and simple method to enforce volume exclusion is employed by McLennan et al. [135]. In this paper, the volume exclusion of collectively migrating neural crest cells in response to chemotactic regulatory signals is implemented by (1) aborting the attempted movement of a cell that would lead to overlap with another cell or a domain boundary and (2) insisting that there is free space before a new particle can be inserted into the domain. Michalski and Loew [146] model the bonds between particles as stiff springs, the net force on a particle due to its bonds with other particles given by:

$$F = \sum_{\substack{i=1 \\ i \neq j}} k_i (r_i - r_{0,i}) \hat{\mathbf{r}}_i,$$

where the sum runs over all bonds, k_i is a spring constant, $\hat{\mathbf{r}}_i$ is the unit vector pointing from the particle to the neighbour with which it shares a bond, r_i is the distance between the particles and $r_{0,i}$ is the equilibrium bond distance. In our work, we chose to use the L-J/Buckingham potential approach rather than the stiff springs method, due to the potentials being simpler to implement and sufficiently accurate.

2.10 Summary

Motivated by the need to study the infection strategy of EPEC in more detail, we have presented in this chapter a review of the biological and mathematical literature and the recent experimental findings relevant to an EPEC-specific infection. The mathematical literature provides tested and approved models and modelling approaches that we use as foundations in the development of our specific models. Incorporating the biological literature in our models ensures they are physiologically realistic.

There is, to our current knowledge, no mathematical models specific to EPEC infections in the literature. Freter et al [147] mathematically model the role of adhesion in colonization of the large intestine by *E. coli*; however, they do not consider the EPEC pathotype specifically or the interaction between *E. coli* and the immune response. We therefore develop novel mathematical models describing an EPEC-specific infection, based on the mathematical and biological literature and the recent experimental findings discussed in this chapter. We account for the mechanisms of the innate immune response, bacterial protein synthesis and replication, effector protein secretion and action, antibiotic administration and action and host pro-inflammatory cytokine manipulation.

Chapter 3

A mathematical model of bacteria-immunity interaction

3.1 Overview

In this chapter we develop and analyse a model aimed at understanding the relationship between a general pathogenic challenge and the resulting immune response from the host. We also consider treatment methods that can lead to the eradication of the infection. The model consists of a system of ODEs (ordinary differential equations) that governs the temporal dynamics of a general pathogenic challenge and the immune response at the cell level. We first formulate the model, accounting for the biological mechanisms described in the literature (see Chapter 2). We investigate this model both analytically and numerically, in order to make physiological statements and predictions regarding the feasibility and efficacy of various possible anti-bacterial treatment strategies and then summarize our findings.

3.2 Model formulation

The formulation of this model for a general bacterial infection is based on the literature discussed in Chapter 2 and on current experimental work being undertaken at the Centre for Biomolecular Sciences at the University of Nottingham. The aim of this model is to identify clinical treatment strategies that will be effective at eradicating or ameliorating infections.

For this model, we focus on 4 variables; the density of bacteria (B), the concentration of pro-inflammatory mediators (P), the concentration of anti-inflammatory mediators (A) and the density of leukocytes (L). Bacteria grow exponentially, with growth slowing down as space and resources run out. There is low level background production of pro-inflammatory mediators along with more prominent stimulated production when the host senses the presence of invaders such as bacteria. Host cells, including leukocytes, are stimulated by the presence of these bacteria to produce pro-inflammatory mediators and this inflammatory response triggers a leukocyte response which aims to eradicate the bacteria via phagocytosis. Anti-inflammatory mediators, whose production is stimu-

lated in the presence of pro-inflammatory mediators, reduce the production rate of pro-inflammatory mediators therefore preventing the environment from becoming too inflamed. We assume that all populations degrade at independent, constant rates. Finally, as the model is population-level, we can construct an ODE representation of the dynamics. Figure 3.1 shows a schematic diagram of the model.

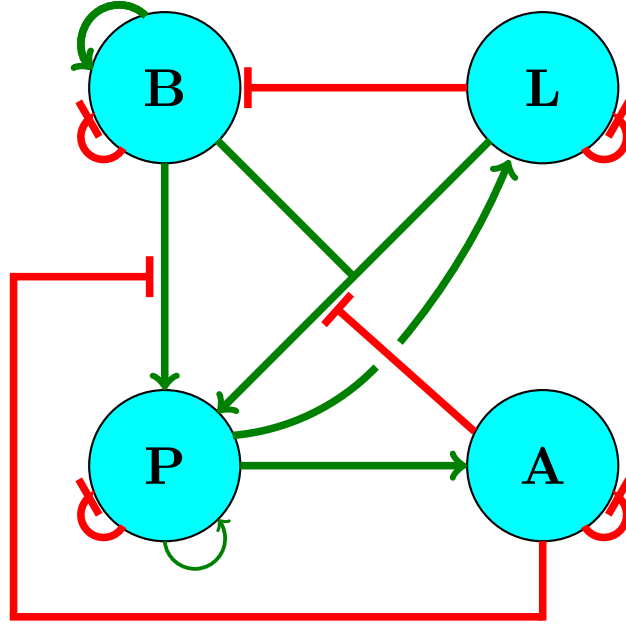


Figure 3.1: A schematic diagram of the model for a general bacterial infection. Green and red arrows indicate up-regulation and down-regulation respectively.

The density of bacteria evolves according to:

$$\frac{dB}{dt} = R_B \frac{B}{x_B + B} - P_B BL - D_B B, \quad (3.1)$$

where the three terms represent growth, phagocytosis and degradation respectively. We model the growth of bacteria using an increasing Hill function with rate parameter R_B and half-maximal parameter x_B . A Hill coefficient of 1 is chosen to ensure that in the absence of any competition, the bacterial population reaches a non-trivial steady state. Hill coefficients greater than 1 yield a stable trivial steady state, and non-integer values are not considered for simplicity. Phagocytosis occurs at a rate that is proportional to the number of encounters between bacteria and leukocytes, therefore the phagocytosis term is proportional to both B and L and has rate coefficient P_B . D_B represents the rate of (linear) bacterial death.

The concentration of pro-inflammatory mediators and anti-inflammatory mediators evolve according to:

$$\frac{dP}{dt} = \left(\Phi + C_B(1 + VL)B \right) \left(\frac{x_A^2}{x_A^2 + A^2} \right) - D_P P, \quad (3.2)$$

$$\frac{dA}{dt} = R_A \left(\frac{P^2}{x_P^2 + P^2} \right) - D_A A, \quad (3.3)$$

where the dynamics of the inflammatory mediators have been based on the activator/inhibitor model developed in [11]. Φ denotes the constant low level background production rate of pro-inflammatory mediators and C_B denotes the rate parameter for the stimulated inflammatory response. The production of pro-inflammatory mediators by host cells that are not leukocytes is proportional to B , while the production by leukocytes is proportional to both B and L . The rate parameter for the stimulated production by leukocytes is defined as $C_B V$, where V is a measure of the relative strength of the production by leukocytes to that by non-phagocytic cells. This way, C_B acts as a parameter that governs the general strength of stimulated pro-inflammatory mediator production by the host immune response. The production of A is stimulated in the presence of P , the rate of which we model using an increasing Hill function in P with rate parameter R_A , half-maximal parameter x_P and Hill coefficient 2, so that A production displays switch-like behaviour around $P = x_P$. All P production terms are then multiplied by an inhibition term involving A . This term is chosen to be a decreasing Hill function with half-maximal parameter x_A and Hill coefficient 2, so that the inhibitory action of A displays switch-like behaviour around $A = x_A$. D_P and D_A represent the rates of (linear) pro- and anti-inflammatory mediator degradation respectively.

The density of leukocytes evolves according to:

$$\frac{dL}{dt} = R_L P - D_L L. \quad (3.4)$$

The first term represents the recruitment of leukocytes by the inflammatory response, the rate of recruitment proportional to P , with rate parameter R_L . The second term represents natural (linear) death, with rate D_L .

The system (3.1)–(3.4) governs the dynamics between a bacterial infection and the immune response. Non-dimensionalising via the scalings $B = x_B b$, $P = x_P p$, $A = x_A a$, $L = \frac{D_B}{P_B} l$ and $t = \frac{\tau}{D_B}$, we obtain the following non-dimensional system of ODEs:

$$\frac{db}{d\tau} = r_b \frac{b}{1+b} - lb - b, \quad (3.5a)$$

$$\frac{dp}{d\tau} = \left[\phi + c_b(1 + vl)b \right] \left(\frac{1}{1+a^2} \right) - d_p p, \quad (3.5b)$$

$$\frac{da}{d\tau} = r_a \frac{p^2}{1+p^2} - d_a a, \quad (3.5c)$$

$$\frac{dl}{d\tau} = r_l p - d_l l, \quad (3.5d)$$

where the dimensionless parameters in (3.5) are given by:

$$r_b = \frac{R_B}{D_B x_B}, \quad \phi = \frac{\Phi}{D_B x_P}, \quad c_b = \frac{x_B C_B}{x_P D_B}, \quad v = \frac{V D_B}{P_B}, \quad d_p = \frac{D_P}{D_B},$$

$$r_a = \frac{R_A}{x_A D_B}, \quad d_a = \frac{D_A}{D_B}, \quad r_l = \frac{R_L x_P P_B}{D_B^2}, \quad d_l = \frac{D_L}{D_B}.$$

Table 3.1 gives a physiological interpretation of these parameters. In the following sections, we provide an exploration of (3.5) by considering biologically realistic steady states, bifurcation analysis, a parameter sensitivity study and numerical simulations. Together, these analyses will aid in illuminating whether or not particular clinical treatments will be effective.

r_b	The growth rate of bacteria.
ϕ	The strength of background production of pro-inflammatory mediators.
c_b	The strength of stimulated production of pro-inflammatory mediators.
v	The ratio of strength of production of pro-inflammatory mediators by leukocytes to that by non-phagocytic host cells.
d_p	The natural degradation rate of pro-inflammatory mediators, relative to the bacterial death rate.
r_a	The production rate of anti-inflammatory mediators.
d_a	The natural degradation rate of anti-inflammatory mediators, relative to the bacterial death rate.
r_l	The recruitment rate of leukocytes to the site of infection.
d_l	The natural death rate of leukocytes, relative to the bacterial death rate.

Table 3.1: Descriptions of the physical interpretation of each parameter for system (3.5).

3.3 Steady state and stability analyses

We now wish to explore the dynamics of system (3.5). We begin by calculating, where possible, the values of the model variables at any physically plausible steady states. In the context of this model, physical plausibility requires that all variables be non-negative. We determine their stability via linear stability analysis, allowing us to explore the effectiveness of particular treatments at fully eradicating infections.

3.3.1 Steady state analysis

The steady states of equations (3.5c) and (3.5d) are given by:

$$\bar{l} = \frac{r_l}{d_l} \bar{p}, \quad \bar{a} = \frac{r_a}{d_a} \frac{\bar{p}^2}{1 + \bar{p}^2}. \quad (3.6)$$

The steady states of equation (3.5a) are given by:

$$0 = r_b \frac{\bar{b}}{1 + \bar{b}} - \bar{l}\bar{b} - \bar{b}. \quad (3.7)$$

From (3.7), it can be shown that either:

$$\bar{b} = 0, \quad \text{or} \quad \bar{b} = \left(\frac{r_b}{\frac{r_l}{d_l}\bar{p} + 1} \right) - 1 > 0. \quad (3.8)$$

In the disease-free state ($\bar{b} = 0$), the steady states of equation (3.5b) are given by:

$$f_1(\bar{p}) = \bar{p} \left(1 + \left(\frac{r_a}{d_a} \frac{\bar{p}^2}{1 + \bar{p}^2} \right)^2 \right) - \frac{\phi}{d_p} = 0, \quad (3.9)$$

and in a diseased state ($\bar{b} > 0$):

$$f_2(\bar{p}) = \frac{\phi}{d_p} + \frac{c_b}{d_p} \left(1 + \frac{vr_l}{d_l} \bar{p} \right) \left(\frac{(r_b - 1) - \frac{r_l}{d_l} \bar{p}}{\frac{r_l}{d_l} \bar{p} + 1} \right) - \bar{p} \left(1 + \left(\frac{r_a}{d_a} \right)^2 \frac{\bar{p}^4}{(1 + \bar{p}^2)^2} \right) = 0. \quad (3.10)$$

Figure 3.2 shows example plots of $f_1(\bar{p})$ and $f_2(\bar{p})$ as the parameters present in $f_1(\bar{p})$ and $f_2(\bar{p})$ are varied in magnitude, the steady state values for p located where the graphs cross the x -axis. It can be seen that in all cases shown, over multiple orders of magnitude, the function either increases or decreases monotonically for any given parameter set and passes through the positive x -axis exactly once, meaning that there are always two biologically realistic steady states; one disease-free state and one diseased state. From the second equation of (3.8), it can be seen that $r_b > 1$ is required for the disease state to have a positive value, since all parameter values are positive¹. In Appendix A.1, we show algebraically that there is exactly one solution to (3.10) with $\bar{p} > 0$.

¹This is not the strictest condition attainable, but it turns out to be useful later.

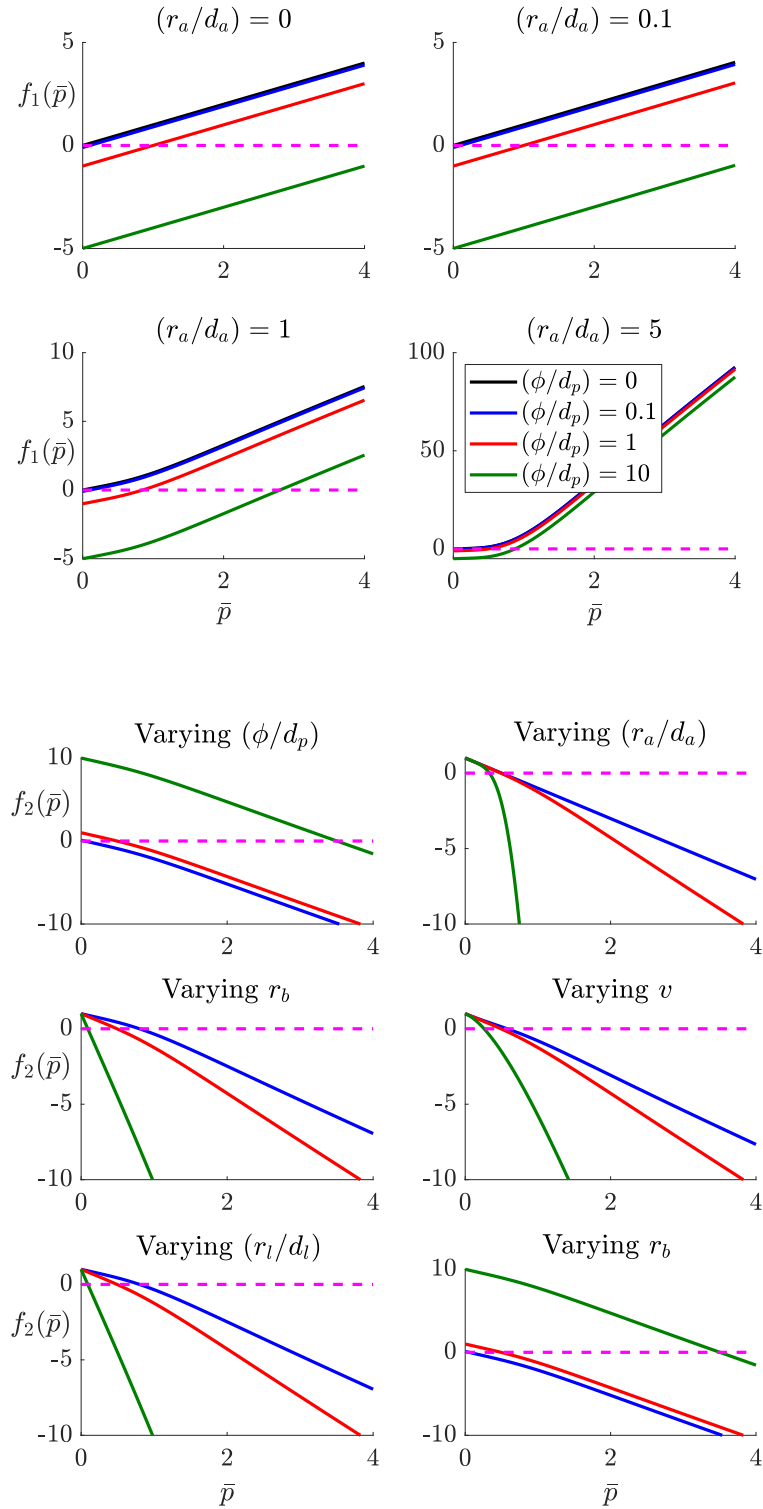


Figure 3.2: Plots of $f_1(\bar{p})$ and $f_2(\bar{p})$, given by (3.9) and (3.10) respectively, as the parameters present in $f_1(\bar{p})$ and $f_2(\bar{p})$ are varied in magnitude. For all graphs, the steady state value for p is located where the graph crosses the x -axis. In the plots of $f_2(\bar{p})$, blue, red and green lines represent $f_2(\bar{p})$ when the parameter combination given in the plot title equals 0.1, 1 and 10 respectively. All other parameter combinations take a value of 1.

Special case: $\phi \ll 1$

It has been shown that pro-inflammatory mediators are detected at higher levels in pathogenically challenged blood [14]. Therefore, we expect for a host with a healthy immune response that the background production of pro-inflammatory mediators is smaller in magnitude than the stimulated production during an infection. The simplest way to incorporate this in the model is to let the background production rate be much smaller than the stimulated production rate — i.e. $\phi \ll c_b$ and $\phi \ll v$. Since we assume that all parameters are $\mathcal{O}(1)$ unless otherwise stated, we must take $\phi \ll 1$. This assumption allows further analytical investigation of the model.

By considering the black and blue lines in all the plots of $f_1(\bar{p})$ (the top four panels in Figure 3.2), we see that, when $\phi \ll 1$, the value for p in the disease-free state is much less than 1 provided that $d_p \sim 1$ and (r_a/d_a) is sufficiently small. By incorporating the condition $0 < \bar{p} \ll 1$ for the p steady state into (3.9), it can be shown that:

$$\bar{p} \approx \frac{\phi}{d_p} \quad (3.11)$$

in the disease-free state. Substituting (3.11) into (3.6) gives:

$$\bar{l} \approx \frac{\phi r_l}{d_p d_l}, \quad \bar{a} \approx \frac{r_a}{d_a} \frac{(\phi/d_p)^2}{1 + (\phi/d_p)^2}. \quad (3.12)$$

Therefore, under the biologically realistic assumption that $\phi \ll 1$ while all other parameter values are $\mathcal{O}(1)$, the two steady states are given by:

$$\text{Disease-free state } (\bar{b}, \bar{p}, \bar{a}, \bar{l}) \approx \left(0, \frac{\phi}{d_p}, \frac{r_a}{d_a} \frac{(\phi/d_p)^2}{1 + (\phi/d_p)^2}, \frac{r_l \phi}{d_l d_p} \right), \quad (3.13)$$

$$\text{Diseased state } (\bar{b}, \bar{p}, \bar{a}, \bar{l}) = \left(\frac{r_b}{(r_l/d_l)\bar{p} + 1} - 1, \bar{p}, \frac{r_a}{d_a} \frac{\bar{p}^2}{1 + \bar{p}^2}, \frac{r_l}{d_l} \bar{p} \right), \quad (3.14)$$

where $\bar{p}$ given by the solution to (3.10). Finally, it is emphasized that the presence of only two biologically relevant steady states implies that there does not exist any bistability in the model. In the next subsection, we explore the stability of steady states (3.13) and (3.14).

3.3.2 Stability analysis

The presence of only two biologically relevant steady states implies that there does not exist any bistability in the model. We are therefore interested in finding bifurcations in stability between these two steady states. By deriving a condition that governs when the disease-free state is stable, we can make predictions on which model parameters to alter in order for the infection to be eradicated.

System (3.5) can be written in the form $\dot{\mathbf{v}} = \mathbf{J}\mathbf{v}$, where the Jacobian $\mathbf{J}$ is given by:

$$\mathbf{J} = \begin{bmatrix} \frac{r_b}{(1+b)^2} - l - 1 & 0 & 0 & -b \\ \frac{c_b(1+vl)}{1+a^2} & -d_p & -\frac{2a}{(1+a^2)^2}[\phi + c_b(1+vl)b] & \frac{c_b vb}{1+a^2} \\ 0 & \frac{2p}{(1+p^2)^2} & -d_a & 0 \\ 0 & r_l & 0 & -d_l \end{bmatrix},$$

the eigenvalues of which determine the stability of the steady states. Consider first the disease-free state. The eigenvalues are determined from:

$$(r_b - 1 - \bar{l} - \lambda)(-d_l - \lambda) \left[\lambda^2 + (d_p + d_a)\lambda + d_p d_a + \frac{4\phi \bar{a} \bar{p}}{(1 + \bar{p}^2)^2(1 + \bar{a}^2)^2} \right] = 0,$$

giving:

$$\lambda_1 = r_b - 1 - \bar{l}, \quad \lambda_2 = -d_l, \quad 0 = \lambda_{3,4}^2 + B\lambda_{3,4} + C,$$

$$\text{where } B = (d_p + d_a), \quad C = d_p d_a + \frac{4\phi \bar{a} \bar{p}}{(1 + \bar{p}^2)^2(1 + \bar{a}^2)^2}.$$

Clearly $\lambda_2 < 0$ and since $B, C > 0$, $\lambda_{3,4}$ have negative real part. Lastly, for λ_1 to be negative, it is required that

$$r_b < 1 + \frac{\phi r_l}{d_p d_l}. \quad (3.15)$$

Therefore, the disease-free state is stable provided that condition (3.15) is met. Physiologically, this condition states that for the infection to be eradicated, the net growth rate of the bacteria $(r_b - 1)$ must be weaker than the basal net recruitment rate of leukocytes. It is clear that $r_b < 1$ guarantees stability of the disease-free state — i.e. if bacteria can not reproduce fast enough, then they will die out. The only bifurcation that can occur that ensures all variables are non-negative is a transcritical bifurcation, occurring when:

$$r_b = 1 + \frac{\phi r_l}{d_p d_l}, \quad (3.16)$$

from which a stable diseased steady state is created. This implies that the disease-free state and the diseased state can not be stable at the same time and that the diseased state is stable when condition (3.15) is not met. It is possible that limit cycles may be created further along the stable diseased state branch. Due to the complexity of the model, the presence of these will not be explored analytically; however, they will be considered in the numerical analysis (see section 3.4).

To summarize:

- The disease-free state is stable provided that $r_b - d_b < \phi r_l / d_l$, assuming that $\phi \ll 1$ and $r_b, d_p, r_l, d_l = \mathcal{O}(1)$.
- There is at least one feasible diseased state and it is stable when $r_b - d_b > \phi r_l / d_l$.

3.3.3 Steady state analysis summary

From the steady state analysis carried out, it can be seen that there are two feasible steady states, one which can be thought of as healthy (zero bacteria) and the other diseased (non-zero bacteria). Due to the absence of bistability, these states cannot coexist stably for any given parameter set. The disease-free state is stable provided that condition (3.15) is true with a bifurcation occurring for parameter values such that equation (3.16) holds leading to a switch in stability so that the diseased state is realized.

The five parameters that play a role in the stability of the disease-free state are: the growth rate of bacteria r_b , the background pro-inflammatory mediator production rate ϕ , the recruitment rate of leukocytes r_l , the degradation rates of pro-inflammatory mediators d_p and the death rate of leukocytes d_l . We assume that the background production of pro-inflammatory mediators is much smaller in magnitude than any stimulated production. If a treatment could alter one or more of these parameters sufficiently to take condition (3.15) from being false to true, then it could make a critical difference in the eradication of an infection. Example treatments include bacteriostatic antibiotics, which reduce the growth rate of bacteria, and a treatment that would increase the production rate or life span of leukocytes. Treatments that boost the immune response must be careful not to send the immune response into a state of overdrive, as this could lead to aseptic health problems such as leukemia or arthritis. In view of their significance highlighted in this section, these parameters will be investigated in more detail in the following sections.

3.4 Sensitivity and bifurcation analyses with numerical simulation

The complexity of the model precludes further analytic exploration, therefore we turn to numerical methods to further our understanding of the model dynamics. First, we carried out a steady state sensitivity analysis to identify parameters that should be explored in detail (in addition to those identified in section 3.3). We then carried out a bifurcation analysis to look for quantitative and qualitative changes in system behaviour when parameters are varied. The results from both of these explorations are compared with the analytic steady state and stability results presented in section 3.3. We carried out analyses in MATLAB and XPPAUT. Numerical simulations are used to explore the dynamics observed in the bifurcation analysis. Finally we draw biological interpretations from the results regarding the infection, the immune response and treatment strategies. Table 3.2 gives the baseline parameter set and the initial conditions that lead to the diseased state and all analysis uses these values unless otherwise stated. Given the absence of relevant experimental data to inform these values, we choose values (through trial and error) that yield qualitatively sensible solutions.

r_b	ϕ	c_b	v	d_p	r_a	d_a	r_l	d_l	$b(0)$	$p(0)$	$a(0)$	$l(0)$
2	0.01	0.25	1	0.3	1	0.3	0.6	0.5	0.01	0	0	0

Table 3.2: The baseline parameter values and the initial conditions used for the numerical simulations of system (3.5).

3.4.1 Steady state sensitivity analysis

In this subsection, we perform a steady state sensitivity analysis to identify the parameters that the infection size in the disease state is most sensitive to. We compute the (diseased) steady state bacterial density via equations (3.5) for the baseline parameter set (see Table 3.2), and denote it $\mathcal{B}_0$. We then undertake a one-at-a-time parameter sensitivity analysis and record the ratio of the new steady state bacterial density, denoted $\mathcal{B}_1$, to $\mathcal{B}_0$. The percentage changes in parameter values we first consider are -20% , -5% , $+5\%$ and $+20\%$ and the results of this analysis are shown in Figure 3.3. We find that the steady state bacterial density is most sensitive to r_b and is second most sensitive to r_l and c_b . Physiologically this means that altering the growth rate of the bacteria has the biggest impact on the size of the infection in the diseased steady state, with alterations to the production/recruitment of pro-inflammatory mediators/leukocytes respectively having the next biggest impact. The steady state bacterial density is much more sensitive to r_b than it is to any other parameter, implying that the growth rate of the bacteria would be the most effective target for a treatment. In view of (3.16), these results are intuitive: given $(\phi r_l / d_p d_l) \ll 1$, a much larger change in r_l is needed to give an effect of the same magnitude as an $\mathcal{O}(1)$ change in r_b . This sensitivity analysis reflects this (comparing the r_b and r_l rows of Figure 3.3).

In a similar manner, we considered a 90% decrease and a 200% increase in parameter values to see how the diseased state bacterial density responds to drastic changes in parameter values. The results for this are shown in Figure 3.4. We see that in both cases, the diseased state bacterial density is still most sensitive to r_b and second most sensitive to r_l and c_b . We also see that the diseased state bacterial density is reduced to 0 for a 90% decrease in r_b . We infer that this also happens at some lower percentage decrease in r_b , given the larger effect of decreasing r_l and c_b , relative to decreasing r_b , on the bacterial density when the parameters are decreased by 90%.

We then carried out a two-parameter sensitivity analysis in which each possible distinct pair of parameters is varied by $\pm 20\%$. We denote the new steady state bacterial density in this case as $\mathcal{B}_2$ and we record the ratio $(\mathcal{B}_2 / \mathcal{B}_0)$. The results for this can be seen in Figure 3.5. The pairs of parameters that give the biggest decrease in the bacterial population in the diseased state when decreased are (r_b, d_l) and (r_b, d_p) , while those that give the biggest decrease when increased are (c_b, r_l) and (v, r_l) . Stability analysis is in agreement with this finding; decreasing r_b , d_p and d_l and increasing ϕ and r_l in some combination results in the condition for the stability of the disease-free state (equation (3.15)) being further from bifurcation (see section 3.3.2). Increasing c_b and v in conjunction with r_l leads to a reduction in pathogen size due to the compounded positive effect on the immune response; a stronger inflammatory response and a faster recruitment rate of leukocytes lead to a large leukocyte population. It is interesting that altering v on its own makes negligible

difference but altering v in conjunction with r_l makes a noticeable difference, showing that synergies are present within the immune response. Together, these results suggest that a combined treatment that simultaneously boosts the immune response and hinders the infection will be an even more effective treatment.

Altering r_b, r_l and c_b gives the largest quantitative change in the infection size in the diseased steady state. We will therefore explore these in more detail in bifurcation analysis and numerical simulations. The parameters involved in equation (3.16) are also important as they are involved in a qualitative change in the bacterial steady state (a stability swap between the disease-free and the diseased states). However, in the stability condition (3.15) and equations (3.13) and (3.14), it is the ratios ϕ/d_p and r_l/d_l that are pivotal. Therefore, only one parameter from each of these ratios needs to be considered, the opposite of any results found being applicable for the respective unchosen parameter. The parameters ϕ and r_l are chosen as we assume that growth/production rates are easier to alter than death/degradation rates in medicinal practice. Therefore, the parameters r_b, r_l, c_b and ϕ are explored in more detail.

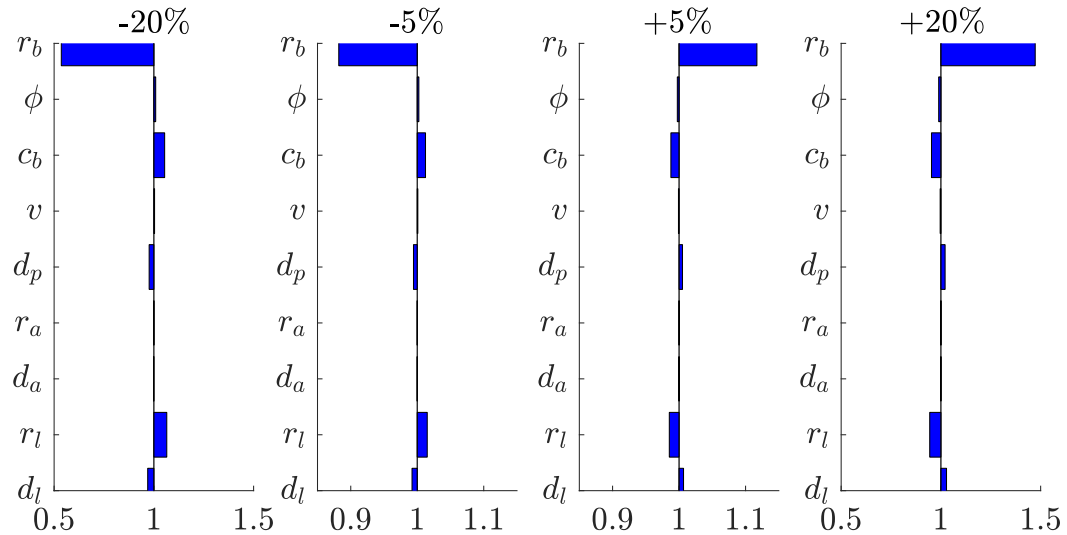


Figure 3.3: A one-at-a-time parameter sensitivity analysis of the bacterial density in the diseased steady state of system (3.5). We show the ratio $(\mathcal{B}_1/\mathcal{B}_0)$ for four different percentage changes in parameter value. Each parameter is changed by the amount given in the respective plot title and all unaltered parameters take their baseline values.

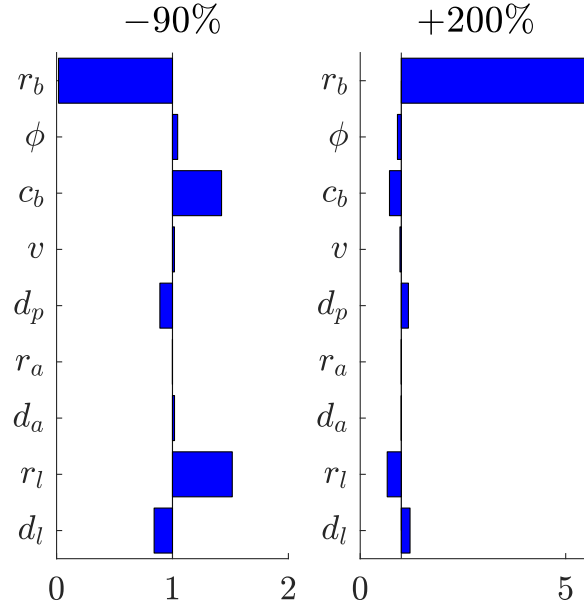


Figure 3.4: A one-at-a-time parameter sensitivity analysis of the bacterial density in the diseased steady state of system (3.5). We show the ratio $(\mathcal{B}_1/\mathcal{B}_0)$ for two different drastic percentage changes in parameter value. Each parameter is changed by the amount given in the respective plot title and all unaltered parameters take their baseline values.

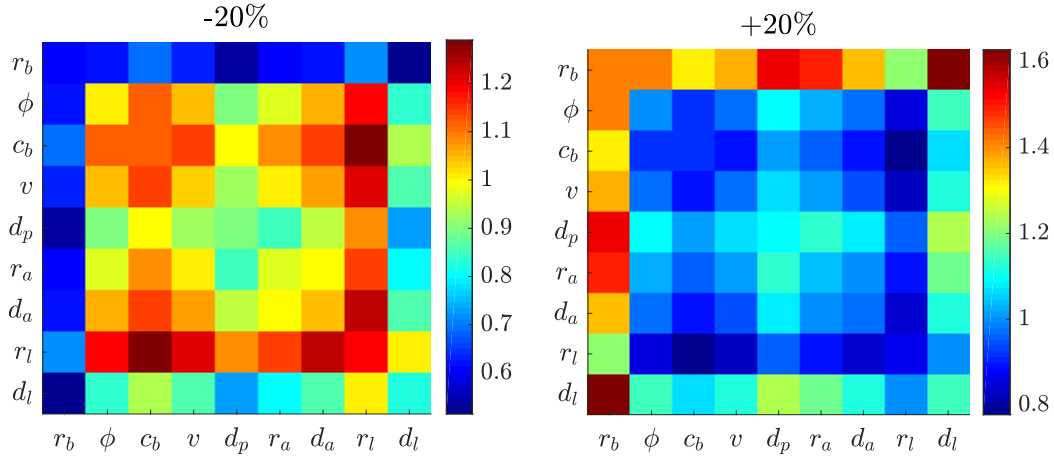


Figure 3.5: A two-at-a-time parameter sensitivity analysis of the bacterial density in the diseased steady state of system (3.5). We show the ratio $(\mathcal{B}_2/\mathcal{B}_0)$ when each possible distinct pair of parameters is changed from the respective baseline values by -20% (left) and $+20\%$ (right). All unaltered parameters take their baseline values. In the cases of repeated parameters, the data from the one-at-a-time parameter sensitivity analysis is used as a baseline reference (see Figure 3.3).

3.4.2 Bifurcation analysis

Bifurcation analysis was undertaken using XPPAUT [148]. The parameters r_b, r_l, c_b and ϕ are varied to look for both quantitative and qualitative changes in the bacterial population steady state, with the aim of giving predictions of potentially successful treatments. As can be shown by equation (3.16), when r_b is varied, there is a transcritical bifurcation at $r_b \approx 1.04$ (Figure 3.6(a)). For higher values of r_b the diseased state is stable and for lower values, for example due to being reduced by a bacteriostatic treatment, it is infeasible (negative). The bacterial population in the steady state then increases approximately linearly with increasing r_b for $r_b \gtrsim 1.04$. We then tracked the position of the bifurcation as a function of r_b and ϕ (Figure 3.6(b)). The position of the bifurcation increases monotonically with ϕ and r_b ; therefore, if bacteria grow faster given all other parameters remain constant, then a stronger background production of pro-inflammatory mediators is required to ensure eradication. For $\phi \ll 1$, the relationship between ϕ and r_b is linear, and the approximate equation for the bifurcation location (equation (3.16)) is accurate. Equation (3.16) becomes very inaccurate for $\phi \geq \mathcal{O}(1)$. We note here that values of $\phi \geq \mathcal{O}(1)$ are not considered in this work, as they represent aseptic autoimmune diseases such as chronic inflammation.

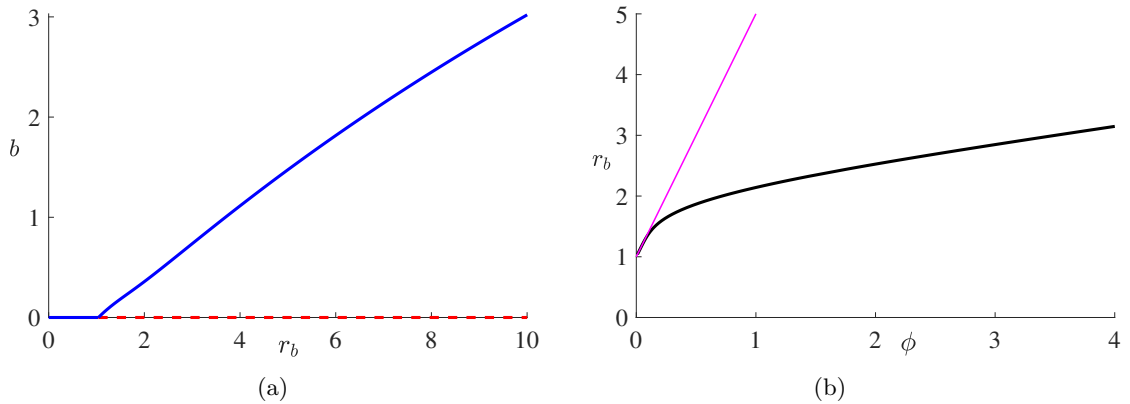


Figure 3.6: (a) Bifurcation diagram of b against r_b for system (3.5) for the baseline parameter set. Blue and red lines represent stable and unstable steady states respectively. (b) Two parameter bifurcation diagram tracking the position of the transcritical bifurcation shown in (a) as a function of r_b and ϕ (black line) and a plot of equation (3.16) as a function of r_b and ϕ (magenta line).

Figure 3.7 shows that when r_l is varied, there is a transcritical bifurcation at $r_l = 15$, as can be shown by equation (3.16). The diseased state is stable for $r_l < 15$ and infeasible for $r_l > 15$. The bacterial population in the diseased steady state is 1 in the absence of leukocytes ($r_l = 0$). The stable diseased state decreases quickly for increasing r_l and goes below 10% of its maximum by $r_l = 2.25$. This demonstrates that increasing the recruitment rate of leukocytes is a viable treatment option. This bifurcation was then tracked for varying r_b and r_l (Figure 3.7(b)). The relationship between r_b and r_l is approximately linear, as reflected by equation (3.16). These results imply that if an infection grows faster, then leukocytes will need to be recruited faster to eradicate the infection. Increasing the recruitment rate of leukocytes is therefore a viable treatment option, particularly when combined with a bacteriostatic treatment.

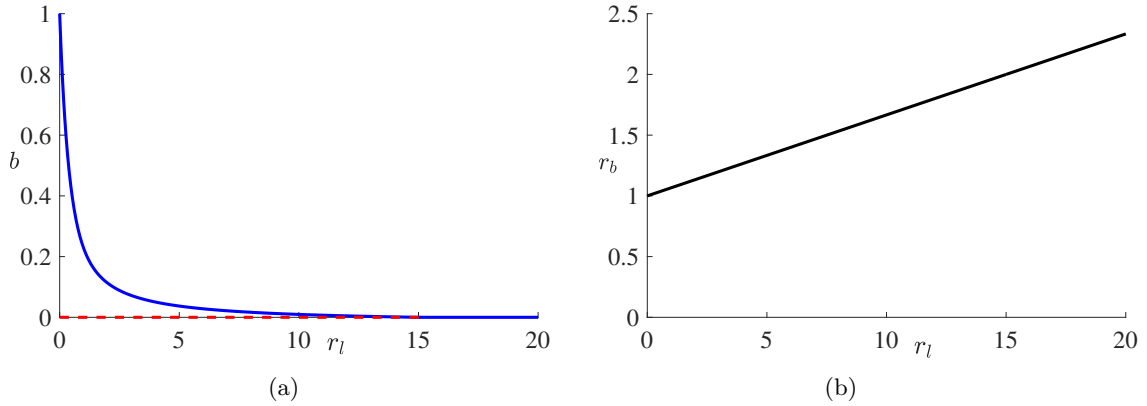


Figure 3.7: (a) Bifurcation diagram of b against r_l for system (3.5) for the baseline parameter set. Blue and red lines represent stable and unstable steady states respectively. (b) Two parameter bifurcation diagram tracking the position of the transcritical bifurcation shown in (a) as a function of r_b and r_l .

When ϕ is varied, there is a transcritical bifurcation at $\phi \approx 0.72$ (Figure 3.8(a)). The diseased state is stable for $\phi < 0.72$ and infeasible for $\phi > 0.72$. This suggests that raising the background levels of pro-inflammatory mediator production sufficiently could eliminate the infection, but this could be considered to be dangerous, as raising the background levels could lead to systemic inflammation and possibly aseptic death. With this in mind, treatments altering ϕ are not recommended. When c_b is varied, there are no qualitative changes in behaviour, which is also predicted by equation (3.16). However, as $c_b \rightarrow \infty$, $\bar{b} \rightarrow 0$ (Figure 3.8(b)). Therefore, for a sufficiently large stimulated production of pro-inflammatory mediators, the immune response is strong enough to reduce the infection to a negligible size, effectively eradicating it. This is an acceptable interpretation, as the continuous ODE representation of the infection breaks down for $b \approx 0$, due to the discreteness of the number of bacteria becoming significant. This viewpoint is adopted in the literature, for example in [149] while considering the dynamics of acute inflammation. Physiologically, if a treatment could temporarily boost the sensitivity of the inflammatory pathways to pathogen stimulus, then that treatment would be effective at reducing the infection size. If it were not temporary and if the pathogen were not completely eradicated, then it could lead to systemic inflammation and possibly septic death.

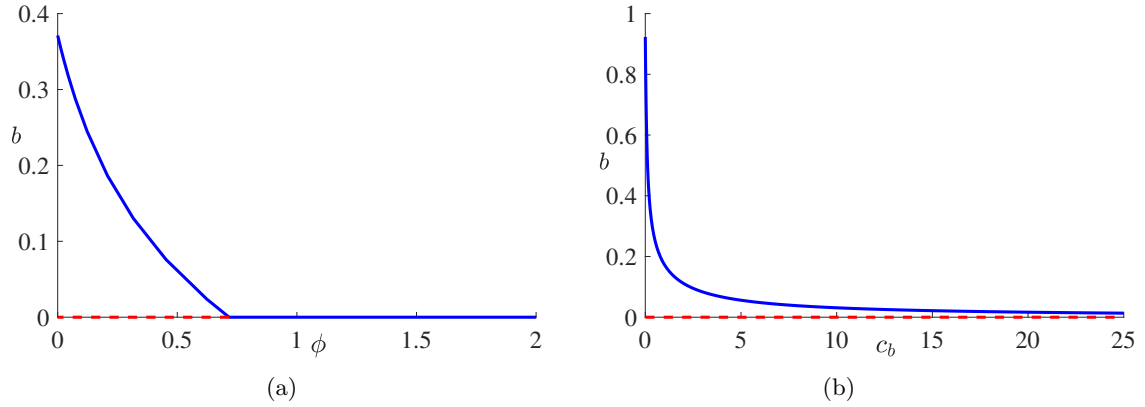


Figure 3.8: (a) Bifurcation diagram of b against ϕ for system (3.5) for the baseline parameter set. (b) Bifurcation diagram of b against c_b for system (3.5) for the baseline parameter set. Blue and red lines represent stable and unstable steady states respectively.

We then consider an alternative parameter set to look for different behaviours. Again, this regime is chosen through trial and error, given the absence of relevant experimental data. This parameter set has more rapid bacterial growth and a stronger immune response than the baseline parameter set, giving more aggressive dynamics at the site of infection. Table 3.3 gives the parameter values used, with those different to the baseline set given in blue. Figure 3.9(a) shows the bifurcation diagram of b for varying r_b . The behaviour here is qualitatively similar to that of the baseline parameter set; a transcritical bifurcation occurring at $r_b \approx 1.067$, as predicted by equation (3.16), and an approximately linear relationship between $\bar{b}$ and r_b for $r_b \gtrsim 1.067$. This again implies that reducing the bacterial growth rate (by a bacteriostatic treatment) is an effective strategy for eradicating the infection. Figure 3.9(b) shows the bifurcation diagram of b for varying r_l . In contrast to the behaviour of the baseline parameter set, there is a stable limit cycle rather than a stable steady state for $1.3 \leq r_l \leq 20.3$ (correct to 1 decimal place). However, the lower bound of this limit cycle is notably low; therefore, we choose to interpret the limit cycle as eradication since $b \approx 0$ for some $t > 0$.

r_b	ϕ	c_b	v	d_p	r_a	d_a	r_l	d_l	$b(0)$	$p(0)$	$a(0)$	$l(0)$
3	0.01	1	1	0.5	1	0.5	1	0.3	0.01	0	0	0

Table 3.3: An alternative parameter and initial conditions regime used for finding alternative system behaviours.

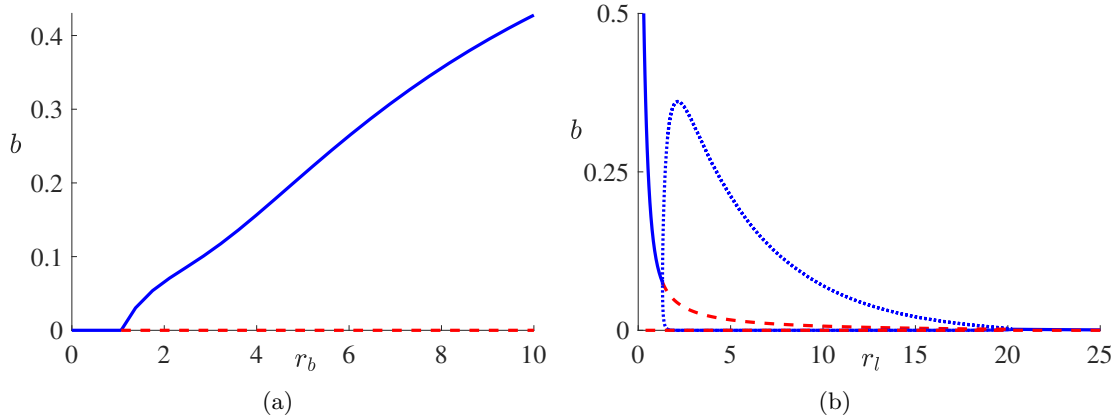


Figure 3.9: Bifurcation diagrams of b against (a) r_b and (b) r_l for system (3.5) for an alternative set of parameters. Solid blue, dotted blue and dashed red lines represent stable steady states, stable limit cycles and unstable steady states respectively.

The dynamics seen in this bifurcation analysis will be explored in more detail in the next section by looking at the corresponding numerical simulations.

3.4.3 Numerical simulations

We executed numerical simulations to observe temporal dynamics and to explore the results obtained in the bifurcation analysis in more detail. For each parameter considered in the bifurcation analysis, numerical simulations at three distinct parameter values are presented, the baseline parameter set explored initially. The trajectories for a are qualitatively similar to those for p , but with a very slight delay. Therefore, trajectories for a have been omitted for brevity.

Figures 3.10 (a)–(c) show simulations for varying r_b . For sufficiently low r_b (panel (a)), the infection is weak enough that it is eradicated, with the immune system variables not getting elevated. For sufficiently large r_b (panel (b)), the infection is not eradicated, since the diseased state is stable. The infection reaches an initial peak before being reduced by the immune system, eventually attaining a non-zero steady state. Heavily damped oscillations are present. The immune system variables remain elevated as they try to, but are unable to, clear the infection. For yet larger r_b (panel (c)), the behaviour is qualitatively similar, but with all variables attaining higher peaks and steady state values. These results yield the intuitive prediction that if a treatment were able to inhibit bacterial proliferation sufficiently, then it would be effective. Figures 3.10 (d)–(f) show time courses for varying r_l . In the absence of leukocytes ($r_l = 0$; panel (d)), the bacteria steadily reaches a steady state due to a lack of external competition. and the inflammatory variables remain elevated. For sufficiently large r_l (panel (f)), the leukocyte recruitment is strong enough to eradicate the infection. The inflammation variables return to low rest states, but the leukocyte population can remain elevated, possibly leading to conditions such as leukocytosis or leukemia. For intermediate r_l (panel (e)), the bacterial population is significantly reduced, but is not completely eradicated, and we regard this as asymptomatic colonisation. There are damped oscillations to this steady state, with the immune system variables remaining elevated. These results predict that if a

treatment strategy could temporarily boost the recruitment rate of leukocytes, it would be effective.

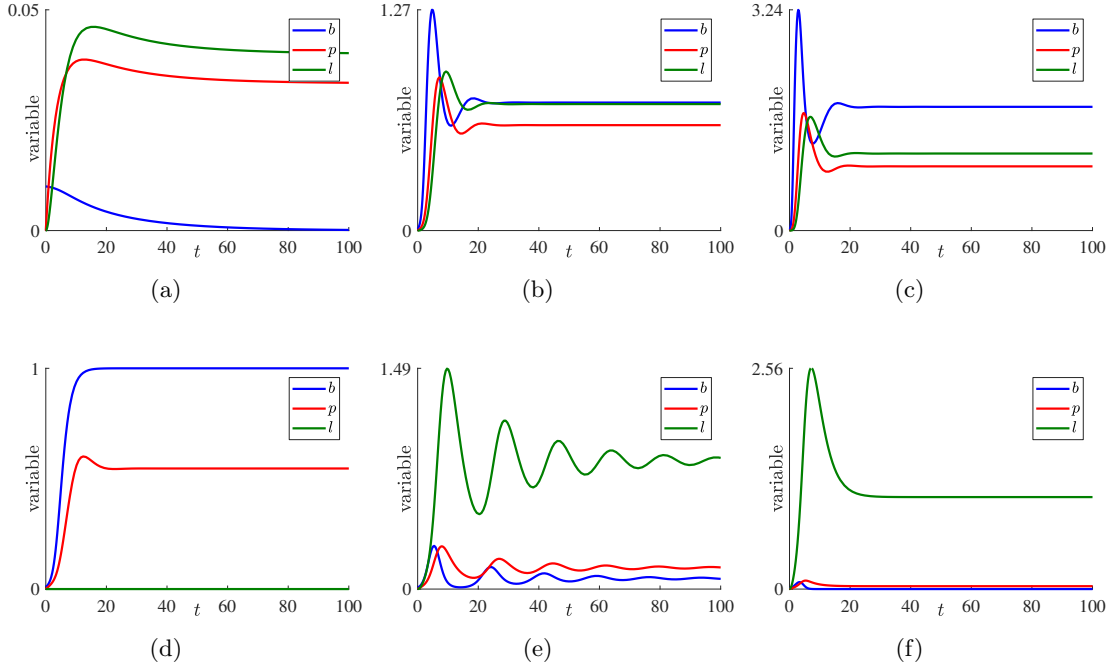


Figure 3.10: Numerical simulations of system (3.5) for (a)–(c) $r_b = 1, 3, 6$ respectively and (d)–(f) $r_l = 0, 3, 16$ respectively, with all other parameters at their baseline values. The variable a has been omitted to make the plots less cluttered.

Figures 3.11 (a)–(c) show time courses for varying c_b . As was found in the bifurcation analysis, it is impossible to attain $b = 0$ simply by altering c_b , but it is possible to attain $b \approx 0$. As discussed earlier, we interpret $b \approx 0$ as eradication, due to the continuous ODE representation of the infection breaking down for very low pathogen numbers. For the baseline c_b value (panel (a)), the resulting immune response is too weak to reduce the bacterial population and all variables remain elevated. As c_b is made larger (panels (b) and (c)), the bacterial population in the steady state is indeed reduced, but the immune variables become more elevated. There are decaying oscillations to the steady state, with the oscillations taking longer to decay for larger c_b . Together, these results predict that temporarily boosting the stimulated production of pro-inflammatory mediators could be an effective strategy. Figures 3.11 (d)–(f) show time courses for varying ϕ . When there is no background production of pro-inflammatory mediators ($\phi = 0$; panel (d)), the immune response is not strong enough to clear the infection. Then as ϕ is increased (panel (e)), the steady state bacterial population decreases while the steady states of the immune system variables all increase. For sufficiently large ϕ (panel (f)), the infection is eradicated, but with the immune system variables remaining elevated. In this case, the background production of pro-inflammatory mediators is of a similar magnitude to that of the stimulated production. While these results predict that temporarily increasing the background production of pro-inflammatory mediators is effective, we do not recommend this treatment style, due to the associated autoimmune health problems discussed earlier.

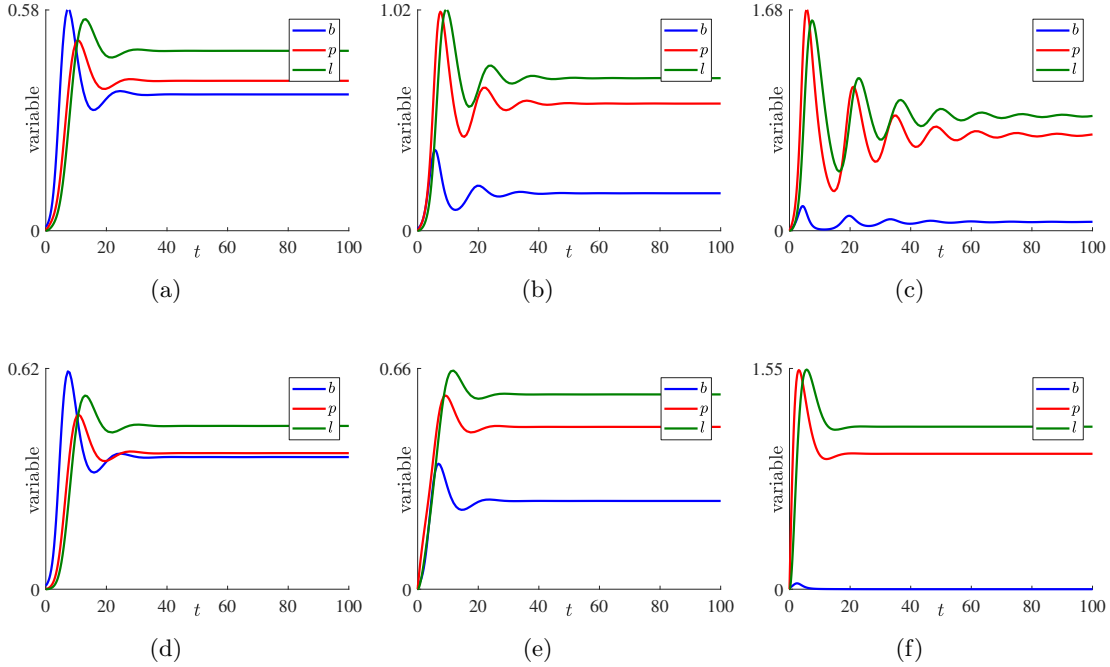


Figure 3.11: Numerical simulations of system (3.5) for (a)–(c) $c_b = 0.25, 1, 4$ respectively and (d)–(f) $\phi = 0, 0.1, 1$ respectively, with all other parameters at their baseline values. The variable a has been omitted to make the plots less cluttered.

Finally, Figure 3.12 gives time courses to explore the behaviour seen in Figure 3.9(b); the analysis here was done with an alternative parameter regime that allowed for a stable limit cycle for a range of r_l values. Under this alternative parameter regime, having a sufficiently low r_l gives lightly damped oscillations to a steady state with a non-zero bacterial population. However, the steady state bacterial density is low (compared to the peak); therefore we regard this as asymptomatic colonisation. For larger r_l , a stable limit cycle is admitted; however, the lower bound of the limit cycle is very low. Figure 3.12(d) shows that the lower bound is much less than 10^{-5} , while the upper bound is ≈ 0.32 . As discussed in the bifurcation analysis (see section 3.4.2), we interpret this limit cycle as eradication, due to the lower bound being sufficiently close to 0. Therefore, the infections shown in Figures 3.12(b)–(d) can be regarded as eradicated. This ultimately leads to a much lower r_l value required to eradicate the infection.

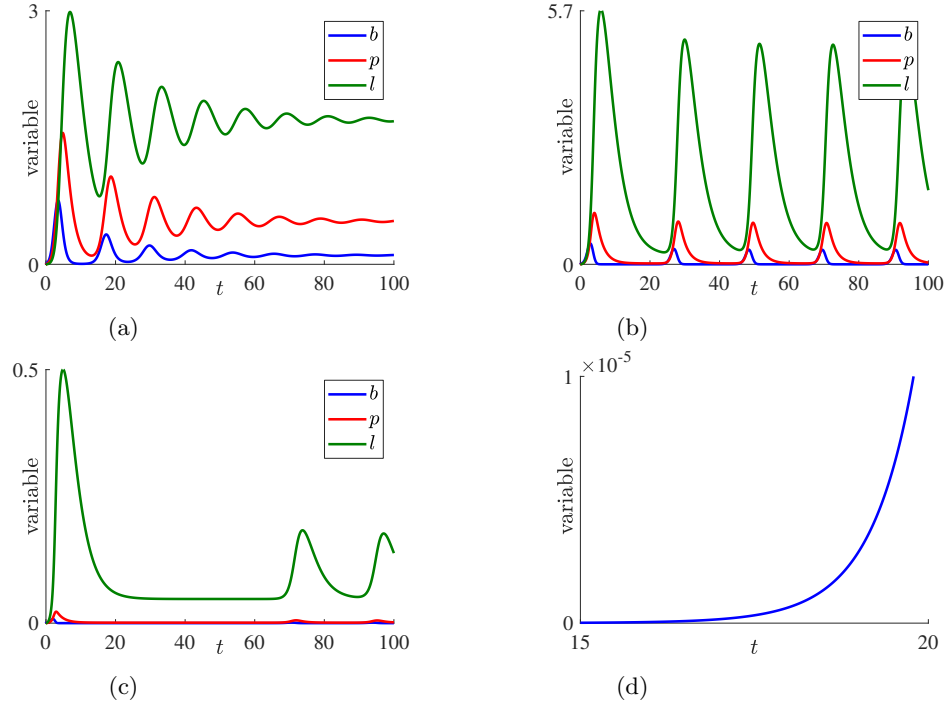


Figure 3.12: (a)–(c) Numerical simulations of system (3.5) for $r_l = 1, 3, 15$ respectively, with all other parameters given by the alternate parameter set (see Table 3.3). (d) A zoom in of panel (b) at $15 \leq t \leq 20$, $0 \leq b \leq 10^{-5}$. The variable a has been omitted to make the plots less cluttered.

Summary

As indicated by steady state and bifurcation analyses, these simulation results show that by either decreasing the replication rate of bacteria (r_b), or increasing the recruitment rate of leukocytes (r_l) and/or stimulated production rate of pro-inflammatory mediators (c_b), the size of the infection can be significantly reduced, potentially eradicating the infection. These results further suggest that a bacteriostatic treatment would be the most effective individual treatment method; however, combined treatments would work synergistically to give even better results. Treatments that boost the immune response can be effective if temporary, and a treatment that increases the stimulated production of pro-inflammatory mediators is favoured over one that increases background production as the effects only occur in the presence of a pathogenic challenge. When the immune response is strong, it can sometimes lead to damped oscillations in the dynamics. Oscillations to a disease-free steady state could be misinterpreted as a recurring infection if the oscillations are sufficiently strong; therefore, care would need to be taken when administering a treatment of this fashion. It is possible to obtain limit cycle behaviour; however, the lower limit of the bacterial population in the limit cycle is often sufficiently low that we regard the infection as eradicated (due to the continuous ODE representation of the infection breaking down).

3.5 Summary

In this chapter, we developed a time dependent, population-level ODE model governing the dynamics of the immune response and a general pathogenic challenge (given by system (3.5)). We

investigated this model by considering biologically realistic steady states, bifurcation analysis, a parameter sensitivity study and numerical simulations, with the aim of identifying clinical treatment strategies that will be effective at eradicating or ameliorating infections. We found that there are always two feasible steady states; a disease-free state (zero bacteria) and a diseased state (non-zero bacteria), and that the model therefore exhibits monostability. We found via linear stability analysis that, for $\phi \ll 1$, $d_p, r_a, d_a = \mathcal{O}(1)$, the disease-free state is stable provided that:

$$r_b < 1 + \frac{\phi r_l}{d_p d_l}, \quad (3.17)$$

i.e. provided the bacteria cannot grow sufficiently fast to overcome the immune response. When r_b is increased to the value at which the condition fails to hold, a transcritical bifurcation takes place where stabilities are exchanged between the diseased state and the disease-free state. Physiologically, if a treatment sufficiently reduces the replication rate of bacteria (r_b), increases the recruitment rate of leukocytes (r_l), decreases the degradation rate of pro-inflammatory mediators (d_p), decreases the death rate of leukocytes (d_l), or is a combination of the above, then that treatment would be effective.

A sensitivity analysis revealed that the diseased state is most sensitive to the growth rate of bacteria r_b , and is next-most sensitive to the recruitment rate of leukocytes r_l and the stimulated production rate of pro-inflammatory mediators c_b . This, combined with the steady state stability condition (3.17), informed the choice of parameters to explore in more detail with further numerical simulations. Bifurcation analysis showed that a transcritical bifurcation takes place when equality holds in (3.17). The bifurcation was shown to occur by making the growth rate of bacteria large enough and by making the recruitment rate of leukocytes and the background production rate of pro-inflammatory mediators ϕ small enough. This further validates the suggestion that a treatment that either reduces the ability of bacteria to grow or boosts the recruitment rate of leukocytes would be an effective strategy in eradicating the infection. We then tracked the location of the bifurcation when pairs of parameters were varied, specifically (r_b, ϕ) and (r_b, r_l) . The relationships between both pairs of parameters obey equation (3.16) for small ϕ , further showing that (3.17) is only valid for a sufficiently small background production rate of pro-inflammatory mediators. The parameter r_b is monotonically increasing in both r_l and ϕ , meaning that if bacteria proliferate faster, then there would need to be a higher background production rate of pro-inflammatory mediators and/or recruitment rate of leukocytes for the stability swap between steady states to occur. An increase in background production of pro-inflammatory mediators is not recommended due to the potential development of autoimmune disorders, such as chronic inflammation and leukocytosis.

Numerical simulations were plotted for the baseline parameter regime and we focused on the density of bacteria, the concentration of pro-inflammatory mediators and the density of leukocytes. For a growth rate of bacteria sufficiently large that the infection can survive, the bacterial population increases quickly from an initially small population, is reduced in size by the resulting immune response and then stabilises at a steady value. For growth rates that are too low, however, the infection cannot survive — potentially even in the absence of an immune response. This result

indicates that a treatment that could reduce the growth rate of bacteria, such as a bacteriostatic drug, could be an effective treatment. For a sufficiently high leukocyte recruitment rate, the infection is cleared, with the density of leukocytes remaining elevated post-infection. This result further suggests that if a treatment could temporarily boost the recruitment rate of leukocytes, it could be an effective strategy for clearing the infection. The treatment must be temporary, however, to prevent the development of health problems, such as leukocytosis and leukemia.

In many cases, the infection size drops sufficiently low that the discreteness of the number of bacteria becomes significant, thus rendering the continuous ODE representation of the infection invalid. When this occurs, we assume that the infection is eradicated. This viewpoint is adopted in the literature, for example in [149] while considering the dynamics of acute inflammation. In adopting this interpretation, additional treatment strategies are considered effective, for example sufficiently increasing the stimulated production rate of pro-inflammatory mediators. This is a preferred strategy to boosting the background production rate, as it is only effective in the presence of the pathogen. In any case, clinical care should be taken to ensure that the effects of a treatment of this fashion are temporary to avoid autoimmune diseases such as chronic inflammation and leukocytosis. Decaying oscillations are observed for all rates of stimulated production of pro-inflammatory mediators, with the oscillations being more pronounced for higher rates. It is therefore important, during a treatment that increases this rate, that these oscillations are not interpreted as treatment failure.

Finally, we considered an alternative parameter regime that represents a stronger infection and a stronger immune system, to look for possible alternate behaviour in the system dynamics. We found in this alternate regime that there is a range of r_l values for which the system exhibits oscillatory behaviour. However, since the lower bound of the bacterial population in the oscillations is very close to zero, we assume the infection is eradicated. Therefore, a treatment that temporarily boosts the recruitment rate of leukocytes is still effective in this parameter regime.

Overall, the model results suggest that reducing the growth rate of the bacteria is the most effective individual treatment method. A two-parameter sensitivity analysis showed that simultaneously decreasing the growth rate of bacteria and either the degradation rate of pro-inflammatory mediators or the death rate of leukocytes is effective. Similarly, simultaneously increasing the recruitment rate of leukocytes and the stimulated production rate of pro-inflammatory mediators by either phagocytic or non-phagocytic cells is effective. These results highlight the synergies present between the inflammatory response and the leukocyte response. It is predicted that a combined treatment would not only be more effective at eradicating a pathogenic challenge, it would also be safer to use than a treatment that purely attacks the pathogen or boosts the immune response, as it would be less likely to lead to the immune response going into over-drive, potentially leading to the described auto-immune health problems.

Chapter 4

A mathematical model of an EPEC infection

4.1 Overview

In this chapter, building on the general bacterial infection model of Chapter 3, we develop and analyse two time-dependent, population-level ODE models to better understand the dynamics of interactions between an EPEC infection, the resulting immune response from the host and aminoglycoside treatments. We are motivated to formulate these models by the prevalence of fatal EPEC infections in countries without adequate healthcare, particularly in children and those with weak immune systems. The aim of these models is to find effective treatment strategies that can lead to the eradication or amelioration of EPEC infections.

We first formulate a basic model that considers the dynamics at the cell level, using the general infection model presented in Chapter 3 (system (3.5)) as a foundation, to which EPEC specific mechanisms are added. We then consider a multi-scale, coupled model that combines the basic model with a sub-cellular gene regulatory network (GRN) governing bacterial protein synthesis and the administration of a specific class of antibiotic (aminoglycosides). In both models, we account for biological mechanisms reported in the literature and on the expertise of, and recent observations by, experimental collaborators (see Chapter 2). We analyse both models by considering biologically realistic steady states, bifurcation analysis, a parameter sensitivity study and numerical simulations. We make comparisons between the models, focusing on the infection size over time in different parameter regimes, and highlight the new dynamics and insights into effective treatment that the inclusion of sub-cellular processes supports. Finally, we make physiological interpretations based on predictions regarding the feasibility and efficacy of various possible anti-bacterial treatment strategies and then summarize our findings.

4.2 Basic model formulation

This model is formulated by adding EPEC-specific mechanisms, based on the literature discussed in the literature review (Chapter 2) and on discussions with experimental collaborators (Delahay et al, Centre for Biomolecular Sciences (CBS), University of Nottingham), to the general infection model presented in the previous chapter (see equations (3.1)–(3.4) in Chapter 3). Figure 4.1 shows a schematic diagram of the model. Many of the added mechanisms focus on how effector proteins subvert the host immune response. Due to the fact that there are many types of effector protein, they are grouped by function and while many effector proteins have overlapping functions physiologically, we assume for simplicity that each group of effector proteins has a single distinct function. We assume that the binding of EPEC to host cells (via intimin and Tir; see section 2.4) has already occurred, therefore the biological mechanisms for bacterial binding are not considered in this model. The effector protein functions that will be considered are (1) the inhibition of pro-inflammatory cell signalling, (2) host cell apoptosis and (3) phagocytosis by leukocytes. The bacterial population is split into two phenotypes: those that have internalized pro-inflammatory mediators and those that have not. Those with internalized pro-inflammatory mediators will typically have effector protein production rates that are dependent on their current environment, whereas those without will not, thus making bacteria with internalized pro-inflammatory mediators more virulent. There are 9 variables in the model;

- the density of bacteria without internalized pro-inflammatory mediators (B_1),
- the density of bacteria with internalized pro-inflammatory mediators (B_2),
- the (external) concentration of pro-inflammatory mediators (P),
- the concentration of anti-inflammatory mediators (A),
- the density of leukocytes (L),
- the density of effector proteins that inhibit pro-inflammatory signalling pathways (E_1),
- the density of effector proteins that inhibit apoptosis of host cells (E_2),
- the density of effector proteins that inhibit phagocytosis (E_3),
- a measure of host cell population necrosis (N).

We now present a description of the dynamics of the model, considering similarities and differences between them and the equivalent dynamics of the general infection model (equations (3.1)–(3.4)). Bacteria grow exponentially quickly, with growth slowing down as space and resources run out. Both phenotypes are removed through phagocytosis by leukocytes and naturally die at the same respective rates as in the general model. The background and immune-stimulated production of pro-inflammatory mediators have the same functional forms as in the general model, and we include an additional source in the form of release due to the necrosis of host cells. The production of pro-inflammatory mediators is inhibited by anti-inflammatory mediators as in the general infection model, but we assume they have no control over necrosis-driven production. The production and degradation of anti-inflammatory mediators have the same functional forms as in the general infection model. Leukocytes are attracted via inflammation and die naturally as in the general infection model, but we also include attraction via the apoptosis markers displayed by damaged host IECs that are undergoing apoptosis. The effector proteins that (1) inhibit pro-inflammatory signalling

(E_1) reduce the strength of programmed pro-inflammatory mediator production, (2) inhibit apoptosis (E_2) reduce the attraction of leukocytes associated with apoptosis markers and (3) inhibit phagocytosis (E_3) reduce the death rate of bacteria associated with phagocytosis. We hypothesize that bacteria of the more virulent phenotype (B_2) can detect local inflammatory conditions via the internalisation of host pro-inflammatory cytokines (Easton et al, CBS, University of Nottingham; unpublished). To reflect this, we assume effector protein production by B_2 is dependent on B_2 and P . Bacteria of the less virulent phenotype (B_1) internalize pro-inflammatory mediators and switch to B_2 when their internalized concentration exceeds a critical threshold. Bacteria cause the necrosis of IECs by directly damaging them and preventing them from undergoing apoptosis. Tissue repair occurs via the replacement of dead cells with new cells. Finally, as the model is population-level, we can construct an ODE representation of the dynamics..

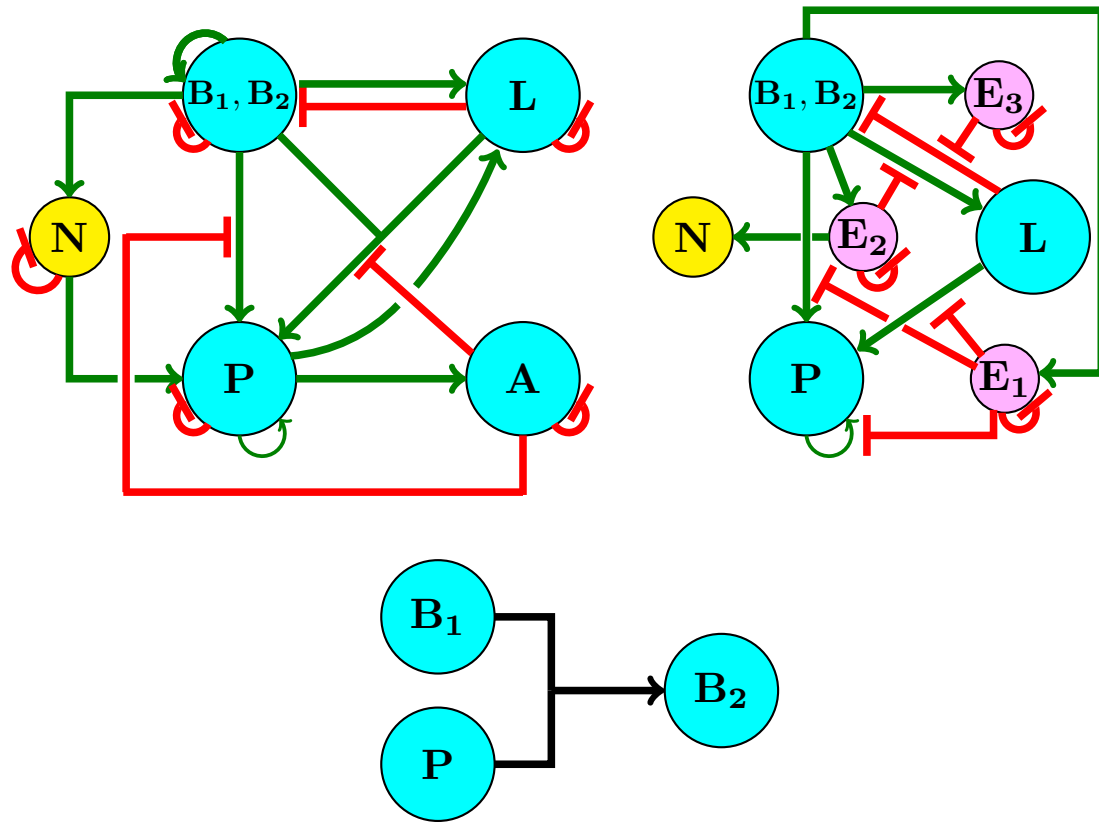


Figure 4.1: Schematic diagrams of the basic model for an EPEC specific infection. Green and red arrows indicate up-regulation and down-regulation respectively and black lines represent variable transformations. Thin arrows represent mechanisms that have effects of a small magnitude. (a) Diagram of all mechanisms excluding those involving effector proteins and the internalisation of pro-inflammatory mediators by EPEC. (b) Diagram showing the mechanisms that effector proteins are involved in. Note that some of the arrows from (a) have been omitted for neatness; only those that effector proteins have a direct effect on are presented. (c) Diagram showing how the two phenotypes of bacteria are connected; bacteria of the less virulent phenotype are converted into the more virulent phenotype when they have internalised pro-inflammatory mediators.

The density of bacteria evolves according to:

$$\frac{dB_1}{dt} = \frac{R_B(B_1 + \lambda B_2)}{x_B + (B_1 + B_2)} - QPB_1 - P_BLB_1 \left(\frac{x_3}{x_3 + E_3} \right) - D_B B_1, \quad (4.1)$$

$$\frac{dB_2}{dt} = \frac{R_B(1 - \lambda)B_2}{x_B + (B_1 + B_2)} + QPB_1 - P_BLB_2 \left(\frac{x_3}{x_3 + E_3} \right) - D_B B_2. \quad (4.2)$$

Both phenotypes grow at the same global rate R_B with half-maximal parameter x_B and undergo phagocytosis and naturally die at rates P_B and D_B respectively. The growth rate of the total bacteria $(B_1 + B_2)$ has the same functional form as that in the general infection model; however, care must be taken when considering the phenotype of the progeny of a replicating bacterium. All B_1 progeny are B_1 , but when a B_2 replicates, the progeny can be either a B_1 or a B_2 depending on how many internalized pro-inflammatory mediators the parent cell had and on how they were divided between the parent and daughter cells. It is not clear biologically that the split is exactly symmetrical and this asymmetry is incorporated into the parameter $\lambda \in [0, 1]$; when $\lambda = 0$, all B_2 progeny are B_2 and when $\lambda = 1$, all B_2 progeny are B_1 . We assume that half of all progeny will be of each phenotype since the *average* concentration of internalized pro-inflammatory mediators is halved and therefore unless stated otherwise, the value of λ will be taken to be 0.5. B_1 internalize P at constant rate Q and this transforms B_1 into B_2 . While it is thought that B_2 continue to internalize P , this has not been included for simplicity. The concentration of internalized P required for a B_1 to become a B_2 is not explicitly defined, but is implicitly incorporated in the parameters λ and Q .

The concentration of pro-inflammatory mediators evolves according to:

$$\frac{dP}{dt} = \left[\Phi + C_B(1 + VL)(B_1 + B_2) \right] \left(\frac{x_A^2}{x_A^2 + A^2} \right) \left(\frac{x_1}{x_1 + E_1} \right) - QPB_1 + S_N N - D_P P. \quad (4.3)$$

The terms for background and stimulated production of pro-inflammatory mediators have the same functional forms as in the general infection model, but have the inhibitory effects of E_1 added. For definitions of Φ, C_B, V, x_A and D_P , see the discussion following (3.2) and (3.3). We model the inhibitory effects of each effector protein group in the model by multiplying the relevant term by a term of the form:

$$\frac{x_i}{x_i + E_i}, \quad i = 1, 2, 3,$$

for the respective effector protein group E_i . This function is a decreasing Hill function in the density of E_i with x_i giving the density of E_i at which their inhibitory effect is half-maximal. The term is equal to 1 when $E_i = 0$ and in this case the term it inhibits is unaltered. As E_i increases the inhibition term decreases, tending to 0 as E_i tends to infinity and therefore the inhibition becomes stronger the more proteins that are present. Returning to (4.3), the term for the internalization of pro-inflammatory mediators is identical to those present in (4.1) and (4.2), thus conserving mass. We model the source due to necrosis as a term linear in the measure of host cell population necrosis

N , with rate coefficient S_N . The concentration of anti-inflammatory mediators evolves in the same way as it does in the general infection model:

$$\frac{dA}{dt} = R_A \frac{P^2}{x_A^2 + P^2} - D_A A. \quad (4.4)$$

For definitions of the parameters in (4.4), see the discussion following (3.2) and (3.3). The density of leukocytes evolves according to:

$$\frac{dL}{dt} = R_L P + S_\alpha (B_1 + B_2) \left(\frac{x_2}{x_2 + E_2} \right) - D_L L, \quad (4.5)$$

R_L and D_L denote the rates of inflammation-stimulated attraction and natural death of leukocytes respectively. Attraction due to the inflammatory response and natural death have the same forms as they do in the general infection model, but there is an additional source term due to attraction to apoptosis markers displayed by host IECs undergoing apoptosis. IECs display apoptosis markers when they are damaged by bacteria and we accommodate this by making this source term linear in the total density of bacteria, with rate parameter S_α .

The densities of the effector protein groups evolve according to:

$$\frac{dE_1}{dt} = X_1 B_1 + X_2 B_2 - D_X E_1, \quad (4.6)$$

$$\frac{dE_2}{dt} = Y_1 B_1 + Y_2 \left(\frac{\Psi_{Y1}^2}{\Psi_{Y1}^2 + P^2} \right) \left(\frac{B_2}{\Psi_{Y2}^2 + B_2^2} \right) - D_Y E_2, \quad (4.7)$$

$$\frac{dE_3}{dt} = \left(Z_1 + \frac{Z_2 P}{\Psi_Z + P} \right) B_2 - D_Z E_3. \quad (4.8)$$

We refer the reader to Figure 4.1 for a schematic of the interactions between effector proteins and the immune response, and Figure 4.2 for schematic and graphical representations of the production rates of effector proteins.

B_1 produce E_1 and E_2 linearly at rates X_1 and Y_1 respectively and do not produce E_3 . B_2 also produce E_1 linearly, but at rate X_2 with $X_2 > X_1$ to reflect that B_2 are more virulent. E_2 become redundant when the environment is highly inflamed, therefore the production of E_2 by B_2 decreases as P increases. To model this, the source term for E_2 by B_2 is multiplied by a decreasing Hill function in P with Hill coefficient 2 and half-maximal parameter Ψ_{Y1} . This allows for switch-like behaviour, where production is low for $P > \Psi_{Y1}$. The rate of production also varies with the population of B_2 such that production is approximately linear in B_2 for low B_2 , and such that there is an optimal production rate for some critical population, denoted Ψ_{Y2} . E_2 production decreases

for higher B_2 populations to minimize damage-induced necrosis of IECs. The global production rate of E_2 is denoted Y_2 .

B_2 produce more E_3 when the environment is more inflamed to protect themselves from the leukocyte response. Therefore, the source term for E_3 by B_2 is the sum of two terms: (1) linear basal production at rate Z_1 and (2) stimulated production at a rate that is linear in B_2 , and is multiplied by an increasing Hill function in P with Hill coefficient 1 and half-maximal parameter Ψ_Z . The global rate of stimulated production is denoted Z_2 . A Hill function in P is used so that the production rate increases with P but plateaus, as we assume that there is a maximal rate at which any individual B_2 can produce E_3 . Finally, all effector proteins degrade linearly, with E_1, E_2 and E_3 degrading at rates D_X, D_Y and D_Z respectively.

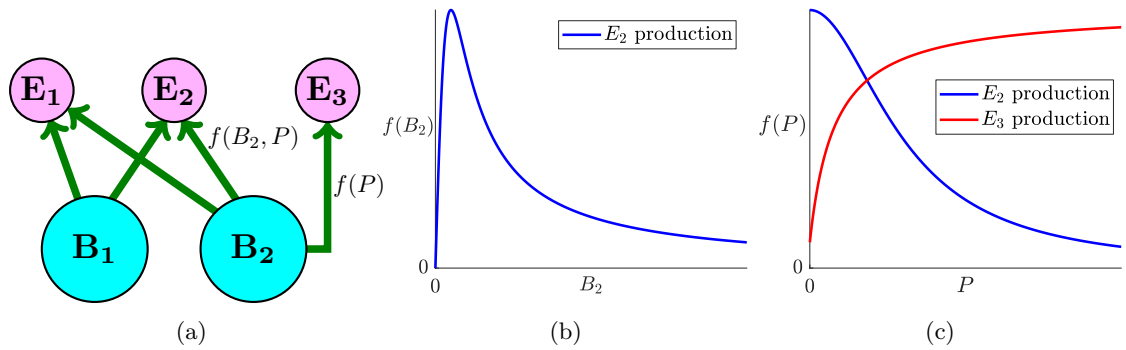


Figure 4.2: (a) Schematic showing how each bacteria phenotype produces each effector protein group (production given by green arrows). Production rates that are not linear are labelled with the variables that the production rate is a function of. (b) Representative functional form of E_2 production as a function of B_2 . (c) Representative functional form of E_2 and E_3 production as a function of P .

The measure of host cell population necrosis evolves according to:

$$\frac{dN}{dt} = \Lambda(B_1 + B_2) \frac{E_2^2}{\Psi_N^2 + E_2^2} - D_N N. \quad (4.9)$$

Since bacteria directly damage host cells, we model the source of necrosis as a term that is linear in the total density of bacteria. Since E_2 inhibit the apoptosis of host cells, they indirectly promote host cell necrosis; therefore, the production rate of host cell necrosis also increases with E_2 . An increasing Hill function in E_2 with Hill coefficient 2 and half-maximal parameter Ψ_N is chosen, giving switch-like behaviour where necrosis is high (above half the maximum) for $E_2 > \Psi_N$. The global rate of host cell necrosis is denoted Λ . Finally, we assume that the host repairs dead host cells at a linear rate, with rate parameter D_N .

Putting (4.1)–(4.9) together, we obtain a nine-dimensional ODE model governing the dynamics between an EPEC infection and the immune response. Non-dimensionalising via the following scalings:

$$B_1 = x_B b_1, \quad B_2 = x_B b_2, \quad P = x_P p, \quad A = x_A a, \quad L = \frac{D_P}{P_B} l,$$

$$E_1 = x_1 e_1, \quad E_2 = x_2 e_2, \quad E_3 = x_3 e_3, \quad N = \frac{\Lambda x_B}{D_P} n, \quad t = \frac{\tau}{D_P},$$

we obtain the following non-dimensional system of ODEs:

$$\frac{db_1}{d\tau} = \frac{r_b(b_1 + \lambda b_2)}{1 + (b_1 + b_2)} - qp b_1 - l b_1 \left(\frac{1}{1 + e_3} \right) - d_b b_1, \quad (4.10a)$$

$$\frac{db_2}{d\tau} = \frac{r_b(1 - \lambda)b_2}{1 + (b_1 + b_2)} + qp b_1 - l b_2 \left(\frac{1}{1 + e_3} \right) - d_b b_2, \quad (4.10b)$$

$$\frac{dp}{d\tau} = \left[\phi + c_b(1 + v l)(b_1 + b_2) \right] \left(\frac{1}{1 + a^2} \right) \left(\frac{1}{1 + e_1} \right) - qu_{bp} p b_1 + s_n n - p, \quad (4.10c)$$

$$\frac{da}{d\tau} = r_a \frac{p^2}{1 + p^2} - d_a a, \quad (4.10d)$$

$$\frac{dl}{d\tau} = r_l p + s_\alpha (b_1 + b_2) \left(\frac{1}{1 + e_2} \right) - d_l l, \quad (4.10e)$$

$$\frac{de_1}{d\tau} = c_{11} b_1 + c_{12} b_2 - d_{e1} e_1, \quad (4.10f)$$

$$\frac{de_2}{d\tau} = c_{21} b_1 + c_{22} \left(\frac{\psi_{y1}^2}{\psi_{y1}^2 + p^2} \right) \left(\frac{b_2}{\psi_{y2}^2 + b_2^2} \right) - d_{e2} e_2, \quad (4.10g)$$

$$\frac{de_3}{d\tau} = \left(c_{31} + \frac{c_{32} p}{\psi_z + p} \right) b_2 - d_{e3} e_3, \quad (4.10h)$$

$$\frac{dn}{d\tau} = (b_1 + b_2) \frac{e_2^2}{\psi_n^2 + e_2^2} - d_n n. \quad (4.10i)$$

where the dimensionless parameters in (4.10) are given by:

$$r_b = \frac{R_B}{x_B D_P}, \quad q = \frac{Q x_P}{D_P}, \quad d_b = \frac{D_B}{D_P}, \quad \phi = \frac{\Phi}{x_P D_P}, \quad c_b = \frac{x_B C_B}{x_P D_P}, \quad v = \frac{V D_P}{P_B},$$

$$u_{bp} = \frac{x_B}{x_P}, \quad s_n = \frac{S_N \Lambda x_B}{x_P D_P^2}, \quad r_a = \frac{R_A}{x_A D_P}, \quad d_a = \frac{D_A}{D_P}, \quad r_l = \frac{R_L x_P P_B}{D_P^2}, \quad s_\alpha = \frac{S_\alpha x_B P_B}{D_P^2},$$

$$d_l = \frac{D_L}{D_P}, \quad c_{11} = \frac{X_1 x_B}{x_1 D_P}, \quad c_{12} = \frac{X_2 x_B}{x_1 D_P}, \quad d_{e1} = \frac{D_X}{D_P}, \quad c_{21} = \frac{Y_1 x_B}{x_2 D_P}, \quad c_{22} = \frac{Y_2}{x_B x_2 D_P},$$

$$\psi_{y1} = \frac{\Psi_{Y1}}{x_P}, \quad \psi_{y2} = \frac{\Psi_{Y2}}{x_B}, \quad d_{e2} = \frac{D_Y}{D_P}, \quad c_{31} = \frac{Z_1 x_B}{x_3 D_P}, \quad c_{32} = \frac{Z_2 x_B}{x_3 D_P}, \quad \phi_z = \frac{\Phi_Z}{x_P},$$

$$d_{e3} = \frac{D_Z}{D_P}, \quad \mu = D_P \Lambda, \quad n_1 = \frac{N_1}{N_2}, \quad \psi_n = \frac{\Psi_N}{x_2}, \quad d_n = \frac{D_N}{D_P}.$$

Table 4.1 gives a physiological interpretation of these parameters. Note that all parameters are non-negative. In sections 4.3 and 4.4, we provide an exploration of (4.10) by considering biologically realistic steady states, bifurcation analysis, a parameter sensitivity study and numerical simulations. Together, these analyses will aid in illuminating whether or not particular clinical treatments will be effective.

r_b	The growth rate of bacteria.
λ	The parameter governing the fraction of b_2 progeny that is b_1 (on average).
q	The rate of pro-inflammatory mediator internalisation by b_1 .
d_b	The natural death rate of bacteria, relative to the natural degradation rate of p .
ϕ	The background production rate of pro-inflammatory mediators.
c_b	The stimulated production rate of pro-inflammatory mediators.
v	The ratio of the production rate of pro-inflammatory mediators by leukocytes to that by non-phagocytic host cells.
u_{bp}	The proportionality factor on pro-inflammatory mediators due to internalization.
s_n	The production rate of pro-inflammatory mediators due to host cell necrosis.
r_a	The production rate of anti-inflammatory mediators.
d_a	The natural degradation rate of anti-inflammatory mediators, relative to the natural degradation rate of p .
r_l	The attraction rate of leukocytes due to inflammation.
s_α	The attraction rate of leukocytes due to apoptosed host cells.
d_l	The natural death rate of leukocytes, relative to the natural degradation rate of p .
c_{11}	The production rate of e_1 by b_1 .
c_{12}	The production rate of e_1 by b_2 .
d_{e1}	The natural degradation rate of e_1 , relative to the natural degradation rate of p .
c_{21}	The production rate of e_2 by b_1 .
c_{22}	The production rate of e_2 by b_2 .
ψ_{y1}	The value of p that gives half-maximal production of e_2 by b_2 .
ψ_{y2}	The value of b_2 that optimizes the production rate of e_2 by b_2 .
d_{e2}	The natural degradation rate of e_2 , relative to the natural degradation rate of p .
c_{31}	The basal production rate of e_3 by b_2 .
c_{32}	The stimulated (by inflammation) production rate of e_3 by b_2 .
ψ_z	The value of p that gives half-maximal production of e_3 by b_2 .
d_{e3}	The natural degradation rate of e_3 , relative to the natural degradation rate of p .
ψ_n	The value of e_2 that gives a half-maximal rate of necrosis of host cells by bacteria.
d_n	The natural repair rate of dead host cells, relative to the natural degradation rate of p .

Table 4.1: Descriptions of the physical interpretation of each parameter for system (4.10).

4.3 Basic model: steady state and stability analyses

We now wish to explore and analyze the dynamics of system (4.10). Similar to the analysis of the general infection model, a sensible starting point is to calculate physically plausible steady states. In the context of this model, physical plausibility requires that steady state values of all variables be non-negative. Once the relevant steady states have been calculated, their linear stability is considered, aiding the exploration of the system dynamics and the search for bifurcations in steady state stability. This analysis will help in elucidating whether particular treatments are effective.

4.3.1 Steady state analysis

Equations (4.10a) and (4.10b) yield $\bar{b}_1 = \bar{b}_2 = 0$, i.e. the disease-free state, as one solution. In this state, the steady state values for e_1, e_2, e_3 and n are also 0. Substituting these values into (4.12c)-(4.12e), the disease-free steady states for p, a and l are given by:

$$f_1(\bar{p}) = \bar{p} \left(1 + \left(\frac{r_a}{d_a} \frac{\bar{p}^2}{1 + \bar{p}^2} \right)^2 \right) - \phi = 0, \quad (4.11a)$$

$$\bar{a} = \left(\frac{r_a}{d_a} \right) \frac{\bar{p}^2}{1 + \bar{p}^2}, \quad (4.11b)$$

$$\bar{l} = \frac{r_l}{d_l} \bar{p}. \quad (4.11c)$$

Equation (4.11a) has the same form as (3.9); the equivalent steady state equation in the general infection model, but with ϕ replacing (ϕ/d_p) . The top four panels of Figure 3.2 are therefore also valid for (4.11a) since $d_p = 1$ for those plots. These plots therefore also show that the value for p in the disease-free state in the basic EPEC model is small provided that $\phi \ll 1$ and (r_a/d_a) is sufficiently small.

We now seek the diseased steady state(s). The diseased steady states are given by:

$$0 = \frac{r_b(\bar{b}_1 + \lambda \bar{b}_2)}{1 + (\bar{b}_1 + \bar{b}_2)} - q\bar{p}\bar{b}_1 - \bar{l}\bar{b}_1 \left(\frac{1}{1 + \bar{e}_3} \right) - d_b\bar{b}_1, \quad (4.12a)$$

$$0 = \frac{r_b(1 - \lambda)\bar{b}_2}{1 + (\bar{b}_1 + \bar{b}_2)} + q\bar{p}\bar{b}_1 - \bar{l}\bar{b}_2 \left(\frac{1}{1 + \bar{e}_3} \right) - d_b\bar{b}_2, \quad (4.12b)$$

$$0 = \left[\phi + c_b(1 + v\bar{l})(\bar{b}_1 + \bar{b}_2) \right] \left(\frac{1}{1 + \bar{a}^2} \right) \left(\frac{1}{1 + \bar{e}_1} \right) - qu_{bp}\bar{p}\bar{b}_1 + s_n\bar{n} - \bar{p}, \quad (4.12c)$$

$$\bar{a} = \frac{r_a}{d_a} \frac{\bar{p}^2}{1 + \bar{p}^2}, \quad (4.12d)$$

$$\bar{l} = \frac{r_l}{d_l} \bar{p} + \frac{s_\alpha}{d_l} (\bar{b}_1 + \bar{b}_2) \left(\frac{1}{1 + \bar{e}_2} \right), \quad (4.12e)$$

$$\bar{e}_1 = d_{e1}^{-1}(c_{11}\bar{b}_1 + c_{12}\bar{b}_2), \quad (4.12f)$$

$$\bar{e}_2 = \frac{c_{21}}{d_{e2}} \bar{b}_1 + \frac{c_{22}}{d_{e2}} \left(\frac{\psi_{y1}^2}{\psi_{y1}^2 + \bar{p}^2} \right) \left(\frac{\bar{b}_2}{\psi_{y2}^2 + \bar{b}_2^2} \right), \quad (4.12g)$$

$$\bar{e}_3 = d_{e3}^{-1} \left(c_{31} + \frac{c_{32} \bar{p}}{\psi_z + \bar{p}} \right) \bar{b}_2, \quad (4.12h)$$

$$\bar{n} = d_n^{-1} (\bar{b}_1 + \bar{b}_2) \frac{\bar{e}_2^2}{\psi_n^2 + \bar{e}_2^2}. \quad (4.12i)$$

We now drop the bars denoting steady state densities for convenience. By adding together equations (4.12a) and (4.12b), the total bacterial population, denoted $B = b_1 + b_2$, satisfies:

$$B = \frac{r_b}{l(1 + e_3)^{-1} + d_b} - 1 \quad (4.13)$$

in a diseased state. In order to obtain a condition for feasibility of diseased steady states, we obtain bounds on the steady states for B and l in (4.13). To obtain bounds on the steady state for B , we observe that:

$$\begin{aligned} \text{for } e_3 = 0, \quad B &= \frac{r_b}{l + d_b} - 1, \\ \text{as } e_3 \rightarrow \infty, \quad B &= \frac{r_b}{d_b} - 1. \end{aligned}$$

Since $(1 + e_3)^{-1}$ is monotonically decreasing in e_3 :

$$\frac{r_b}{l + d_b} - 1 \leq B < \frac{r_b}{d_b} - 1. \quad (4.14)$$

We see that $r_b > d_b$ is required for a positive infection size and that e_3 acts to decrease the minimum net bacterial growth rate (r_b/d_b) required for a feasible diseased state as well as increase the infection size at the respective diseased state. (4.12e) can be written as:

$$l = \frac{r_l}{d_l} p + \frac{s_\alpha}{d_l} \frac{B}{1 + e_2}.$$

Again, to obtain bounds on the steady state for l , we observe that:

$$\begin{aligned} \text{for } e_2 = 0, \quad l &= \frac{r_l}{d_l} p + \frac{s_\alpha}{d_l} B, \\ \text{as } e_2 \rightarrow \infty, \quad l &= \frac{r_l}{d_l} p. \end{aligned}$$

Since $(1 + e_2)^{-1}$ is monotonically decreasing in e_2 , bounds on l are given by:

$$\frac{r_l}{d_l}p < l \leq \frac{r_l}{d_l}p + \frac{s_\alpha}{d_l}B. \quad (4.15)$$

Substituting the maximum possible diseased steady state l density (the right-hand side of (4.15)) into the minimum possible diseased steady state B density (the left-hand side of (4.14)), the minimum feasible infection size in a diseased state is given by:

$$B^2 + \left(1 + \frac{r_l}{s_\alpha}p + \frac{d_b d_l}{s_\alpha}\right)B + \left(\frac{r_l p - (r_b - d_b)d_l}{s_\alpha}\right) \geq 0. \quad (4.16)$$

The roots of the quadratic in (4.16) such that equality holds are given by:

$$2B^\pm = -\left(1 + \frac{r_l}{s_\alpha}p + \frac{d_b d_l}{s_\alpha}\right) \pm \sqrt{\left(1 + \frac{r_l}{s_\alpha}p + \frac{d_b d_l}{s_\alpha}\right)^2 - 4\left(\frac{r_l p - (r_b - d_b)d_l}{s_\alpha}\right)}. \quad (4.17)$$

B^- will be negative if it is real since all parameters are positive, therefore all $B \leq B^-$ must also be negative and are therefore ignored. For $B \geq B^+$:

$$B^+ \geq -\left(1 + \frac{r_l}{s_\alpha}p + \frac{d_b d_l}{s_\alpha}\right) + \sqrt{\left(1 + \frac{r_l}{s_\alpha}p + \frac{d_b d_l}{s_\alpha}\right)^2 - 4\left(\frac{r_l p - (r_b - d_b)d_l}{s_\alpha}\right)}.$$

In order for this value to be positive, we require:

$$r_b - d_b > \frac{r_l}{d_l}p. \quad (4.18)$$

From (4.18), one obtains a condition for the feasibility of diseased steady states. Physiologically, this condition states that, in the absence of e_2 and e_3 , bacterial growth needs to be sufficiently strong to overcome the inflammation-stimulated host immune response. We infer that the equivalent condition should the densities of e_2 and/or e_3 be non-zero is less strict on the net bacterial growth rate. The dynamics of e_1 are encapsulated implicitly within p in (4.18). Due to the intractability of solving (4.12c) analytically, it is not possible to obtain a usable expression for p only in terms of model parameters, therefore (4.18) cannot be further simplified. While we have not proven the existence of only one diseased state, numerical simulations of this model (see section 4.4) suggest the absence of multiple diseased states.

Condition (4.18) is not satisfied if the net growth rate of the bacteria is sufficiently reduced, the attraction rate or the life span of leukocytes is sufficiently increased, or the concentration of pro-inflammatory mediators is sufficiently increased. A bacteriostatic drug reduces r_b and a bactericidal drug essentially increases d_b ; therefore, both of these treatment strategies are predicted by (4.18) to be effective provided that their effects are strong enough. Similarly, treatments that sufficiently increase the pro-inflammatory mediator production rate and/or the leukocyte recruitment rate are

also predicted to be effective.

Special case: $\phi \ll 1$

As in the general infection model (see section 3.3), to model a healthy immune response, we assume that $\phi \ll 1$ and that all other model parameters are $\mathcal{O}(1)$. We showed earlier in this section that, under this assumption, $0 < \bar{p} \ll 1$ provided that (r_a/d_a) is sufficiently small. Substituting $0 < \bar{p} \ll 1$ and $(r_a/d_a) \sim 1$ into (4.11a) reduces it to the approximate steady state:

$$\bar{p} \approx \phi. \quad (4.19)$$

Substituting (4.19) into (4.11b) and (4.11c) yields:

$$\bar{a} \approx \frac{r_a \phi^2}{d_a(1 + \phi^2)}, \quad \bar{l} \approx \frac{r_l \phi}{d_l}. \quad (4.20)$$

Summary

- In the disease-free state:

$$\bar{p} \left(1 + \left(\frac{r_a}{d_a} \frac{\bar{p}^2}{1 + \bar{p}^2} \right)^2 \right) - \phi = 0, \quad \bar{a} = \left(\frac{r_a}{d_a} \right) \frac{\bar{p}^2}{1 + \bar{p}^2}, \quad \bar{l} = \frac{r_l}{d_l} \bar{p}.$$

- The total bacteria (B) in the diseased state satisfies

$$\frac{r_b}{\bar{l} + d_b} - 1 \leq \bar{B} < \frac{r_b}{d_b} - 1,$$

- The minimum feasible total bacteria in the diseased state is positive provided that

$$r_b - d_b > \frac{r_l}{d_l} \bar{p},$$

and occurs when $\bar{e}_2, \bar{e}_3 = 0$.

- We let $\phi \ll 1$ and $(r_a/d_a) \sim 1$ to model a healthy immune response. Under these assumptions, the disease-free state is given by:

$$(\bar{b}_1, \bar{b}_2, \bar{p}, \bar{a}, \bar{l}, \bar{e}_1, \bar{e}_2, \bar{e}_3, \bar{n}) \approx \left(0, 0, \phi, \frac{r_a \phi^2}{d_a(1 + \phi^2)}, \frac{r_l \phi}{d_l}, 0, 0, 0, 0 \right). \quad (4.21)$$

The next subsection explores the stability of these steady states.

4.3.2 Stability analysis

By deriving a condition that governs when the disease-free state is stable, we can make predictions on which model parameters to alter to lead to the eradication of the infection. This will be achieved

via linear stability analysis. The Jacobian of system (4.10) is given by:

$$\mathbf{J} = \begin{bmatrix} J_{11} & J_{12} & J_{13} & 0 & J_{15} & 0 & 0 & J_{18} & 0 \\ J_{21} & J_{22} & J_{23} & 0 & J_{25} & 0 & 0 & J_{28} & 0 \\ J_{31} & J_{32} & J_{33} & J_{34} & J_{35} & J_{36} & 0 & 0 & J_{39} \\ 0 & 0 & J_{43} & J_{44} & 0 & 0 & 0 & 0 & 0 \\ J_{51} & J_{52} & J_{53} & 0 & J_{55} & 0 & J_{57} & 0 & 0 \\ J_{61} & J_{62} & 0 & 0 & 0 & J_{66} & 0 & 0 & 0 \\ J_{71} & J_{72} & J_{73} & 0 & 0 & 0 & J_{77} & 0 & 0 \\ 0 & J_{82} & J_{83} & 0 & 0 & 0 & 0 & J_{88} & 0 \\ J_{91} & J_{92} & 0 & 0 & 0 & 0 & 0 & 0 & J_{99} \end{bmatrix}, \quad (4.22)$$

where $J_{i,j}$ is the entry in the i th row and j th column of the Jacobian. The elements of the Jacobian are given in Appendix section A.2.

Evaluating $\det[\mathbf{J} - s\mathbf{I}] = 0$ at the disease-free state gives, after reduction:

$$2s_{1,2} = (J_{11} + J_{22}) \pm \sqrt{(J_{11} + J_{22})^2 - 4(J_{11}J_{22} - J_{12}J_{21})}, \quad (4.23a)$$

$$2s_{3,4} = (J_{33} + J_{44}) \pm \sqrt{(J_{33} + J_{44})^2 - 4(J_{33}J_{44} - J_{34}J_{43})}, \quad (4.23b)$$

$$s_5 = -d_l, \quad s_7 = -d_{e2}, \quad s_8 = -d_{e3}, \quad s_9 = -d_n, \quad s_6 = -d_{e1}, \quad (4.23c)$$

where s denote the eigenvalues of (4.22). For a detailed description of this reduction, see Appendix section A.3. We see that s_5, s_6, s_7, s_8 and s_9 are all negative, thus the disease-free state is stable provided that:

$$J_{11} + J_{22} < 0, \quad (4.24a)$$

$$J_{33} + J_{44} < 0, \quad (4.24b)$$

$$J_{11}J_{22} - J_{12}J_{21} > 0, \quad (4.24c)$$

$$J_{33}J_{44} - J_{34}J_{43} > 0. \quad (4.24d)$$

Equation (4.24b) is always satisfied since $J_{33} = -1$ and $J_{44} = -d_a$, and (4.24d) is always satisfied

since:

$$J_{33}J_{44} - J_{34}J_{43} = d_a + \frac{4\phi r_a \bar{p} \bar{a}}{(1 + \bar{p}^2)^2(1 + \bar{a}^2)^2} > 0.$$

In order to obtain expressions for (4.24a) and (4.24c), we note here that, in the disease-free state:

$$J_{11} = r_b - q\bar{p} - \bar{l} - d_b,$$

$$J_{12} = r_b \lambda,$$

$$J_{21} = q\bar{p},$$

$$J_{22} = (1 - \lambda)r_b - \bar{l} - d_b.$$

Substituting these Jacobian element expressions into (4.24a) and (4.24c) gives:

$$\left(1 - \frac{\lambda}{2}\right) r_b - \left(d_b + \bar{l} + \frac{q\bar{p}}{2}\right) < 0, \quad (4.25a)$$

$$\left(A - r_b\right) \left(A - r_b + \lambda r_b + q\bar{p}\right) > 0. \quad (4.25b)$$

where $A = (\bar{l} + d_b) > 0$. (4.25b) is satisfied when either; (1) $A - r_b > 0$ or (2) $A - r_b < -(\lambda r_b + q\bar{p})$. These conditions give, respectively:

$$r_b - d_b < \bar{l}, \quad (4.26a)$$

$$\left(1 - \frac{\lambda}{2}\right) r_b - \left(d_b + \bar{l} + \frac{q\bar{p}}{2}\right) > \frac{1}{2}(\lambda r_b + q\bar{p}). \quad (4.26b)$$

For stability of the disease-free steady state, we require that (4.25a) and one of (4.26a) or (4.26b) are satisfied. We see that (4.25a) and (4.26b) contradict each other, therefore we must take (4.26a). By re-writing (4.25a) in the form:

$$r_b - d_b < \bar{l} + \frac{q\bar{p}}{2} + \frac{\lambda r_b}{2}, \quad (4.27)$$

we see that if (4.26a) is true, then (4.25a) must also be true. Therefore for the disease-free state to be stable, condition (4.26a) must be satisfied. The disease-free state becomes unstable when condition (4.26a) is not satisfied, and the only bifurcation that can occur that ensures all steady states are non-negative is a transcritical bifurcation, occurring when equality holds in (4.26a).

When $\phi \ll 1$ and $(r_a/d_a) \sim 1$, (4.26a) reduces to:

$$r_b - d_b < \frac{\phi r_l}{d_l}. \quad (4.28)$$

Physiologically, (4.28) suggests that for the infection to be eradicated, the net growth rate of the bacteria must be weaker than the basal net attraction rate of leukocytes. This stability condition is identical to that found in the general infection model. This implies that the added EPEC specific dynamics (effector proteins, host cell death and p internalization) make a negligible contribution to the stability of the disease-free state.

In order to maximise the possibility of a return to health, condition (4.28) must be satisfied. It is clear that $r_b < d_b$ guarantees stability of the disease-free state – i.e. if bacteria cannot reproduce fast enough, then they will die out. A bacteriostatic drug reduces r_b and a bactericidal drug essentially increases d_b , therefore these treatment strategies are effective; however, the effects of antibiotic resistance have not been explicitly included in the model. Having leukocytes recruited faster and last longer raises the minimum growth rate required by the bacteria to survive which is a feasible treatment strategy. However, care needs to be taken so as not to induce other chronic aseptic diseases such as leukemia, once the infection has been eradicated.

Summary

- We calculated the Jacobian and the eigenvalues for the disease-free state of (4.10). The disease-free state is stable provided that $r_b - d_b < \bar{l}$
- Under the assumption that $\phi \ll 1$ and $(r_a/d_a) \sim 1$:
 - the disease-free state is stable provided that $r_b - d_b < (\phi r_l/d_l)$.
 - There is at least one feasible diseased state and it is stable when $r_b - d_b > (\phi r_l/d_l)$.
 - The EPEC specific dynamics (effector proteins, host cell death and internalization of p) make a negligible contribution to the stability of the disease-free state.

4.3.3 Steady state analysis summary

Many of the results obtained from the analysis of this model match those found in the general infection model (Chapter 3) showing that the general infection model is powerful in determining the long term infection dynamics, particularly regarding the return to health. From the steady state analysis carried out, we see that there are at least two feasible steady states: a disease-free (healthy) state and at least one diseased states. The healthy state is stable provided that condition (4.26a) is satisfied; therefore, there is at least one stable diseased state when (4.26a) is no longer satisfied.

We assume that the background production of pro-inflammatory mediators is much smaller in magnitude than any stimulated production to model a healthy immune response. The healthy state is then stable provided that condition (4.28) holds; therefore, there is at least one stable diseased state when (4.28) is no longer satisfied.

The five parameters that play a role in the stability of the disease-free state are: the growth rate of bacteria r_b ; the background pro-inflammatory mediator production rate ϕ ; the attraction rate of leukocytes r_l ; and the death rates of bacteria and leukocytes d_b and d_l respectively. If a treatment can alter one or more of these parameters sufficiently to take condition (4.28) across equality, then it will make a critical difference in the eradication of a potent infection. Example treatments include bacteriostatic/bactericidal antibiotics, which reduce the growth rate and increase the death rate of bacteria respectively and a treatment that increases the production rate or life span of leukocytes. The effector protein parameters, while not involved in the stability of the healthy state, do impact the feasibility of diseased states implicitly via the p term of condition (4.18) and the variable values in any diseased states. The model suggests that in order to avoid potential aseptic health problems, an effective treatment strategy is to reduce the efficacy of effector proteins allowing the immune response to return to its usual strength, rather than boosting the immune response to try and overpower the effector proteins as this can lead to the immune response being in a state of overdrive once the infection has been eradicated. The parameters mentioned above, or ratios of them, will be explored in more detail in subsequent analysis since they play a role in governing the stability of the steady states.

4.4 Basic model: sensitivity, bifurcation and numerical simulation analyses

4.4.1 Overview

The complexity of the model precludes further analytic exploration, therefore we turn to numerical methods to further our understanding of the model dynamics. We carry out the same analyses that were carried out for the general infection model (see Chapter 3). First, we carried out a steady state sensitivity analysis to identify parameters that should be explored in detail (in addition to those identified in section 4.3). We then carried out a bifurcation analysis to look for quantitative and qualitative changes in system behaviour when parameters are varied. The results from both of these explorations are compared with the analytic steady state and stability results. We carried out analyses in MATLAB and XPPAUT. Time-dependent simulations are used to explore the dynamics observed in the bifurcation analysis. We adopt the interpretation (as in the general infection model) that the infection is eradicated if the density of bacteria becomes sufficiently low, as the continuous ODE representation of the infection breaks down when the discreteness of the number of bacteria becomes significant. Finally we draw biological interpretations from the results regarding the infection, the immune response and treatment strategies. Table 4.2 gives the baseline parameter set and the initial conditions that lead to the diseased state and all analysis uses these values unless otherwise stated. Given the absence of relevant experimental data to inform these values, we choose values (through trial and error) that yield qualitatively sensible solutions.

r_b	λ	q	d_b	ϕ	c_b	v	u_{bp}	s_n	r_a
1.5	0.5	3	0.75	0.03	2	1	1	0.6	1.5
d_a	r_l	s_α	d_l	c_{11}	c_{12}	d_{e1}	c_{21}	c_{22}	ψ_{y1}
1	1	1	1	10	12	2	10	10	0.5
ψ_{y2}	d_{e2}	c_{31}	c_{32}	ψ_z	d_{e3}	ψ_n	d_n		
0.5	2	10	10	0.6	2	1	1		
$b_1(0)$	$b_2(0)$	$p(0)$	$a(0)$	$l(0)$	$e_1(0)$	$e_2(0)$	$e_3(0)$	$n(0)$	
0.01	0	0	0	0	0	0	0	0	

Table 4.2: The baseline parameter values and the initial conditions used in the numerical simulations of system (4.10).

4.4.2 Steady state sensitivity analysis

The large number of model parameters means that a full time-dependent parameter investigation is computationally intensive. Therefore, a steady state sensitivity analysis was undertaken to determine those parameters to which the diseased state bacterial density is most sensitive. These parameters, in addition to those that play a role in the stability of the steady states, will be explored in more detail.

We first compute the (diseased) steady state bacterial density via equations (4.10) for the baseline parameter set (see Table 4.2), and denote it $\mathcal{B}_0$. We then undertake a one-at-a-time parameter sensitivity analysis and record the ratio of the new steady state bacterial density, denoted $\mathcal{B}_1$, to $\mathcal{B}_0$. The percentage changes in parameter chosen are -20% , -5% , $+5\%$ and $+20\%$ to allow us to consider different magnitude increases and decreases in parameter values. The results of this analysis are shown in Figure 4.3. The six parameters that the diseased state bacterial density is most sensitive to are: $r_b, d_b, d_l, r_l, d_{e3}$ and c_{31} , with the disease state bacterial density being significantly more sensitive to r_b and d_b than all other parameters. We also found that (1) for a 90% parameter decrease, the diseased state bacterial density is significantly more sensitive to d_b than any other parameter, but is reduced to 0 when r_b and d_l are decreased and (2) for a 200% parameter increase, the diseased state bacterial density is significantly more sensitive to r_b than any other parameter, but is reduced to 0 when d_b is increased (results not shown for brevity). Only one parameter from each of the ratios (r_b/d_b) , (r_l/d_l) and (c_3/d_{e3}) is considered, since an equivalent change in ratio can be achieved for an inverse change in the respective other parameter. The parameters r_l, r_b and c_3 are chosen as we assume that growth/production rates are easier to alter than death/degradation rates in practice.

We then carried out a two-parameter sensitivity analysis in which each possible distinct pair of parameters is varied by $\pm 20\%$. We denote the new steady state bacterial density in this case as $\mathcal{B}_2$ and we record the ratio $(\mathcal{B}_2/\mathcal{B}_0)$. The results for this can be seen in Figure 4.4. The pairs of parameters that give the biggest decrease in the diseased state bacterial density when decreased

are (r_b, d_l) and (r_b, c_{31}) , while those that give the biggest decrease when increased are (d_b, r_l) and (d_b, d_{e3}) . These six parameters are also the parameters that the bacterial steady state is most sensitive to when altered individually. Stability analysis is in agreement with this finding as both decreasing r_b and d_l and increasing d_b and r_l act to move the condition for stability of the disease-free state (4.26a) further from bifurcation (see section 4.3.2). This suggests that a combination treatment that simultaneously hinders the growth of the infection and boosts the attraction/longevity of leukocytes may be more effective than either individual treatment. It is also worth noting that the steady state bacterial density is more sensitive to r_l than s_α , implying that more leukocytes are attracted via inflammatory pathways than via apoptosis pathways. Decreasing the net e_3 production rate in conjunction with decreasing the net growth rate of bacteria significantly reduces the infection size in the diseased state, suggesting that another effective combination treatment style is one that directly affects the bacteria by inhibiting their growth as well as indirectly affecting them by inhibiting their virulence. We now explore the chosen parameters in a numerical bifurcation analysis.

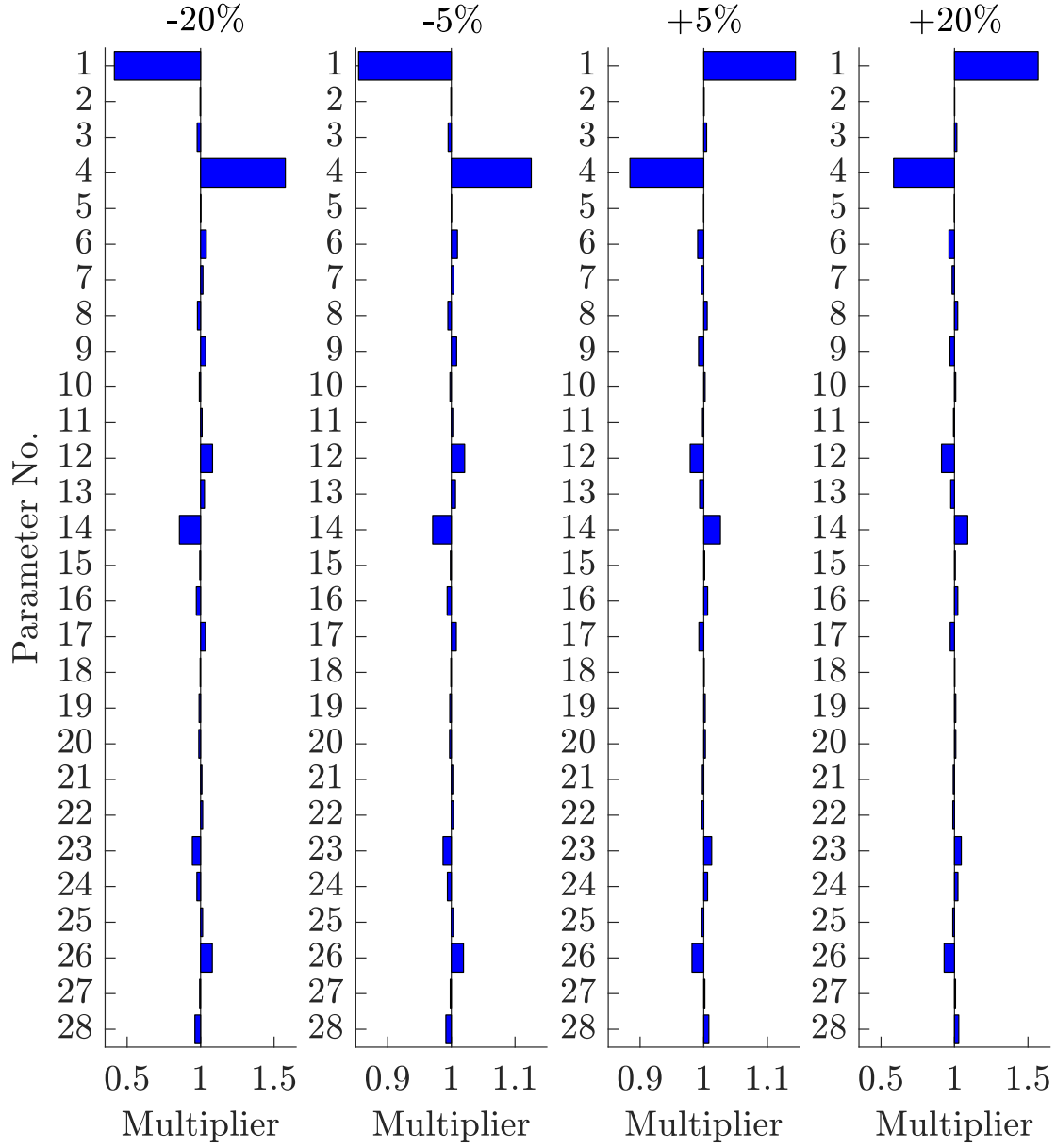


Figure 4.3: A one-at-a-time parameter sensitivity analysis of the bacterial density in the diseased steady state of system (4.10). We show the ratio $(\mathcal{B}_1/\mathcal{B}_0)$ for four different percentage changes in parameter value. The parameters are numbered according to the order they appear in Table 4.2. Each parameter is changed by the amount given in the respective plot title and all unaltered parameters take their baseline values.

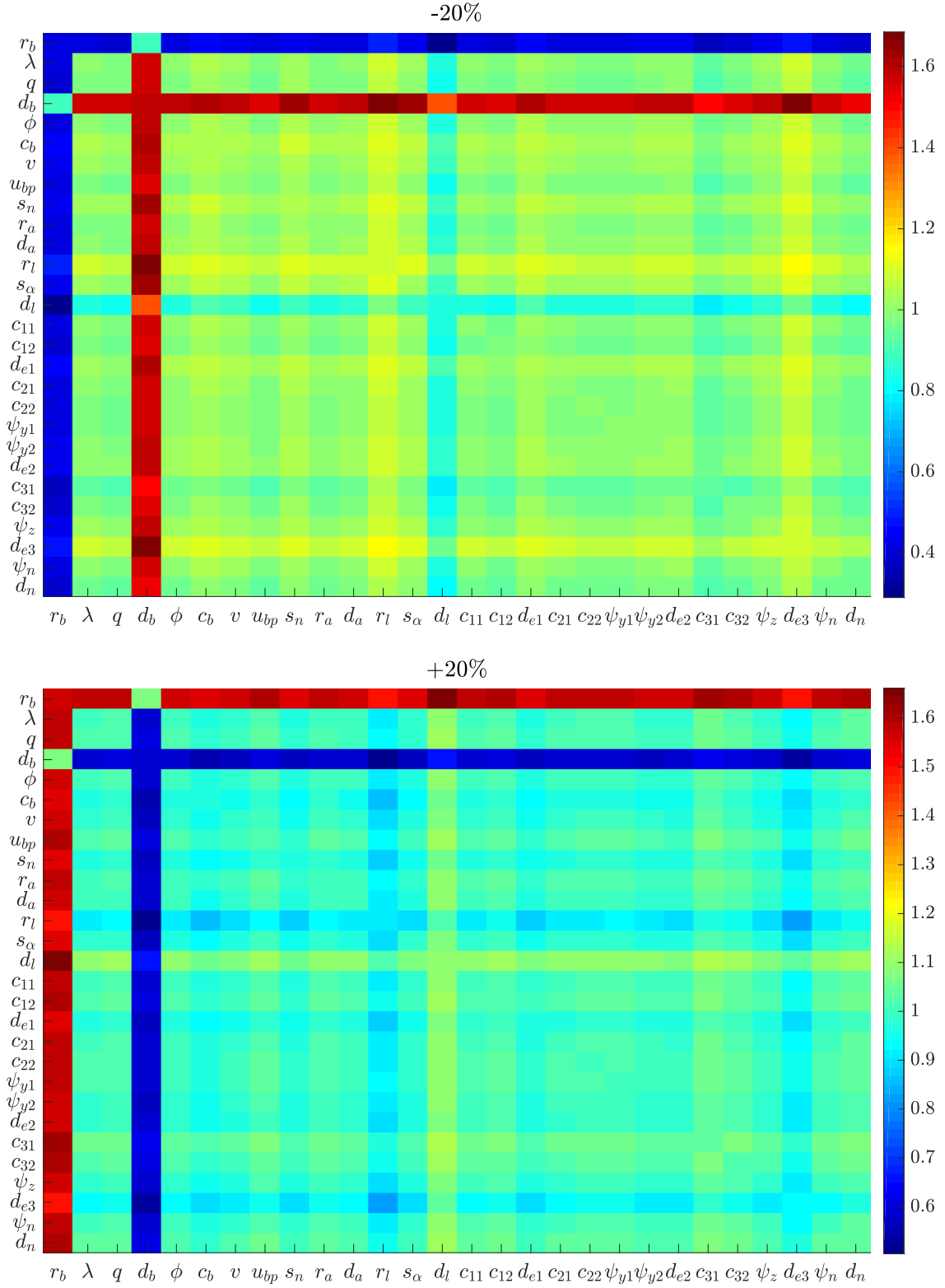


Figure 4.4: A two-at-a-time parameter sensitivity analysis of the bacterial density in the diseased steady state of system (4.10). We show the ratio $(\mathcal{B}_2/\mathcal{B}_0)$ when each possible distinct pair of parameters is changed from the respective baseline values by -20% (top) and $+20\%$ (bottom). All unaltered parameters take their baseline values. In the cases of repeated parameters, the data from the one-at-a-time parameter sensitivity analysis is used as a baseline reference (see Figure 4.3).

4.4.3 Bifurcation analysis

Bifurcation analysis was undertaken using XPPAUT [148]. The parameters $r_b, r_l, \phi, \lambda, q, s_\alpha, s_n, c_1, c_2, c_3$ and d_n are varied so as to look for both quantitative and qualitative changes in the bacterial population in the steady state. We choose many of these parameters for either appearing in bifurcation equation (4.28) or being the parameters to which the bacterial population steady state is most sensitive to. The parameters c_1, c_2, q and λ are chosen to explore the EPEC specific dynamics in more detail and s_α, s_n and d_n are chosen to explore host cell death in detail.

The diseased steady state becomes infeasible for sufficiently low r_b and sufficiently high r_l or ϕ : Figure 4.5(a) shows a bifurcation diagram of the diseased state bacterial population as the growth rate of bacteria (r_b) is varied. There is a transcritical bifurcation at $r_b \approx 0.78$, as predicted by condition (4.28). When the diseased state exists, the total bacteria steady state increases approximately linearly with r_b . Figure 4.5(b) shows a bifurcation diagram of the bacterial population as the inflammation-stimulated attraction rate of leukocytes (r_l) is varied. The density of bacteria decreases sharply as r_l increases from 0 and there is a transcritical bifurcation at $r_l \approx 25$, as predicted by condition (4.28). For higher values of r_l , the disease is completely eradicated. In the absence of stimulated attraction of l by inflammation, the bacterial population is ≈ 0.9 and it is worth noting that in the total absence of leukocytes, the bacterial population is equal to $(r_b/d_b) - 1 = 1$. This implies that the majority of leukocytes are attracted by inflammation and only a small number by apoptosis markers. Figure 4.6(a) shows a bifurcation diagram of the bacterial population as the background production rate of pro-inflammatory mediators (ϕ) is varied. There is a transcritical bifurcation at $\phi \approx 0.97$ and a saddle-node bifurcation at $\phi \approx 3.70$. The system exhibits bi-stability between these two values, with a disease-free state and a diseased state coexisting. However, these values do not satisfy $0 < \phi \ll 1$, so we do not consider this behaviour in further detail; a background pro-inflammatory mediator production rate this high would represent an autoimmune disorder. Figure 4.6(b) gives a two parameter bifurcation diagram showing the transcritical bifurcation shown in Figure 4.6(a) for varying r_b and ϕ (black line). We see that there is a positive trend between the two — if bacteria proliferate faster, then a higher p is needed to mount a sufficiently strong immune response to eradicate the infection. The magenta line shows condition (4.28) plotted for varying values of r_b and ϕ . We see that this condition is a good approximation to the location of the bifurcation for sufficiently small ϕ , but becomes inaccurate for larger ϕ ($\phi > 1.5$). Since we are only concerned with $0 < \phi \ll 1$, the approximation is sufficiently accurate.

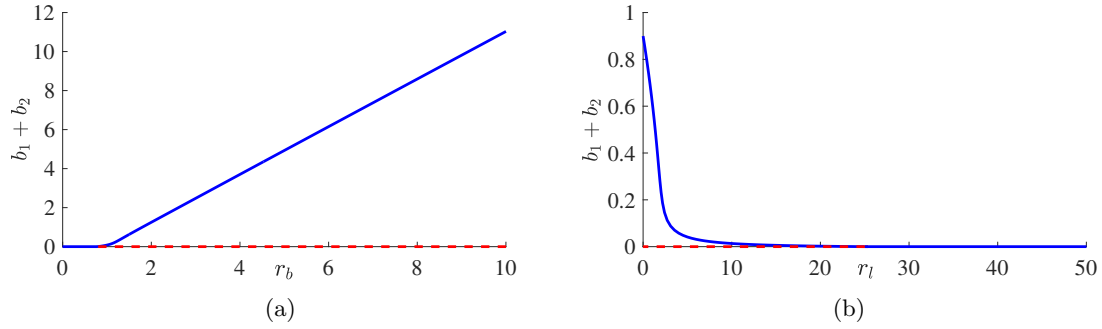


Figure 4.5: Bifurcation diagram for the total bacterial population in the steady states of system (4.10) for (a) varying r_b and (b) varying r_l for the baseline parameter set. Blue and dashed red lines represent stable and unstable steady states respectively.

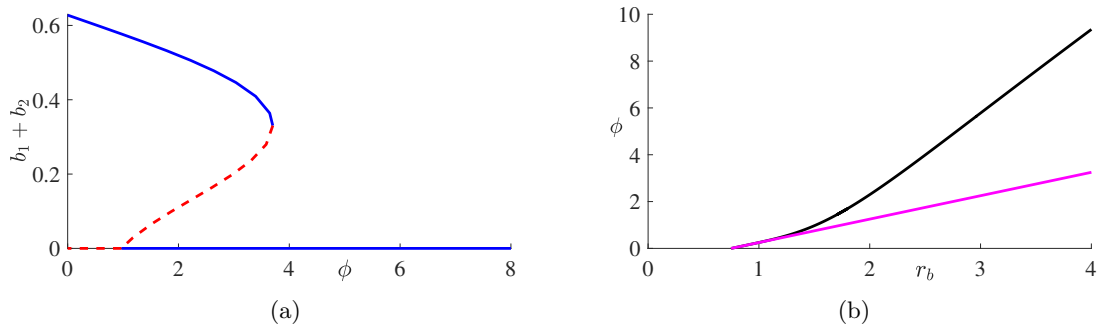


Figure 4.6: (a) Bifurcation diagram for the total bacterial population in the steady states of system (4.10) for varying ϕ for the baseline parameter set. Blue and dashed red lines represent stable and unstable steady states respectively. (b) Two parameter bifurcation diagram showing the position of the transcritical bifurcation shown in (a) as a function of r_b and ϕ (black line) and a plot of equation (4.28) as a function of r_b and ϕ (magenta line).

Differences in the phenotypes of bacteria progeny do not significantly affect the infection size in the diseased steady state: Figure 4.7(a) shows the steady state values of b_1 , b_2 and the total bacterial population as the parameter governing the phenotypes of b_2 progeny (λ) is varied, to explore how the split in internalized p during bacterial replication affects the potency of the infection. We remind the reader that $\lambda = 0$ and $\lambda = 1$ result in all b_2 progeny being b_2 and b_1 respectively. The b_1 and b_2 populations increase and decrease respectively for increasing λ . The total bacterial population remains approximately unchanged for varying λ . This is an unexpected result; we expected the infection to be more successful when more progeny are of the virulent phenotype b_2 . However, having progeny created as b_1 allows more p to be internalized and removed from the environment, leading to reduced attraction of leukocytes.

The infection benefits from the internalization of pro-inflammatory mediators: Figure 4.7(b) shows the steady state values of b_1 , b_2 and the total bacterial population as the pro-inflammatory mediator internalisation rate (q) is varied. b_1 and b_2 decrease and increase respectively for increasing q and plateau for large q . There is a small initial increase in b_1 from $q = 0$ with b_1 peaking at $q \approx 0.63$. We speculate this to be due to the reduced rate of removal of b_1 by the immune response,

caused by the sequestration of p from the environment, being of a slightly larger magnitude than the conversion of b_1 into b_2 . This explanation is consistent with the fact that the total population of bacteria increases (and then plateaus) for increasing q . Together, these results confirm that the infection benefits from the internalization of pro-inflammatory mediators and that inhibiting this mechanism could be an effective treatment strategy.

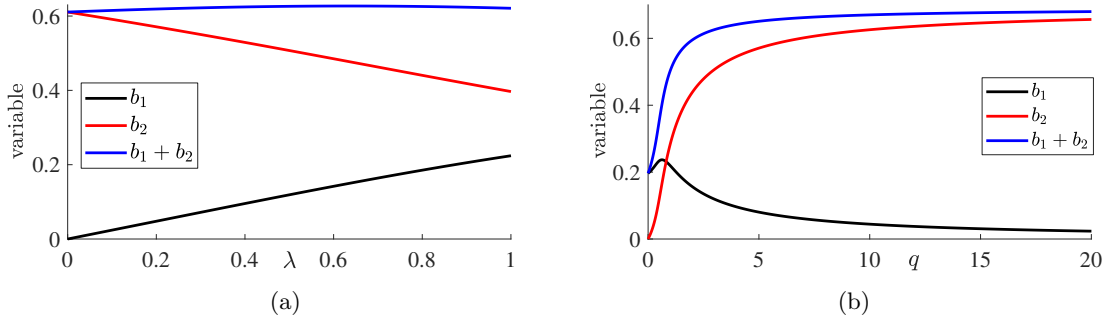


Figure 4.7: Diagrams for the bacterial populations in the diseased steady state of system (4.10) for (a) varying λ and (b) varying q for the baseline parameter set. No bifurcations occur as λ or q are varied.

The infection can be eradicated for a sufficiently large rate of apoptosis-stimulated leukocyte attraction or necrosis-driven pro-inflammatory mediator production: Figure 4.8(a) shows a bifurcation diagram of the bacterial population as the apoptosis-stimulated attraction rate of leukocytes (s_α) is varied. As s_α increases, the steady state bacterial population decreases and sharply initially, tending to zero for large s_α . For large enough s_α , the bacterial population is sufficiently reduced that it is regarded as eradicated. The parameters r_l and s_α are the two rates of attraction of leukocytes. It is worth noting that the infection can be totally eradicated for large enough r_l (see Figure 4.5(b)), but not for s_α and that the population of bacteria decreases faster for increasing r_l than it does for increasing s_α . This indicates, that r_l is a more important parameter to alter than s_α to achieve eradication. This result is in agreement with the sensitivity analysis (see Figures 4.3 and 4.4) — that the majority of leukocytes are attracted by inflammation and only a small number by apoptosis markers. Figure 4.8(b) shows a bifurcation diagram of the bacterial population as the production rate of pro-inflammatory mediators due to host cell necrosis (s_n) is varied. The results here are qualitatively similar to those for varying s_α (Figure 4.8(a)) — for large enough s_n , the bacterial population is sufficiently reduced that it is regarded as eradicated.

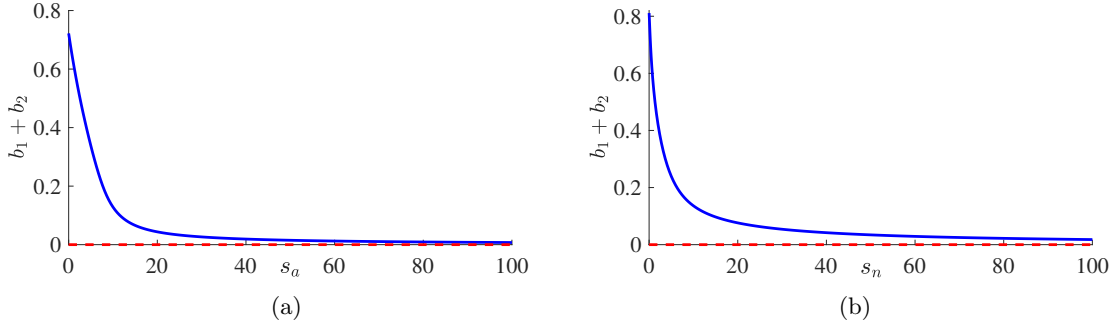


Figure 4.8: Diagrams for the total bacterial population in the steady states of system (4.10) for (a) varying s_α and (b) varying s_n for the baseline parameter set. No bifurcations occur as s_α or s_n are varied. Blue and dashed red lines represent stable and unstable steady states respectively.

The effector proteins that inhibit phagocytosis have a greater effect on the bacterial population in the diseased state than the other effector protein groups: To make it easier to investigate the effects of the global production rates of effector proteins on bacterial growth, we assume that the production rates by both bacterial phenotypes are equal; i.e. $c_{11} = c_{12} = c_1$, $c_{21} = c_{22} = c_2$ and $c_{31} = c_{32} = c_3$, where c_{ij} is the production rate of e_i by b_j . Figure 4.9(a) shows the effects on the bacterial population in the steady state when the global production rate of each effector protein group is varied individually whilst keeping the others fixed at their baseline values. Figure 4.9(b) shows the same but whilst the other groups are absent. Both figures show that varying e_3 produces the biggest effect on the bacterial population in the steady state which is supported by the results of the sensitivity analysis (c_3 and d_{e3} are two of the eight parameters that the diseased state is most sensitive to, while c_1, c_2, d_{e1} and d_{e2} are not). It appears that e_2 has the least prominent effect on the bacterial population in the steady state and makes surprisingly little difference when e_1 and e_3 are switched off, implying that e_2 may only work in synergy with the other groups.

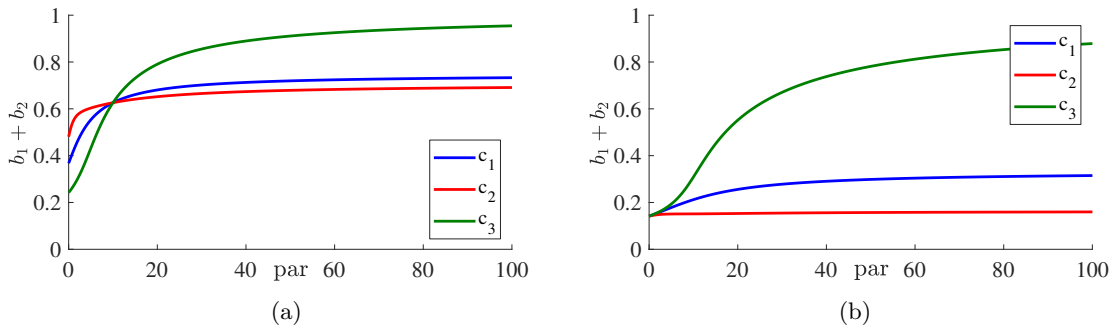


Figure 4.9: Diagrams for the total bacterial population in the diseased steady state of system (4.10) as c_1, c_2, c_3 are varied individually with the other two (a) fixed at their baseline values and (b) set to zero. All other parameters take their baseline values. No bifurcations occur as c_1, c_2 or c_3 are varied.

Effector proteins are worthwhile targets for novel anti-virulence treatment strategies: Figure 4.10(a) shows how the p concentration in the diseased state varies with c_1 , highlighting that e_1 reduces it by over 80% when $c_1 \gg 1$. Together, Figures 4.10(a) and 4.9 suggest that inhibiting pro-

inflammatory mediator production and phagocytosis are effective virulence mechanisms, with the latter being more effective. Given that treatments that indirectly hinder infections are less likely to promote genetic resistance (see Chapter 2), these mechanisms could be worthwhile targets for novel anti-virulence treatment strategies.

The infection size oscillates when dead host cells take a long time to repair: Figure 4.10(b) shows a bifurcation diagram of the bacterial population as the repair rate of dead host cells (d_n) is varied. For large d_n , the steady state bacterial population is approximately 0.8. As d_n decreases, the steady state bacterial population decreases, implying that the infection is less potent when dead host cells take longer to repair. The finding that a slower rate of host repair is beneficial to the host is counter-intuitive, but not illogical; a more inflamed environment elicits a stronger leukocyte response. Requiring the maintenance of the immune response above a critical response is a result that is also in keeping with other studies, including Ternent et al. [85] and Gjini et al. [86]. The diseased state becomes unstable at $d_n \approx 0.17$, where there is a Hopf bifurcation creating a stable limit cycle for d_n below this value. In this parameter region, the infection size oscillates; however for d_n sufficiently small, the lower bound of the oscillations is sufficiently low that we assume the infection is eradicated (see section 4.4.1). The peak size of the recurrent infection is approximately half that when d_n is large.

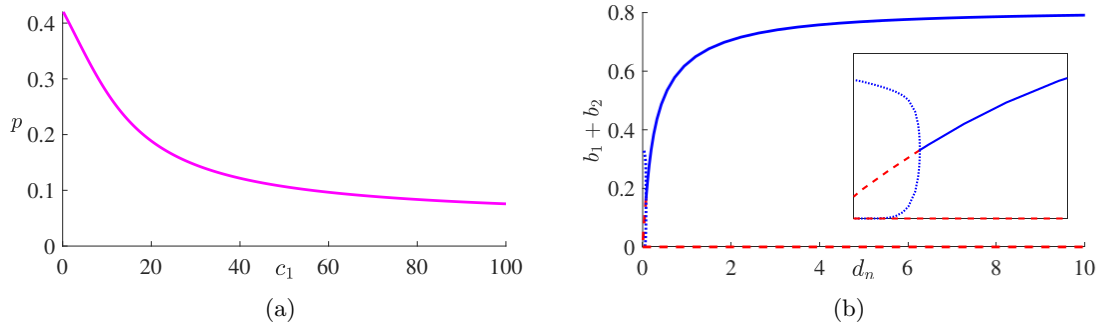


Figure 4.10: (a) Graph showing the diseased state p population of system (4.10) for varying c_1 for the baseline parameter set. (b) Bifurcation diagram for the total bacterial population in the steady states of system (4.10) for varying d_n for the baseline parameter set. The overlaid plot zooms in on the bifurcation at $d_n \approx 0.17$, highlighting the presence of a stable limit cycle. Full blue, dashed red and dotted blue lines represent stable steady states, unstable steady states and stable limit cycles respectively.

Summary

We carried out a sensitivity analysis to determine which parameters the diseased state is most sensitive to. We considered these parameters, along with those present in the disease-free stability condition (equation (4.28)), for bifurcation analysis. If the growth and death parameters of a variable were present, we chose the growth parameter as we assume it is easier to alter in clinical practice. Conclusions obtained are reversed for the corresponding death rates. We also chose to explore the EPEC specific dynamics in detail.

A bifurcation analysis of system (4.10) found that treatments that sufficiently:

1. inhibit the growth or promote the death of bacteria,
2. increase the attraction rate or survival time of leukocytes,
3. increase the rate of apoptosis-stimulated leukocyte attraction,
4. increase the rate of pro-inflammatory mediator production due to host cell necrosis,

result in the eradication of the infection. Increasing the background pro-inflammatory mediator production rate achieves a similar result, but is not a recommended strategy as it leads to auto-immune health problems such as leukemia and inflammatory diseases. Care must be taken when altering the parameters governing the strength of the stimulated immune response (via both inflammation and host cell death) to reduce the risk of similar health problems. Inhibiting the production of, or promoting the removal of, effector proteins, particularly those that inhibit phagocytosis, reduces the steady state infection size but not enough to eradicate the infection. We also found that:

1. differences in the phenotypes of bacteria progeny do not significantly affect the infection size in the diseased steady state,
2. the infection benefits from the internalization of pro-inflammatory mediators,
3. the pro-inflammatory mediator concentration in the diseased steady state is significantly reduced when the production of the effector proteins that inhibit their production is high,
4. the infection is less potent when dead host cells take longer to repair.

A two-parameter sensitivity analysis predicts that combined treatments are more effective than individual treatments, in particular treatments that both inhibit the net growth of bacteria and either inhibit the net production of effector proteins or boost the attraction rate or longevity of leukocytes. Indirect treatments, such as those targeting the production or removal of effector proteins, can compliment the actions of more direct treatments, allowing direct treatments to be used at lower intensities, which are likely to be safer for the host.

The behaviours seen in this section will now be explored in more detail by considering numerical simulation of system (4.10).

4.4.4 Numerical simulations

We now present numerical simulations to observe transient behaviours and to explore the results obtained from the bifurcation analysis in more detail. For each parameter explored in the bifurcation analysis, except the background production rate of pro-inflammatory mediators (ϕ), numerical simulations at three distinct parameter values are presented to give a broad display of the possible temporal dynamics. ϕ is omitted from this analysis as it is not a sensible treatment strategy due to the auto-immune health problems it leads to. In Figures 4.12–4.17, only the total density of bacteria (B), the concentration of pro-inflammatory mediators (p) and the density of leukocytes (l) are plotted for brevity.

Figure 4.11 shows numerical simulations for all variables under the baseline parameter regime. Under this regime, the infection survives well and an equilibrium between the immune response and the infection is reached. The effector proteins all peak at slightly different times and values.

The density of e_2 peaks and then decreases due to b_2 and p continuing to increase to levels that give sub-optimal production of e_2 . The density of dead host cells increases and plateaus at a similar time to the density of bacteria and the concentration of pro-inflammatory mediators.

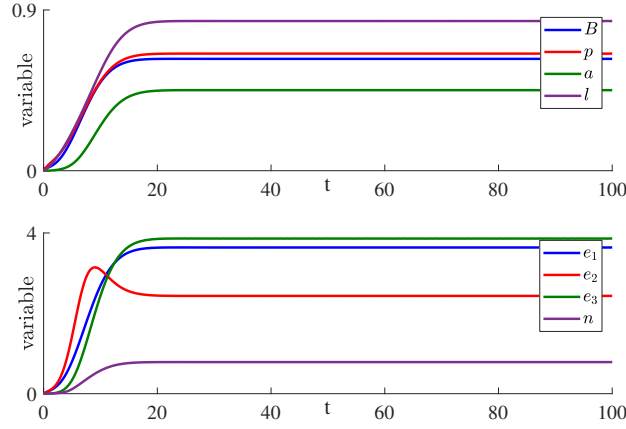


Figure 4.11: Numerical simulations of system (4.10) for the baseline parameter set.

Hindering the net growth of bacteria is an effective treatment strategy: Figure 4.12(a) shows numerical simulations for B , p and l for various r_b values. For $r_b < d_b$ (top panel), the infection dies out regardless of the immune response, as the population dies faster than it grows. For intermediate r_b (middle panel), the infection survives at a small population and we regard this as asymptomatic colonisation. For larger r_b values (bottom panel), the infection survives and thrives, as the immune response is not strong enough to overcome the growth of the bacteria. The variables of the immune response also remain elevated as they continue to fight the infection. Together, these results suggest that hindering the growth rate or reducing the survival time of bacteria could be an effective treatment strategy.

Boosting the inflammation-stimulated leukocyte response is an effective treatment strategy: Figure 4.12(b) shows numerical simulations for B , p and l for various r_l values. When attraction of l due to p is absent (top panel), the bacteria thrive as not enough leukocytes are present to overcome them. For intermediate r_l (middle panel), the bacterial population is reduced to a low level, with the density of leukocytes remaining elevated. For sufficiently large r_l (top panel), the infection is completely cleared, but there is a very high peak in the density of leukocytes which then settles to a steady state value that is still elevated. These results predict that temporarily boosting the inflammation-stimulated leukocyte response is an effective treatment strategy.

Altering the pro-inflammatory mediator apportioning of replicating bacteria does not significantly affect the infection size: We remind the reader here that all b_1 progeny are b_1 , while the proportion of b_2 progeny that are b_1 is governed by $\lambda \in [0, 1]$; $\lambda = 1$ results in all b_2 progeny being b_1 . Figure 4.13(a) shows numerical simulations for b_1 , b_2 and p for various λ values. We found in the bifurcation analysis that the total density of bacteria does not vary much with λ , therefore we consider only the effects on the individual phenotypes. When all b_2 progeny are b_2 (top panel), all bacteria end

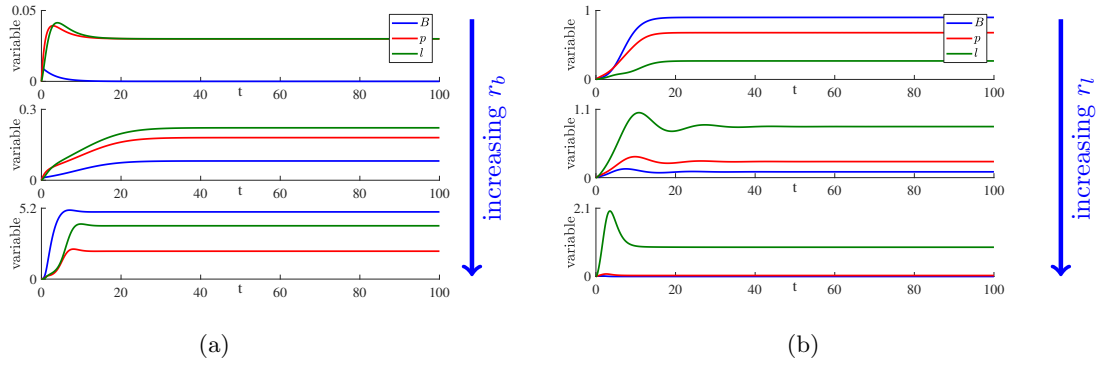


Figure 4.12: Numerical simulations of system (4.10) for (a) $r_b = 0.5, 1, 5$ respectively and (b) $r_l = 0, 3, 30$ respectively, with all other parameters at their baseline values.

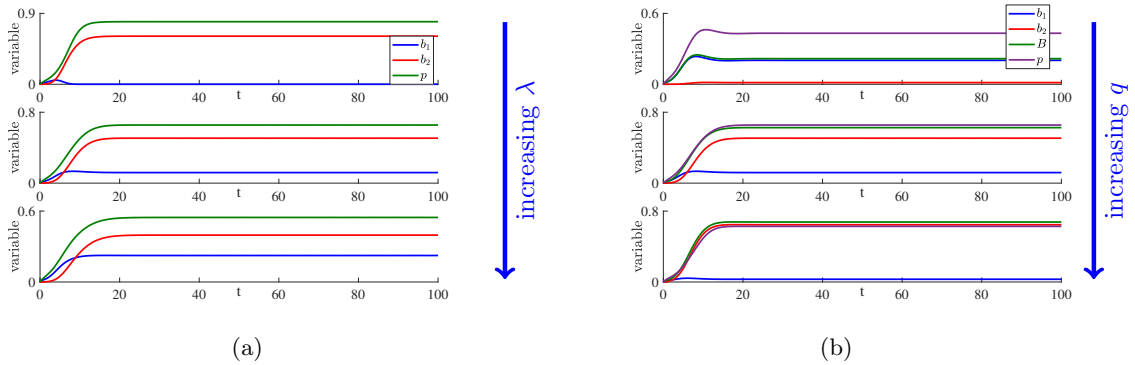


Figure 4.13: Numerical simulations of system (4.10) for (a) $\lambda = 0, 0.5, 1$ respectively and (b) $q = 0.1, 3, 15$ respectively, with all other parameters at their baseline values.

up as b_2 very quickly. When half of b_2 progeny are b_2 (middle panel), there is ultimately a fraction ($\approx 17\%$) of the total bacteria that are b_1 ; however, the majority are b_2 . When all b_2 progeny are b_1 (bottom panel), then approximately 35% of all bacteria are b_1 . This implies that the majority of bacteria are always b_2 . The concentration of p goes down as λ increases due to more offspring being b_1 , allowing for more internalization of p . These results suggest that shifting the p apportioning of replicating bacteria may not be an effective treatment strategy.

Inhibiting the internalization of pro-inflammatory mediators by bacteria contributes to eradication: Figure 4.13(b) shows numerical simulations for b_1 , b_2 , B and p for various q values. As q increases, b_1 and p decrease while b_2 increases as expected. The total density of bacteria increases and plateaus with increasing q , demonstrating that the infection benefits from the internalization of p . Inhibiting this mechanism is an effective treatment strategy; however, it cannot lead to eradication of the infection on its own – a treatment of this form will act as a supplementary treatment to a more direct treatment strategy.

Boosting the apoptosis-stimulated leukocyte response is an effective treatment strategy: Figure 4.14(a) shows numerical simulations for B , p and l for various s_α values. In the absence of l attraction via apoptosis (top panel), the immune response from inflammation is not strong enough

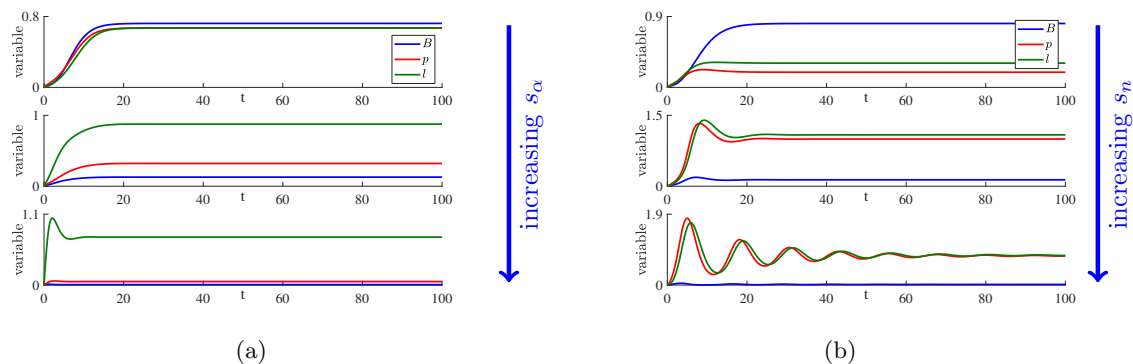


Figure 4.14: Numerical simulations of system (4.10) for (a) $s_\alpha = 0, 10, 100$ respectively and (b) $s_n = 0, 10, 100$ respectively, with all other parameters at their baseline values.

to overcome the infection and the infection thrives. As s_α increases (middle and bottom panels), the bacterial population decreases and is reduced to a negligible size for a sufficiently large value; therefore, the infection is eradicated. l increases with increasing s_α as expected, but remains elevated post-infection, similar to the case of high r_l . The peak density of l is considerably lower for high s_α than it is compared with high r_l , suggesting that altering s_α might be the safer of the two treatment styles in order to avoid self-injurious disease. Together, these results suggest that temporarily boosting the apoptosis-stimulated leukocyte response is an effective treatment strategy.

Increasing the pro-inflammatory mediator release during host cell necrosis is an effective treatment strategy: Figure 4.14(b) shows numerical simulations for B , p and l for various s_n values. In the absence of p production from dead host cells (top panel), the infection is not eradicated. For intermediate s_n (middle panel), the bacterial population is reduced to a negligible size, while the immune response remains elevated. For large s_n (bottom panel), p and l are elevated quickly and exhibit decaying oscillation down to steady state values that are surprisingly lower than they are for intermediate s_n (although not significantly low). These results suggest that increasing the concentration of p released during host cell necrosis is an effective treatment strategy.

Inhibiting the effector proteins that inhibit pro-inflammatory signalling and phagocytosis contributes to eradication: Figure 4.15(a) shows numerical simulations for B , p and l for various c_1 values. In the absence of e_1 (top panel), the infection persists at a low level (≈ 0.37). For increasing c_1 (middle and bottom panels), B increases and plateaus at a higher steady state. p decreases with increasing c_1 as its production is inhibited and the consequential reduction in l results in the observed larger infection size. Figure 4.15(b) shows numerical simulations for B , p and l for various c_2 values. In the absence of e_2 (top panel), the infection persists at a low level that is higher than that in the absence of e_1 (≈ 0.48 compared with 0.37). B increases with increasing c_2 (middle and bottom panels) and plateaus at a value comparable with that for large c_1 (0.69 vs 0.73 respectively). While p increases with e_2 due to more host cells necrosing, l decreases slightly, implying that the net effect of more inflammation and fewer apoptosis markers benefits the infection. Figure 4.16(a) shows numerical simulations for B , p and l for various c_3 values. In the absence of e_3 (top panel), the infection persists at a low level that is lower than that in the absence of e_1 or e_2 (≈ 0.24 compared

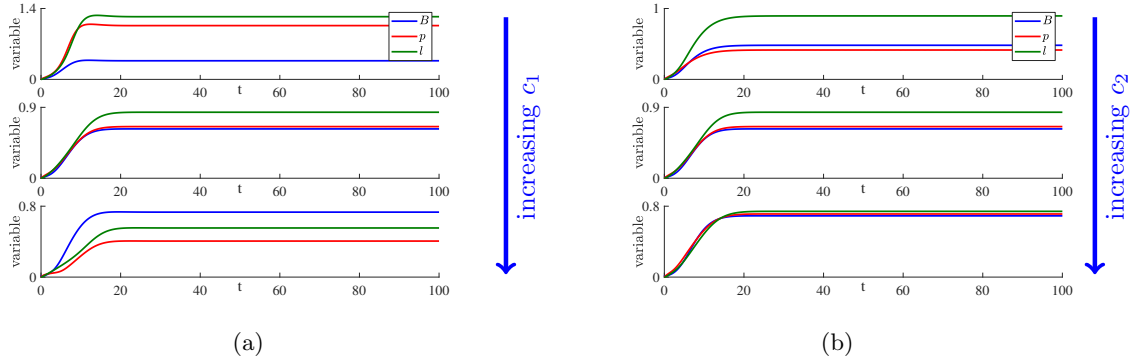


Figure 4.15: Numerical simulations of system (4.10) for (a) $c_1 = 0, 10, 100$ respectively and (b) $c_2 = 0, 10, 100$ respectively, with all other parameters at their baseline values.

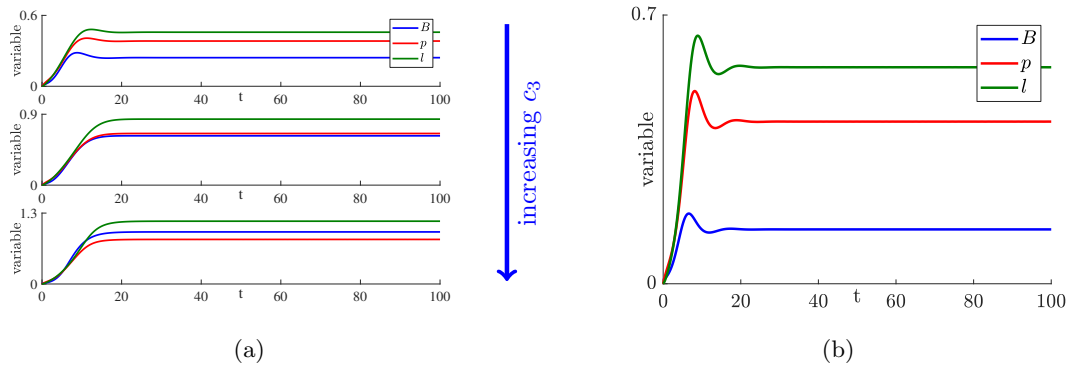


Figure 4.16: Numerical simulations of system (4.10) for (a) $c_3 = 0, 10, 100$ respectively and (b) $c_1, c_2, c_3 = 0$ respectively, with all other parameters at their baseline values.

with 0.37 or 0.48). B increases with increasing e_3 and reaches a value higher than for increasing e_1 or e_2 (≈ 0.95 compared with 0.73 or 0.69 respectively). p and l increase as e_3 increases, as a reaction to the increase in B . Figure 4.16(b) shows numerical simulations for B , p and l when $c_1, c_2, c_3 = 0$. The bacterial steady state in this case is approximately 0.14, which is lower than that for any of the cases with only one effector protein group turned off and the steady state values for p and l are also lower. Together, these results imply that inhibiting the production or reducing the survival time of any effector proteins reduces the infection size. By comparing the differences in the infection size at the steady state, we see that removing e_3 has the biggest negative effect on the infection size. This implies that inhibiting phagocytosis is the most effective virulence mechanism of EPEC; therefore, in agreement with results from the bifurcation analysis, inhibiting this group of effector proteins may be the most effective individual anti-effector protein treatment. Treatments that target different groups will work in synergy, so by combining anti-effector protein treatments, the virulence of the infection can be vastly reduced. However, even in the absence of all effector proteins the infection is not eradicated, therefore anti-effector protein treatments will need to be combined with other treatment strategies to achieve eradication. This is in keeping with other anti-virulence studies in the literature, both mathematical [85, 150] and experimental [151, 152].

The infection is less potent when dead host cells take longer to repair: Figure 4.17 shows numerical

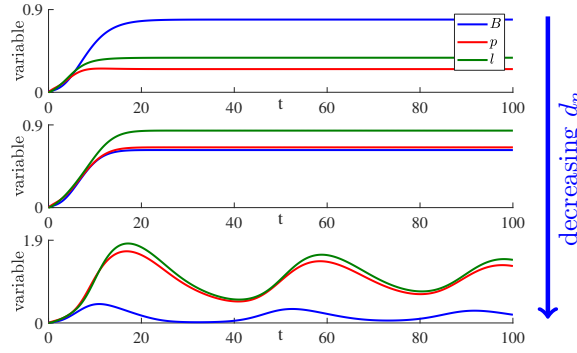


Figure 4.17: Numerical simulations of system (4.10) for $d_n = 10, 1, 0.08$, with all other parameters at their baseline values.

simulations for B , p and l for various d_n values. For large d_n (top panel), the quick repair of dead host cells leads to a less inflamed environment and so a weaker immune response, allowing the infection to thrive. For intermediate d_n (middle panel), the environment becomes more inflamed leading to a stronger immune response, leading to higher values for p and l . This then leads to the bacterial population being reduced in size. For sufficiently low d_n (bottom panel), the bacterial population oscillates; however, the lower bound of the oscillations is sufficiently low that the infection is regarded as eradicated. These results imply that the infection is less potent when dead host cells take longer to repair, since a more inflamed environment elicits a stronger leukocyte response.

Summary

Numerical simulations have highlighted many potential treatment styles, some of which reduce the virulence of an infection while others work to reduce the infection size. The results suggest that some treatment strategies can work individually to eradicate an infection but some work well in concert, particularly a combination of an anti-virulence treatment combined with a bacteriostatic or a bactericidal treatment. The results presented here are in agreement with those found in the bifurcation analysis.

The most effective individual EPEC infection treatment predicted by this model is either a bacteriostatic (reduces growth rate) or a bactericidal (increases death rate) treatment. This implies that, regardless of additional virulence mechanisms such as the secretion of effector proteins, the best results are attained by directly attacking the bacteria. Antibiotic resistance has not been considered in this model, therefore while these treatment strategies may work well on many strains, they may not work well on more resistant strains. Direct treatments such as these are also likely to promote antibiotic resistance being passed on to future generations of bacteria which is an undesirable consequence. Temporarily boosting the leukocyte attraction rate, both by inflammation and host cell death pathways, is predicted to be an effective strategy; however, care must be taken to ensure that the white blood cell count does not reach dangerous levels during the course of the treatment. Boosting the leukocyte attraction via host cell death can result in a comparable reduction in infection size to that via inflammation pathways, but does not result in as high a peak in the density of leukocytes implying that this method may be safer for the host. Inhibiting the ability of EPEC to

internalize host pro-inflammatory mediators is an effective anti-virulence strategy; however, it will need to act in concert with another more direct treatment strategy to achieve full eradication of the infection. It works well as an additional treatment as it does not particularly elevate the immune response, therefore it is less likely to lead to the immune response going into a state of overdrive when combined with other treatment strategies. Treatment strategies that reduce the production of any effector proteins are beneficial, but targeting the production of those that inhibit phagocytosis is the most effective individual strategy. Combining treatment methods in such a way that all effector proteins have reduced production leads to a large reduction in the infection size; however, this anti-virulence treatment method is not strong enough to eradicate the infection and needs to be combined with a more direct treatment such as a bacteriostatic or a bactericidal treatment. It is thought that anti-virulence treatments such as the inhibition of effector protein production or the internalization of pro-inflammatory mediators are unlikely to promote antibiotic resistance due to their indirect nature, which makes them appealing as accessory treatments. While each of the discussed treatment styles has its benefits and drawbacks, the best treatment or combination of treatments to take may depend on the circumstances of the individual patient. For example, a patient with a weak immune response may respond better and more safely to a treatment that boosts the attraction rate of leukocytes than a patient with a strong immune response.

4.4.5 EPEC basic model summary

The analytical and numerical explorations of the EPEC basic model have revealed several potentially effective treatment strategies which are given, along with their benefits and drawbacks, in Table [4.3](#).

Treatment strategy	Benefits	Drawbacks
Reducing the growth rate or increasing the death rate of bacteria	Can eradicate the infection and does not cause a dangerously strong immune response	Susceptible to and likely to promote, antibiotic resistance
Temporarily increasing the attraction rate of leukocytes via inflammation	Can eradicate the infection	Can lead to a dangerously strong immune response, which can lead to autoimmune diseases
Temporarily increasing the attraction rate of leukocytes via apoptosis markers	Can eradicate the infection (but not as well as via inflammation)	Can lead to a dangerously strong immune response, which can lead to autoimmune diseases (but not as severe as via inflammation)
Inhibiting the ability of EPEC to internalize host pro-inflammatory mediators	Does not elicit a strong immune response and is not likely to promote antibiotic resistance	Cannot eradicate the infection on its own
Inhibiting the production of effector proteins	Does not elicit a strong immune response and is not likely to promote antibiotic resistance	Cannot eradicate the infection on its own

Table 4.3: The potentially effective treatment strategies given by the EPEC basic model along with their benefits and drawbacks.

The main finding from the analysis of our basic EPEC model (equations (4.10)) is that a combination of direct anti-bacterial treatments and indirect anti-virulence or immune-boosting treatments is likely to yield the best results both short term and long term. We now move on to discuss and analyze a multi-scale coupled EPEC model (referred to as the coupled model from here on) that includes a sub-cellular GRN governing bacterial protein synthesis as well as a class of antibiotics (aminoglycosides) that acts to inhibit this protein synthesis.

4.5 A subcellular gene network (GRN) for bacterial protein synthesis

4.5.1 Introduction

First, we formulate a sub-cellular GRN that governs bacterial protein synthesis, comprising a population-level ODE model and accomodating the effects of aminoglycosides (a class of antibiotic) on bacterial protein synthesis. Analysis of this GRN illuminates potentially effective treatment strategies that target the mechanisms of the GRN. In section 4.6, we incorporate this GRN into the basic EPEC model to yield a more biologically detailed model for an EPEC infection.

4.5.2 Formulation of the GRN

The GRN is formulated based on the literature discussed in the literature review (Chapter 2) and the recent discovery by experimental collaborators that EPEC become less sensitive to aminoglycosides when they have internalized host pro-inflammatory mediators (see section 2.5.1). This model has six variables: the numbers of unbound mRNA (M_0), bound mRNA (M_1), functioning proteins (Γ) and mistranslated proteins (Γ_X) and the (internalised) concentrations of aminoglycosides (A) and pro-inflammatory mediators (P). As the model is population-level, we can construct an ODE representation of the dynamics. Figure 4.18 shows a schematic diagram of the GRN dynamics.

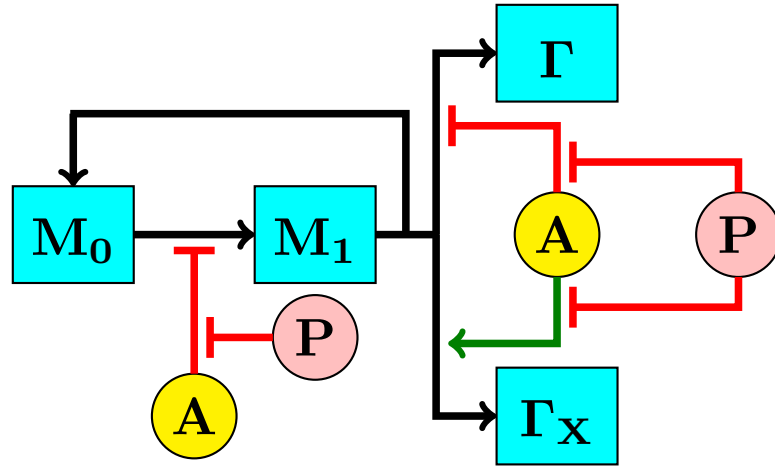


Figure 4.18: Schematic diagram of the GRN dynamics. Black, green and red arrows represent reactions, up-regulation and down-regulation respectively.

The GRN evolves according to:

$$\dot{M}_0 = R_0 + R_\Gamma \bar{M}_1 - R_1(1 - f_M)\bar{M}_0 - K_M \bar{M}_0, \quad (4.29a)$$

$$\dot{M}_1 = R_1(1 - f_M)\bar{M}_0 - R_\Gamma \bar{M}_1, \quad (4.29b)$$

$$\dot{\Gamma} = R_\Gamma(1 - f_\Gamma)\bar{M}_1 - K_\Gamma \bar{\Gamma}, \quad (4.29c)$$

$$\dot{\Gamma}_X = R_\Gamma f_\Gamma \bar{M}_1 - K_{\Gamma X} \bar{\Gamma}_X, \quad (4.29d)$$

where:

$$f_M(A, P) = \left(\frac{A}{Q_M + A} \right) \left(\frac{Q_P}{Q_P + P} \right), \quad (4.30a)$$

$$f_\Gamma(A, P) = \left(\frac{A}{Q_\Gamma + A} \right) \left(\frac{Q_P}{Q_P + P} \right). \quad (4.30b)$$

M_0 is synthesized by the bacterium at a constant rate R_0 from nutrients sourced from its environment and ribosomes bind to M_0 at rate R_1 , converting them into M_1 . Translation occurs at

rate R_Γ and results in the production of proteins (either Γ or Γ_X). We assume that M_0, Γ and Γ_X degrade naturally at rates K_M, K_Γ and K_{Γ_X} respectively, where $K_{\Gamma_X} > K_\Gamma$ since mistranslated proteins are broken down more frequently to make amino acids available for the assembly of other proteins. We assume for simplicity that bound mRNA do not degrade. Aminoglycosides inhibit (1) the binding of ribosomes to M_0 , reducing the conversion rate of M_0 to M_1 and (2) ribosomal proof-reading, resulting in more of the proteins created being Γ_X . We incorporate both of these actions by making the conversion rate of M_0 to M_1 a decreasing function of A , and the production rates of Γ and Γ_X decreasing and increasing functions of A respectively. We choose these functions to be Hill functions with coefficient 1 and half-maximal concentrations Q_M and Q_Γ for mRNA binding and proof-reading respectively.

As discussed in the literature review (see section 2.5.1), it has been found experimentally that internalized pro-inflammatory mediators make EPEC less sensitive to the action of aminoglycosides, but the mechanism of action is not known. Following discussion with our experimental collaborators, we hypothesize that internalized pro-inflammatory mediators inhibit the action of the antibiotic via competition for binding sites. We therefore introduce a P -dependent inhibition term in the form of a decreasing Hill function, with coefficient 1 and half-maximal concentration Q_P . This term decreases monotonically as P increases and multiplies the aminoglycoside action terms, thus inhibiting them. We assume for simplicity that the inhibitory effect of P is the same strength on both aminoglycoside actions. In the absence of antibiotic, correctly translated protein synthesis occurs unaltered and for a sufficiently large concentration of antibiotic, all protein production is switched off. However, for a sufficiently large concentration of internalized pro-inflammatory mediators, the effects of the antibiotic are completely inhibited regardless of its concentration. The total rate of protein production is conserved under proof-reading inhibition.

We now move to explore the dynamics of (4.29). In sections (4.5.3) and (4.5.4), we consider a constant antibiotic concentration and a concentration that varies over time respectively, in both cases analyzing the effects on the protein populations. The concentration of internalized pro-inflammatory mediators is treated as a constant parameter in this analysis since mechanisms for internalization are not considered; however, the concentration will be treated as time-dependent in the coupled model (see section 4.6).

4.5.3 Constant antibiotic concentration

We emphasize here that since P and A are constant, f_M and f_Γ are constant. The steady states of (4.29) are given by:

$$0 = R_0 + R_\Gamma \bar{M}_1 - R_1(1 - f_M) \bar{M}_0 - K_M \bar{M}_0, \quad (4.31a)$$

$$0 = R_1(1 - f_M) \bar{M}_0 - R_\Gamma \bar{M}_1, \quad (4.31b)$$

$$0 = R_\Gamma(1 - f_\Gamma) \bar{M}_1 - K_\Gamma \bar{\Gamma}, \quad (4.31c)$$

$$0 = R_\Gamma f_\Gamma \bar{M}_1 - K_{\Gamma_X} \bar{\Gamma}_X. \quad (4.31d)$$

After some algebra, these provide:

$$(\bar{M}_0, \bar{M}_1, \bar{\Gamma}, \bar{\Gamma}_X) = \left(\frac{R_0}{K_M}, (1 - f_M) \frac{R_0 R_1}{R_\Gamma K_M}, (1 - f_M)(1 - f_\Gamma) \frac{R_0 R_1}{K_M K_\Gamma}, (1 - f_M) f_\Gamma \frac{R_0 R_1}{K_M K_{\Gamma X}} \right). \quad (4.32)$$

As for the basic model (section 4.4), we choose parameter values that give qualitatively sensible results, given the absence of relevant experimental data. Figure 4.19 shows example numerical simulations of (4.29) and the corresponding steady states given by (4.32) when $P, A = 0$, the remaining parameter values given in the figure caption. We see that all variables increase monotonically from 0 to the respective steady state value and that the numerical simulation steady state values are in agreement with (4.32).

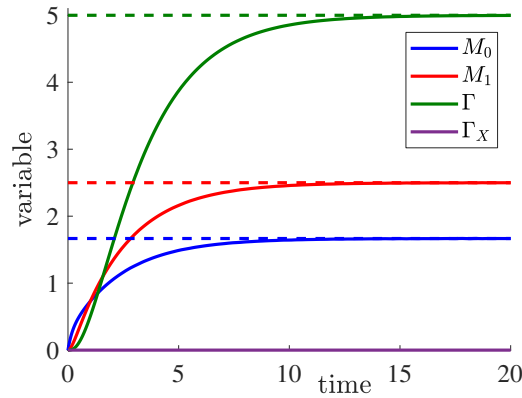


Figure 4.19: Numerical simulations of (4.29) (full lines) and the corresponding steady states given by (4.32) (dashed lines) when $P, A = 0$. Other parameter values: $R_0 = 2, R_1 = 3, R_\Gamma = 2, K_M = 1.2, K_\Gamma = 1, K_{\Gamma X} = 1.5$.

We are interested in the number of correctly translated proteins being produced by the GRN, as the coupled model assumes that the production rates of bacteria and effector proteins are functions of Γ (see section 4.6). Figure 4.20 shows numerical simulations of system (4.29) for various (constant) values of P and A with all other parameter values equal to their values in Figure 4.19. In the absence of pro-inflammatory mediators and with a constant supply of antibiotic (Figure 4.20(a)), the steady state Γ population is vastly reduced. This shows that the antibiotic can effectively reduce the density of correctly translated proteins produced in the absence of pro-inflammatory mediators. In the presence of pro-inflammatory mediators (Figure 4.20(b)), the action of the antibiotic is inhibited and as a result, the steady state Γ population is not as sharply reduced. For the same concentration of pro-inflammatory mediators but a much higher antibiotic concentration (Figure 4.20(c)), the steady state Γ population is decreased less than in Figure 4.20(a) but more than in Figure 4.20(b). When both concentrations are higher (Figure 4.20(d)), the dynamics tend to what they are in the absence of the antibiotic, since the action of the antibiotic becomes fully inhibited when P is large, regardless of concentration. Figure 4.21 shows a surface plot of the steady state Γ population as a function of P and A , further demonstrating the results seen in Figure 4.20.

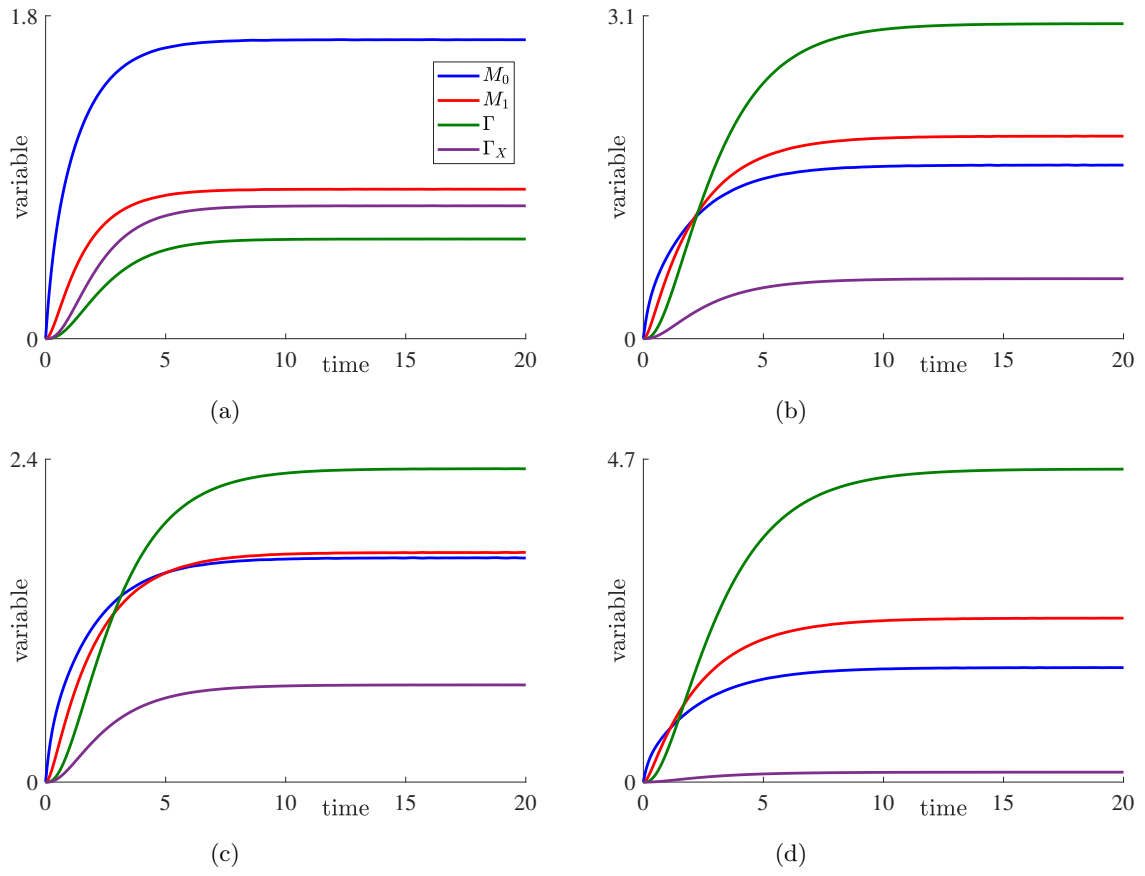


Figure 4.20: Numerical simulations of system (4.29) for various (constant) values of P and A . (a) $A = 1, P = 0$, (b) $A = 1, P = 1$, (c) $A = 10, P = 1$, (d) $A = 10, P = 10$.

Relevant parameter values: $R_0 = 2, R_1 = 3, R_\Gamma = 2, K_M = 1.2, K_\Gamma = 1, K_{\Gamma X} = 1.5$.

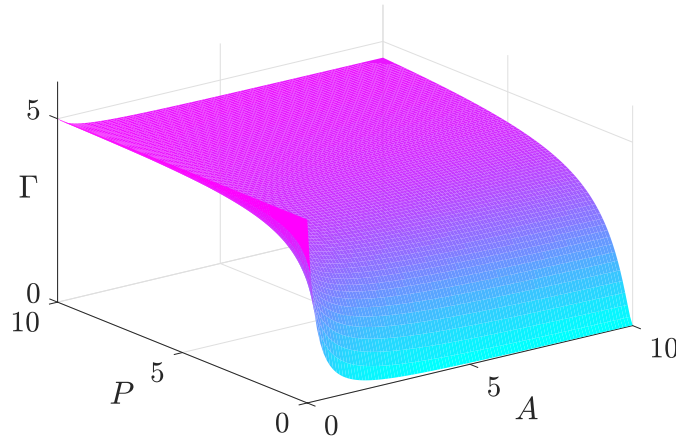


Figure 4.21: Surface plot of Γ as a function of A and P .

Parameter values: $R_0 = 2, R_1 = 3, R_\Gamma = 2, K_M = 1.2, K_\Gamma = 1, K_{\Gamma X} = 1.5, Q_M = Q_\Gamma = Q_P = 0.5$.

4.5.4 Varying antibiotic concentration

We now present the formulation of a time-dependent antibiotic concentration, allowing more accurate modelling of the inhibitory effects of the antibiotic. For the antibiotic to be effective, it must first cross the cell membrane. We therefore introduce a measure of the permeability or ‘leakiness’ of bacterial cell membranes, denoted L . A more permeable membrane allows more antibiotic to enter the bacterium, therefore A is an increasing function of L . We define Z_0 and Z_1 as the basal and maximum internalized concentrations of antibiotic respectively, assuming that there is a maximum possible concentration of antibiotic that can be internalized due to the finite volume of the bacterium. We assume for simplicity that once an antibiotic molecule enters a bacterium, it stays within the bacterium. We also assume that the degradation times of the antibiotic molecules are normally distributed. The equation governing the concentration of internalized antibiotic over time is:

$$A = \left(Z_0 + (Z_1 - Z_0) \frac{L}{\phi_L + L} \right) \exp\left(-K_A(t - t_{\text{inj}})^2 \right) H(t - t_{\text{inj}}).$$

We assume that the antibiotic is injected instantaneously at time $t = t_{\text{inj}} \geq 0$ and we model this using a Heaviside step function with argument $(t - t_{\text{inj}})$. We model the increase in internalized antibiotic due to permeability using a Hill function, ϕ_L denoting the permeability at which the antibiotic concentration is halfway between the basal and maximum concentrations. We model the degradation of antibiotic molecules using a decreasing exponential function with argument $-K_A(t - t_{\text{inj}})^2$, K_A governing the rate at which antibiotic molecules degrade on average.

We model the permeability of bacterial membranes using the equation:

$$L = \frac{W_0 \Gamma_X}{W_1 + \Gamma_X}.$$

Physiologically, we assume bacterial membranes become permeable when mistranslated proteins are part of their composition. Leakiness is minimal in the absence of mistranslated proteins and a membrane is fully permeable when all of the proteins in it are mistranslated, the leakiness of a fully permeable membrane denoted W_0 . W_1 denotes the density of mistranslated proteins at which membrane permeability is half-maximal. The model for the GRN with a time-dependent antibiotic concentration therefore has the form:

$$\dot{M}_0 = R_0 + R_\Gamma M_1 - R_1(1 - f_M)M_0 - K_M M_0, \quad (4.33a)$$

$$\dot{M}_1 = R_1(1 - f_M)M_0 - R_\Gamma M_1, \quad (4.33b)$$

$$\dot{\Gamma} = R_\Gamma(1 - f_\Gamma)M_1 - K_\Gamma \Gamma, \quad (4.33c)$$

$$\dot{\Gamma}_X = R_\Gamma f_\Gamma M_1 - K_{\Gamma X} \Gamma_X, \quad (4.33d)$$

$$A = \left(Z_0 + (Z_1 - Z_0) \frac{L}{\phi_L + L} \right) \exp \left[-K_A(t - t_{\text{inj}})^2 \right] H(t - t_{\text{inj}}), \quad (4.33e)$$

$$L = \frac{W_0 \Gamma_X}{W_1 + \Gamma_X}, \quad (4.33f)$$

where $f_M(A, P)$ and $f_\Gamma(A, P)$ are given by (4.30). Non-dimensionalising (4.33) via the scalings $M_0 = W_1 m_0$, $M_1 = W_1 m_1$, $\Gamma = W_1 \gamma$, $\Gamma_X = W_1 \gamma_X$, $A = Z_1 \alpha$, $L = \phi_L \Lambda$, $P = x_P p$, $t = \tau/D_P$, we obtain the following non-dimensional system:

$$\dot{m}_0 = r_0 + r_\gamma m_1 - r_1(1 - f_m)m_0 - k_m m_0, \quad (4.34a)$$

$$\dot{m}_1 = r_1(1 - f_m)m_0 - r_\gamma m_1, \quad (4.34b)$$

$$\dot{\gamma} = r_\gamma(1 - f_\gamma)m_1 - k_\gamma \gamma, \quad (4.34c)$$

$$\dot{\gamma}_X = r_\gamma f_\gamma m_1 - k_{\gamma X} \gamma_X, \quad (4.34d)$$

$$\alpha = \left(z_0 + (1 - z_0) \frac{\Lambda}{1 + \Lambda} \right) \exp \left[-k_\alpha(\tau - \tau_{\text{inj}})^2 \right] H(\tau - \tau_{\text{inj}}), \quad (4.34e)$$

$$\Lambda = \frac{w_0 \Gamma_X}{1 + \Gamma_X}, \quad (4.34f)$$

where:

$$f_m(\alpha, p) = \left(\frac{\alpha}{q_m + \alpha} \right) \left(\frac{q_p}{q_p + p} \right), \quad (4.35a)$$

$$f_\gamma(\alpha, p) = \left(\frac{\alpha}{q_\gamma + \alpha} \right) \left(\frac{q_p}{q_p + p} \right). \quad (4.35b)$$

The dimensionless parameters in (4.34) are given by:

$$r_0 = \frac{R_0}{D_P}, \quad r_1 = \frac{R_1}{D_P}, \quad r_\gamma = \frac{R_\Gamma}{D_P}, \quad q_m = \frac{Q_M}{Z_1}, \quad q_p = \frac{Q_P}{x_P}, \quad q_\gamma = \frac{Q_\Gamma}{Z_1}, \quad k_m = \frac{K_M}{D_P},$$

$$k_\gamma = \frac{K_\Gamma}{D_P}, \quad k_{\gamma X} = \frac{K_{\Gamma X}}{D_P}, \quad z_0 = \frac{Z_0}{Z_1}, \quad w_0 = \frac{W_0}{\phi_L}, \quad k_\alpha = \frac{K_A}{D_P^2}, \quad \tau_{\text{inj}} = t_{\text{inj}} D_P.$$

Table 4.4 gives a physiological interpretation of these parameters and Table 4.5 gives the baseline value of each parameter and initial condition of each variable. As for the basic model (section 4.4), we choose parameter values that give qualitatively sensible results, given the absence of relevant experimental data. Under this non-dimensionalisation, the maximum antibiotic concentration is equal to 1. In the following sections, we provide an exploration of (4.34) by considering numerical simulations and parameter variations. These analyses will aid in illuminating whether or not particular clinical treatments that target bacteria protein synthesis and/or aminoglycoside action will be effective.

r_0	The synthesis rate of mRNA (transcription).
r_1	The binding rate of ribosomes to mRNA.
r_γ	The production rate of proteins (translation).
k_m	The natural degradation rate of (unbound) mRNA.
k_γ	The natural degradation rate of correctly translated proteins.
$k_{\gamma X}$	The natural degradation rate of mistranslated proteins.
q_m	The antibiotic concentration at which the effect of ribosome blockage is half-maximal.
q_γ	The antibiotic concentration at which the effect of ribosomal proof-reading is half-maximal.
q_p	The pro-inflammatory mediator count at which their inhibitory effect on the antibiotic is half-maximal.
p	The concentration of internalized pro-inflammatory mediators.
z_0	The concentration of antibiotic that can enter a fully functioning bacterial membrane.
k_α	The natural degradation rate of the antibiotic.
τ_{inj}	The time at which the antibiotic is injected (the infection starts at time $\tau = 0$).
w_0	The leakiness of a fully permeable bacterial membrane.

Table 4.4: A physical interpretation of each parameter in system (4.34).

r_0	r_1	r_γ	k_m	k_γ	$k_{\gamma X}$	q_m	q_γ	q_p
2	3	2	1.2	1	1.5	0.5	0.5	0.5

p	z_0	k_α	τ_{inj}	w_0	$m_0(0)$	$m_1(0)$	$\gamma(0)$	$\gamma_X(0)$
1	0.1	0.001	10	1	0	0	0	0

Table 4.5: The baseline parameter values and the initial conditions used in the numerical simulations of system (4.34).

Figure 4.22 shows numerical simulations of (4.34) for varying q_m, q_γ and p , where q_m and q_γ denote the antibiotic concentrations at which ribosomal blockage and proof-reading are half-maximal respectively. In the absence of p (Figure 4.22(a)), Γ is reduced to below 50% of the drug-free steady state. This temporary reduction of the density of correctly translated proteins can potentially give the immune response enough of an advantage to overcome the infection (this will be explored in more detail in the analysis of the coupled model). When there is a non-zero concentration of p (Figure 4.22(b)), Γ is reduced but much less so than in the absence of p (Figure 4.22(a)). When the action of the antibiotic is more effective (Figure 4.22(c)), Γ is reduced more than it is in Figure 4.22(b), but not as much as in Figure 4.22(a). This suggests that the internalization of host pro-inflammatory mediators by EPEC effectively inhibits the functions of the aminoglycoside. When the action of the antibiotic is even more effective (Figure 4.22(d)), Γ is reduced to a density comparable to that in Figure 4.22(a), but also stays close to the minimum for a longer time. This gives the immune system more time to overcome the infection, increasing the potential for clearance of the infection. When the action of the antibiotic is as effective as it is in Figure 4.22(d), and there is a high concentration of p (Figure 4.22(e)), the antibiotic has a negligible effect on the GRN, demonstrating the importance of the ability of EPEC to internalize host pro-inflammatory mediators to the virulence of the infection.

Figure 4.23 shows surface plots of the minimum correctly translated protein density that occurs after aminoglycoside administration (see black circles in Figure 4.22) when selected pairs of parameters are varied. We now present some interesting results that are yielded by analysing these plots.

Treatments that alter the effectiveness of aminoglycosides or internalized pro-inflammatory mediators only have a significant effect when the bacteria have sufficiently low concentrations of internalized pro-inflammatory mediators: Figure 4.23(a) shows that the minimum γ decreases as k_α decreases when p is small, but varies negligibly with k_α when p is large. Figure 4.23(b) shows that the minimum γ decreases significantly as q_m and q_γ decrease when p is small, however as with k_α , altering q_m and q_γ makes negligible difference to the minimum γ when p is large. Figure 4.23(c) shows that altering q_p only makes a significant difference to the minimum γ if p is sufficiently small. These findings imply that increasing the longevity of α , making the bacteria more sensitive to the inhibitory action of α and making α less sensitive to the inhibitory action of internalized p will only be effective if the concentration of internalized p is sufficiently low, further demonstrating the importance of the ability of EPEC to internalize host pro-inflammatory mediators to the success of the infection.

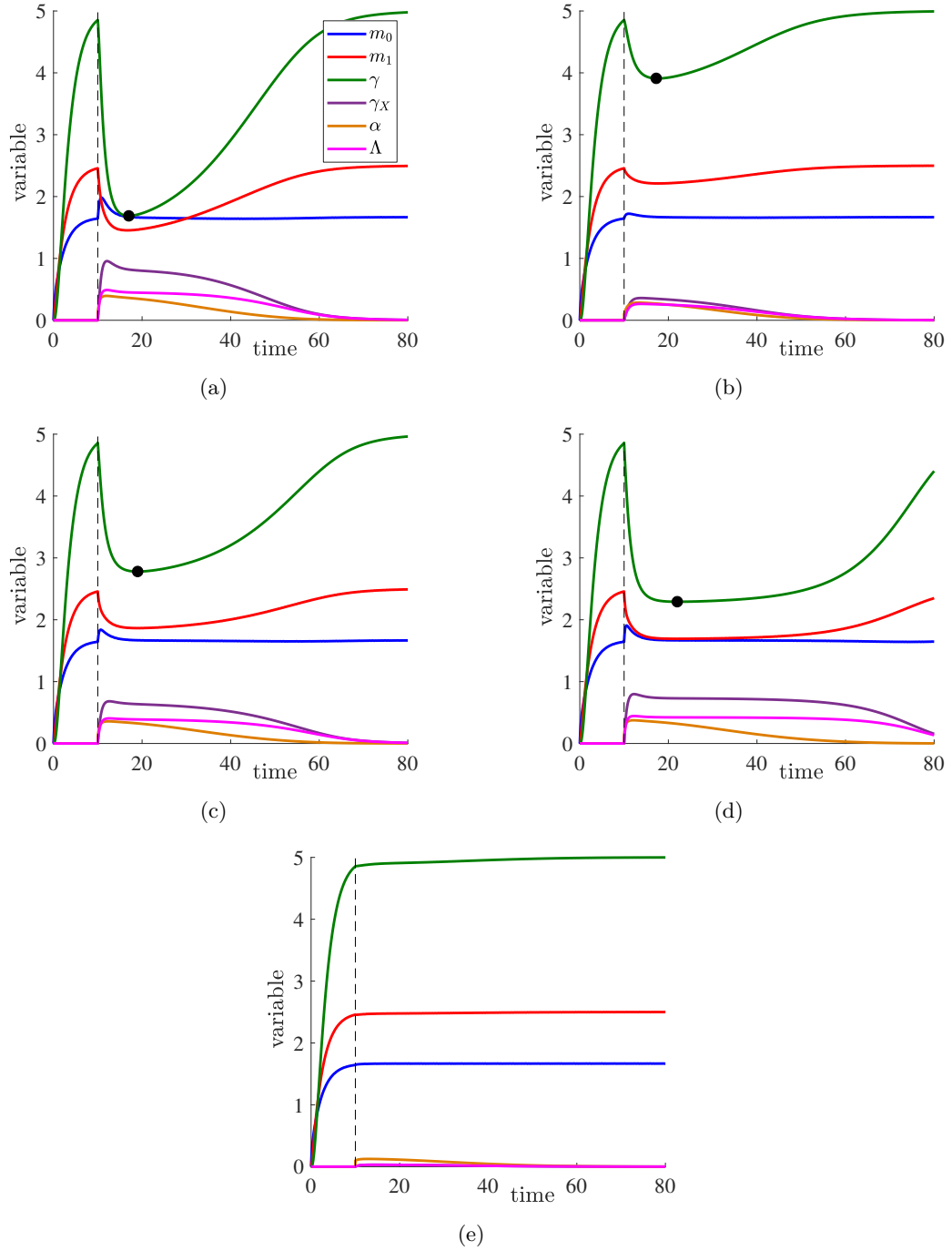


Figure 4.22: Numerical simulations of system (4.34). (a) $p = 0$, (b) $p = 1$, (c) $q_m, q_\gamma = 0.1, p = 1$, (d) $q_m, q_\gamma = 0.01, p = 1$, (e) $q_m, q_\gamma = 0.05, p = 10$. The black circles represent the minimum density of correctly translated proteins after aminoglycoside administration. Initial conditions and all other parameter values are given in Table 4.5.

Protein synthesis is significantly reduced if antibiotic treatment is commenced sufficiently early: Figure 4.23(d) shows that the minimum γ decreases as the delay in the antibiotic injection decreases, regardless of p . This suggests that quick diagnosis and commencement of treatment can be pivotal to the successful eradication of the infection.

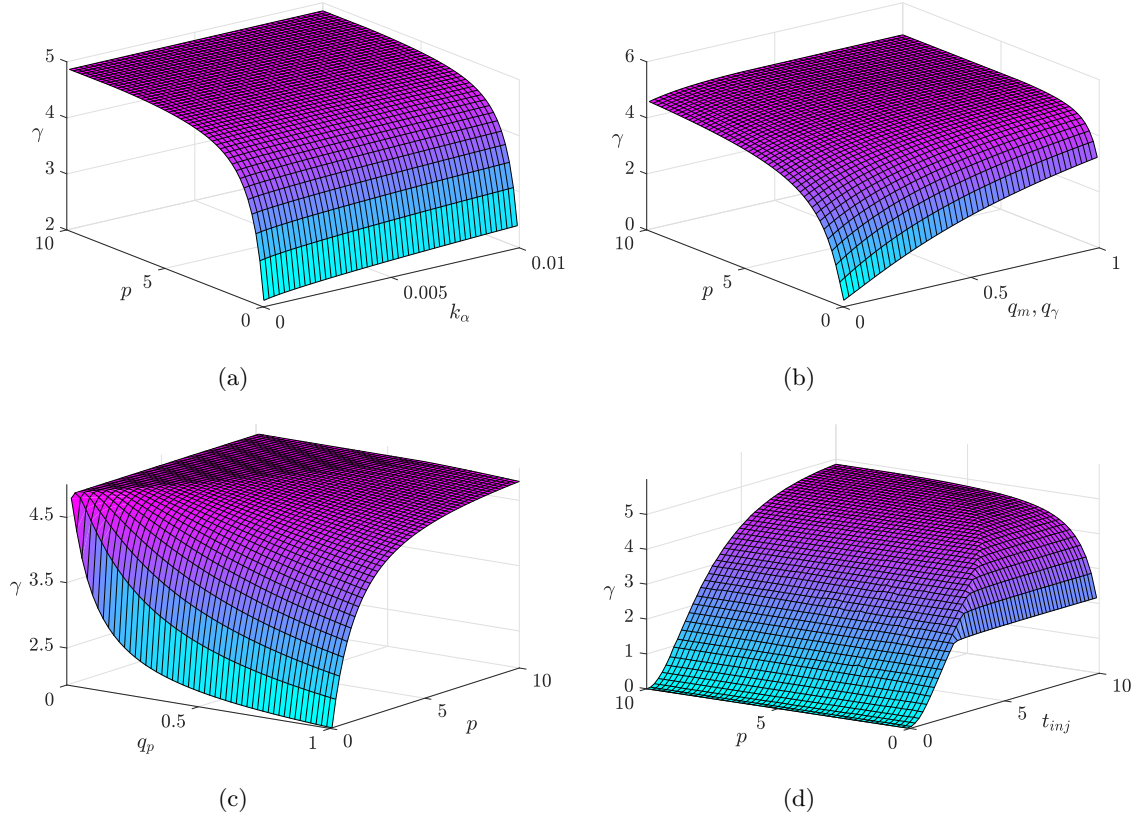


Figure 4.23: Numerical simulations of system (4.34) showing $\min(\gamma)$ for: (a) varying k_α and p , (b) varying (q_m, q_γ) and p , (c) varying t_{inj} and p , (d) varying (q_m, q_γ) and q_p . All other parameters take their baseline values.

Summary

Feasible strategies that reduce the density of correctly translated proteins being made by the bacteria, potentially inhibiting the infection sufficiently that the immune response can eliminate it, are:

- commencing an aminoglycoside treatment as soon as possible.
- reducing the internalization of pro-inflammatory mediators and
 - making the aminoglycoside naturally last longer,
 - making the bacteria more sensitive to the inhibitory action of the aminoglycoside.
 - making the antibiotic less sensitive to the inhibitory action of pro-inflammatory mediators.

Now that we have explored how the density of proteins produced by bacteria is affected by the action of aminoglycosides, we wish to see how this affects the success of the infection. We therefore couple the sub-cellular GRN with the EPEC-specific basic model.

4.6 The coupled EPEC model

4.6.1 Introduction

The basic model governs the dynamics between the bacteria, its secreted effector proteins and the immune response, while the sub-cellular GRN governs bacterial protein synthesis. We wish to combine these models to obtain a more realistic description of an EPEC infection and, therefore, more accurate treatment predictions. The resulting combination of the basic model and the GRN is referred to as the coupled model.

4.6.2 Formulating the model

In the coupled model, $(m_0, m_1, \gamma, \gamma_X, \alpha$ and $\Lambda)$ are the *average* quantities of the respective variables per bacterium, yielding an average growth rate of bacteria and average production rates of effector proteins. Since bacteria and effector proteins are formed from the correctly translated proteins (γ) produced by the GRN, we make their production rates proportional to the density of correctly translated proteins. By combining equations (4.10) and (4.34), the coupled model is given by:

$$\frac{db_1}{d\tau} = r_b \gamma \frac{b_1 + \lambda b_2}{1 + (b_1 + b_2)} - qp b_1 - lb_1 \left(\frac{1}{1 + e_3} \right) - d_b b_1, \quad (4.36a)$$

$$\frac{db_2}{d\tau} = r_b \gamma \frac{(1 - \lambda)b_2}{1 + (b_1 + b_2)} + qp b_1 - lb_2 \left(\frac{1}{1 + e_3} \right) - d_b b_2, \quad (4.36b)$$

$$\frac{dp}{d\tau} = \left[\phi + c_b(1 + vl)(b_1 + b_2) \right] \left(\frac{1}{1 + a^2} \right) \left(\frac{1}{1 + e_1} \right) - qu_{bp} p b_1 + s_n n - p, \quad (4.36c)$$

$$\frac{da}{d\tau} = r_a \frac{p^2}{1 + p^2} - d_a a, \quad (4.36d)$$

$$\frac{dl}{d\tau} = r_l p + s_\alpha (b_1 + b_2) \left(\frac{1}{1 + e_2} \right) - d_l l, \quad (4.36e)$$

$$\frac{de_1}{d\tau} = \left(c_{11} b_1 + c_{12} b_2 \right) \gamma - d_{e1} e_1, \quad (4.36f)$$

$$\frac{de_2}{d\tau} = \left(c_{21} b_1 + c_{22} \left(\frac{\psi_{y1}^2}{\psi_{y1}^2 + p^2} \right) \left(\frac{b_2}{\psi_{y2}^2 + b_2^2} \right) \right) \gamma - d_{e2} e_2, \quad (4.36g)$$

$$\frac{de_3}{d\tau} = \left(c_{31} + \frac{c_{32} p}{\psi_z + p} \right) b_2 \gamma - d_{e3} e_3, \quad (4.36h)$$

$$\frac{dn}{d\tau} = (b_1 + b_2) \frac{e_2^2}{\psi_n^2 + e_2^2} - d_n n, \quad (4.36i)$$

$$\frac{dm_0}{d\tau} = r_0 + r_\gamma m_1 - r_1 \left[1 - \left(\frac{\alpha}{q_m + \alpha} \right) \left(\frac{q_p}{q_p + p} \right) \right] m_0 - k_m m_0, \quad (4.36j)$$

$$\frac{dm_1}{d\tau} = r_1 \left[1 - \left(\frac{\alpha}{q_m + \alpha} \right) \left(\frac{q_p}{q_p + p} \right) \right] m_0 - r_\gamma m_1, \quad (4.36k)$$

$$\frac{d\gamma}{d\tau} = r_\gamma \left[1 - \left(\frac{\alpha}{q_\gamma + \alpha} \right) \left(\frac{q_p}{q_p + p} \right) \right] m_1 - k_\gamma \gamma, \quad (4.36l)$$

$$\frac{d\gamma_X}{d\tau} = r_\gamma \left(\frac{\alpha}{q_\gamma + \alpha} \right) \left(\frac{q_p}{q_p + p} \right) m_1 - k_{\gamma_X} \gamma_X, \quad (4.36m)$$

$$\alpha = \left(z_0 + (1 - z_0) \frac{\Lambda}{1 + \Lambda} \right) \exp \left[-k_A (\tau - \tau_{\text{inj}})^2 \right] H(\tau - \tau_{\text{inj}}), \quad (4.36n)$$

$$\Lambda = \frac{w_0 \Gamma_X}{1 + \Gamma_X}, \quad (4.36o)$$

The coupling of the two models allows the concentration of pro-inflammatory mediators (p) to vary with time in the GRN. The next section is concerned with the analysis of (4.36).

4.6.3 Steady state and stability analysis

The steady state equations for m_0, m_1 and γ are given by:

$$0 = r_0 + r_\gamma \bar{m}_1 - r_1 \bar{m}_0 - k_m \bar{m}_0,$$

$$0 = r_1 \bar{m}_0 - r_\gamma \bar{m}_1,$$

$$0 = r_\gamma \bar{m}_1 - k_\gamma \bar{\gamma},$$

since $\alpha \rightarrow 0$ for large τ . Solving these equations yields:

$$\bar{m}_0 = \frac{r_0}{k_m}, \quad \bar{m}_1 = \frac{r_0 r_1}{r_\gamma k_m}, \quad \bar{\gamma} = \frac{r_0 r_1}{k_m k_\gamma}.$$

The same parameter values from the basic and GRN models are used in the coupled model, allowing comparison between the models. The growth rates for bacteria (r_b) and effector proteins (c_{ij} , for $i = 1, 2, 3$ and $j = 1, 2$) are divided by $\bar{\gamma}$, allowing them to be comparable to their equivalent rates in equations (4.10) and (4.34) when the GRN has reached its steady state. Table 4.6 gives the baseline parameter values and the initial conditions used in the coupled model analysis. The injection delay time t_{inj} is given a baseline value of 30 to allow time for the infection to grow close to the carrying capacity before antibiotic treatment commences. We assume the GRN is at its steady state before the infection occurs and numerical simulations (not included for brevity) show that with these parameter values and initial conditions, the coupled model in the absence of antibiotic has identical dynamics over time to the basic model. As in the basic model, we define $B = b_1 + b_2$. Figure 4.24 shows numerical simulations for B, p, l and α for this baseline parameter set. For this parameter set, the infection is almost eradicated with the aid of the antibiotic but the infection recurs once the transient antibiotic concentration becomes sufficiently low.

As in the basic model (see section 4.4.4), we assume that the infection is eradicated for $B \approx 0$. As the concentration of antibiotic in (4.36n) is transient, the infection always recovers in numerical simulations. Therefore, we concentrate on the minimum infection size, $\min(B(t))$, rather than the steady state infection size, and apply the above eradication assumption. In this analysis, we let $q_m = q_\gamma = q_\alpha$ so that an overall antibiotic susceptibility (q_α) can be obtained and its effect on the infection dynamics measured. We now consider $\min(B(t))$ when several different model parameters are varied.

r_b	λ	q	d_b	ϕ	c_b	v	u_{bp}	s_n	r_a	d_a
0.3	0.5	3	0.75	0.03	2	1	1	0.6	1.5	1

r_l	s_α	d_l	c_{11}	c_{12}	d_{e1}	c_{21}	c_{22}	ψ_{y1}	ψ_{y2}	d_{e2}
1	1	1	2	2.4	2	2	2	0.5	0.5	2

c_{31}	c_{32}	ψ_z	d_{e3}	ψ_n	d_n	r_0	r_1	r_γ	k_m	k_γ
2	2	0.6	2	1	1	2	3	2	1.2	1

$k_{\gamma X}$	q_m	q_γ	q_p	z_0	k_α	τ_{inj}	w_0		$b_1(0)$	$b_2(0)$
1.5	0.5	0.5	0.5	0.1	0.001	30	1		0.01	0

$p(0)$	$a(0)$	$l(0)$	$e_1(0)$	$e_2(0)$	$e_3(0)$	$n(0)$	$m_0(0)$	$m_1(0)$	$\gamma(0)$	$\gamma_X(0)$
0	0	0	0	0	0	0	5/3	2.5	5	0

Table 4.6: The baseline parameter values and the initial conditions used in the numerical simulations of system (4.36).

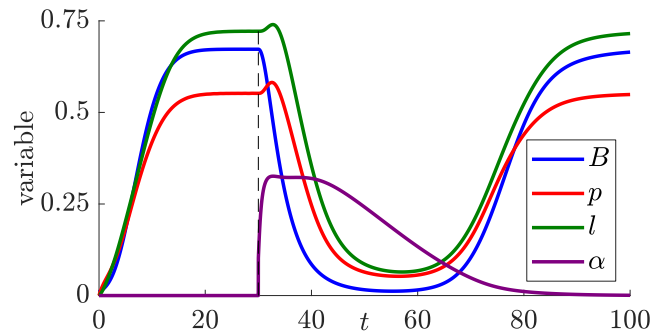


Figure 4.24: Numerical simulations of system (4.36) for the total density of bacteria, the concentration of pro-inflammatory mediators, the density of leukocytes and the aminoglycoside concentration for the baseline parameter set.

4.6.4 Numerical simulations

1-parameter analysis

In this investigation, we compare the minimum infection size to the initial infection size as chosen parameters are varied. For each parameter, we:

1. find the parameter value that gives $\min(B(t > 0)) = B(t = 0)$,
2. compute the parameter value that gives a 90% decrease in $\min(B)$,
3. calculate the percentage change in parameter.

Figure 4.25 provides a visual aid for this process. We then compare the required parameter changes and deduce which parameters the minimum infection size is most sensitive to. We will later combine parameter alterations to see which combinations are the most effective at eradicating the infection (reducing $\min(B)$ below a critical threshold).

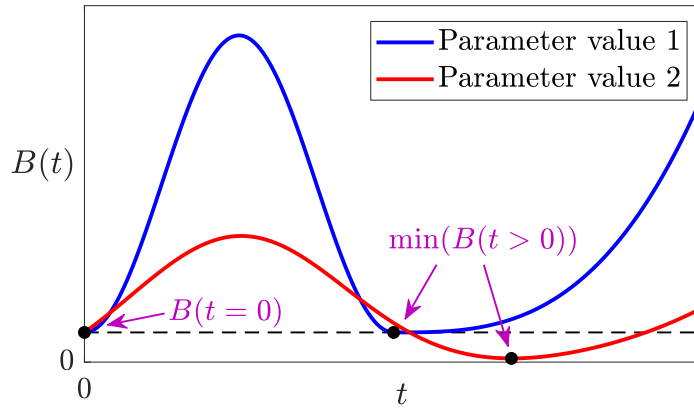


Figure 4.25: Example graph showing how the parameter sensitivity analysis of the coupled EPEC model is undertaken. From the parameter value that gives $\min(B(t > 0)) = B(t = 0)$ (blue line), we compute the parameter value that gives a 90% reduction in $\min(B)$ (red line). We then calculate the percentage change in parameter required for this 90% reduction.

During this analysis, $c_{i1} = c_{i2} = c_i$ for $i = 1, 2, 3$ so that the global effector protein production rates can be considered and $q_\alpha = q_m = q_\gamma$ so that the global antibiotic effectiveness can be considered. The chosen parameters are $q_\alpha, k_\alpha, t_{\text{inj}}, q_p, w_0$ and r_0 from the GRN and r_b, r_l, c_3 and c_b from the basic model. The parameters w_0 and r_0 are chosen as we want to explore how altering the susceptibility of the bacterial membrane to permeability and the efficacy of mRNA synthesis respectively affect the success of the infection. The basic model parameters r_b, r_l and c_3 are chosen as they are involved in the 3 most sensitive net growth rates (regarding the steady state infection size) and c_b is chosen to explore the effects of altering the concentration of pro-inflammatory mediators on the success of the infection.

Figure 4.26(a)–(j) shows $\min(B(t))$ for varying $q_\alpha, k_\alpha, t_{\text{inj}}, q_p, w_0, r_0, r_b, r_l, c_3$ and c_b respectively. We see that $\min(B(t))$ becomes negligibly small for sufficiently low q_α, k_α, r_0 and r_b and sufficiently

high q_p, w_0 and r_l . The infection can therefore be eradicated by sufficiently reducing (1) the sensitivity of antibiotics to the inhibitory effects of internalized pro-inflammatory mediators, (2) the degradation rate of aminoglycosides, (3) the synthesis rate of mRNA strands or (4) the growth rate of bacteria, or by sufficiently increasing (1) the sensitivity of bacteria to aminoglycosides, (2) the leakiness of a fully permeable membrane or (3) the inflammation-stimulated attraction rate of leukocytes.

We see from Figure 4.26(c) that while the minimum infection size cannot be made negligibly small compared to $B(t = 0)$ by altering t_{inj} , a 90% reduction can be achieved by a sufficient reduction in t_{inj} . This suggests that an antibiotic treatment is more likely to be successful if the treatment is commenced quickly after the initial infection; however, a quick diagnosis is impractical if the symptoms that lead to diagnosis do not manifest themselves sufficiently early in the infection.

Figure 4.26(i) shows that the minimum infection size cannot be decreased by 90% by only decreasing c_3 , implying that the minimum infection size is not sensitive to c_3 . These results suggest that while inhibiting the growth rate of effector proteins is helpful, there are more sensitive infection dynamics to target that will achieve better results.

Figure 4.26(j) shows that $\min(B(t); c_b) < B(t = 0) \forall c_b$. Since we cannot consider a 90% reduction in $\min(B)$ from $B(t = 0)$, we instead consider a 90% reduction from the peak $\min(B)$. We find that a 700% increase in c_b is required, implying that the minimum infection size is not sensitive to this parameter. Increasing the production rate of pro-inflammatory mediators many-fold is dangerous to the host and can lead to auto-immune diseases such as chronic inflammation and leukocytosis.

Table 4.7 gives a list of the chosen parameters, ordered by required percentage change, that the minimum infection size is most sensitive to, along with physiological descriptions of the parameter changes and ways they can be implemented clinically. We see that the three most effective individual treatments are decreasing the synthesis rate of bacterial mRNA, reducing the growth rate of the bacteria and making the bacteria more sensitive to aminoglycosides. Other treatments are found to be less effective, for example for a 90% decrease in the minimum infection size, the delay in antibiotic delivery must be almost halved or the attraction rate of inflammation-stimulated leukocytes must be increased by three quarters. In the basic model, for the baseline parameter set, the steady state infection size is not particularly sensitive to c_b (see Figure 4.3) and cannot be reduced to a negligible size by solely varying c_3 (see Figure 4.9); results that are consistent with those from the coupled model (Figure 4.26). Furthermore, both models agree that by sufficiently reducing r_b or raising r_l , the infection is eradicated.

Par.	Par. change	Physiological description	Clinical application
r_b	-13%	Reduce the growth rate of bacteria	Bacteriostatic / bactericidal antibiotics
r_0	-13%	Reduce the synthesis rate of bacterial mRNA	Sequester free nutrients (internally and externally), such as sugars, phosphates and nitrogen
q_α	-22%	Increase the sensitivity of bacteria to aminoglycosides	Create a higher affinity for binding
w_0	+64%	Increase the leakiness of a fully permeable membrane	Force mistranslated proteins to fold into shapes that have a greater span
k_α	-67%	Decrease the degradation rate of aminoglycosides	Improve the longevity / robustness of individual molecules
r_l	+74%	Increase the inflammation-stimulated recruitment rate of leukocytes	Increase the sensitivity of leukocyte recruitment signalling pathways to pro-inflammatory mediator presence
t_{inj}	-90%	Decrease the delay in antibiotic treatment	Diagnose the infection sooner
q_p	+108%	Decrease the sensitivity of aminoglycosides to internalized pro-inflammatory mediators	Create a lower affinity for binding
c_b	+700%	Increase the stimulated production rate of pro-inflammatory mediators	Increase the sensitivity of pro-inflammatory signalling pathways to pathogen presence

Table 4.7: A list of the chosen parameters that the minimum infection size, $\min(B)$, in (4.36) is most sensitive to, ordered by the percentage change in parameter value required to give a 90% decrease in $\min(B)$, from the value that gives $\min(B(t > 0)) = B(t = 0)$. Physiological descriptions of the parameter changes, and ways they can be implemented clinically, are also given.

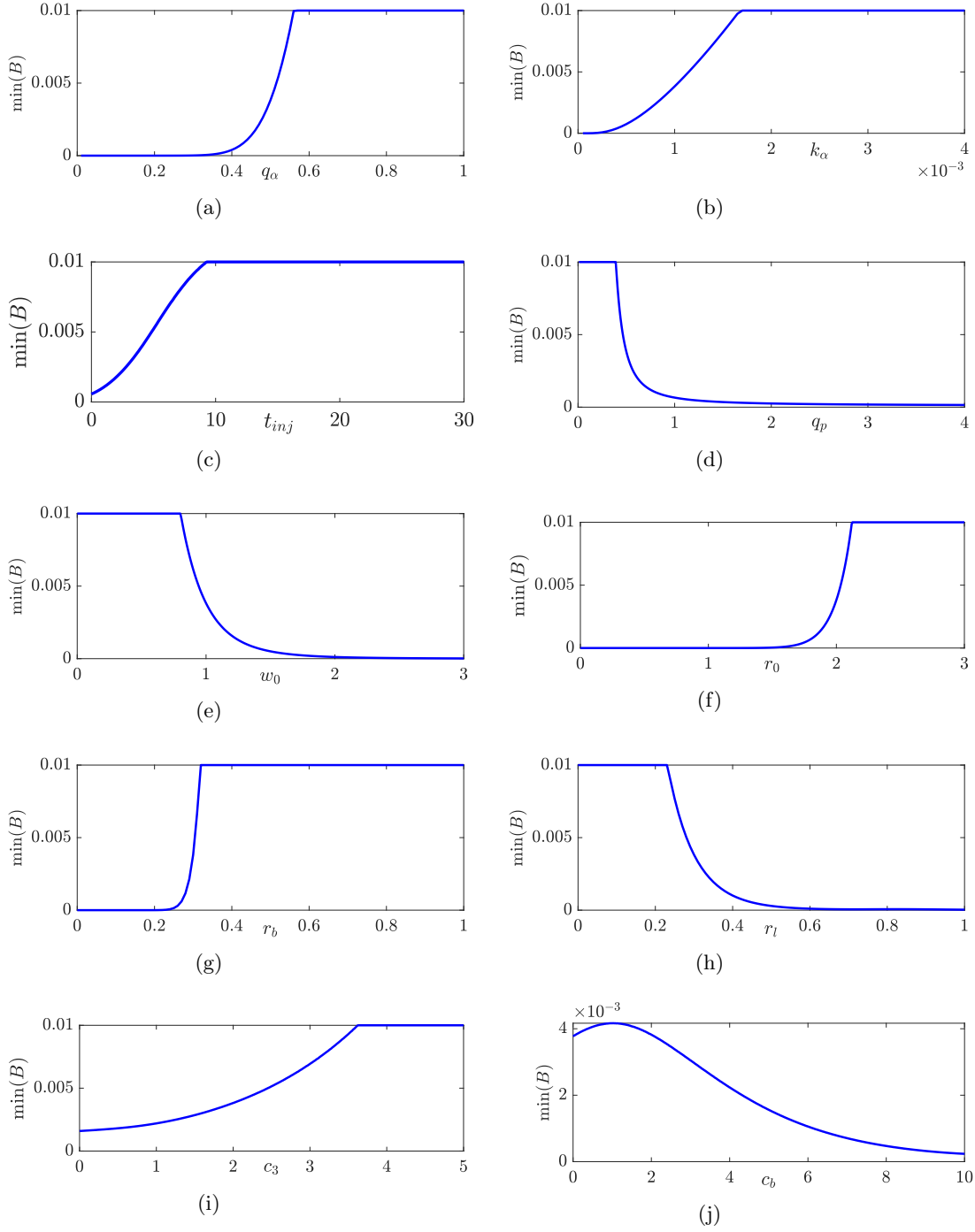


Figure 4.26: Graphs showing the minimum infection size in (4.36) for varying (a) q_α , (b) k_α , (c) t_{inj} , (d) q_p , (e) w_0 , (f) r_0 , (g) r_b , (h) r_l , (i) c_3 , (j) c_b . All other parameters and initial conditions are given in Table 4.6.

2-parameter analysis

We now consider varying pairs of parameters to look for effective synergistic treatment strategies. We consider all of the parameters from the 1 parameter analysis that can give a 90% reduction

in the minimum infection size for parameter changes that are below 200%. Table 4.8 shows the minimum infection size as a percentage of the initial infection size ($B(t = 0) = 0.01$) when each chosen pair of parameters is altered by 25% from their baseline values in the direction that leads to a reduction in the minimum infection size.

The most effective individual parameter change is reducing the growth rate of bacteria, leading to the minimum infection size being 0.17% of the initial infection size. The biggest decrease in the minimum infection size occurs when both the net growth rate of the bacteria and the production rate of bacterial mRNA are decreased, the minimum infection size being 10,000 times smaller than the initial infection size. This implies that a treatment combination that inhibits both the net growth rate of the bacteria and the production rate of bacterial mRNA will be a successful treatment. The second biggest decrease in the minimum infection size occurs when both the net growth rate of the bacteria is decreased and the susceptibility of the bacteria to the antibiotic is increased (a smaller q_α implies a greater susceptibility), the minimum infection size being approximately 83 times smaller than the initial infection size. This implies that a treatment combination that both inhibits the net growth rate of the bacteria and increases the susceptibility of the bacteria to the antibiotic will be a successful treatment. Other treatment combinations that the model predicts to be effective are:

- decreasing the production rate of bacterial mRNA and increasing the susceptibility of the bacteria to the antibiotic,
- decreasing both the net growth rate of the bacteria and the degradation rate of the antibiotic,
- decreasing the net growth rate of the bacteria and increasing the susceptibility of bacterial membranes to permeability,

the minimum infection sizes being 42, 11 and 10 times smaller than the initial infection size respectively. Altering parameters such as the inflammation-stimulated attraction rate of leukocyte and the susceptibility of the antibiotic to pro-inflammatory mediators helps reduce the minimum infection size in each case, but not significantly. Altering the delay in the injection time makes surprisingly little difference when altered from the baseline parameter set. This implies that for a noticeable effect, a much larger reduction in the antibiotic delay time must be used but as discussed, this is possibly infeasible due to the possible absence of symptoms early in the infection.

	None	q_α	k_α	t_{inj}	q_p	r_b	r_l	w_0
None	100							
q_α (-)	7.7							
k_α (-)	68	2.5						
t_{inj} (-)	100	7.7	68					
q_p (+)	64	3.6	35	64				
r_b (-)	0.35	0.012	0.095	0.34	0.23			
r_l (+)	60	3.4	33	60	34	0.17		
w_0 (+)	46	2.1	22	46	23	0.097	22	
r_0 (-)	0.73	0.024	0.23	0.73	0.51	1.0×10^{-4}	0.39	0.21

Table 4.8: The minimum infection size as a percentage of the initial infection size ($B(t=0) = 0.01$) when each chosen pair of parameters is altered by 25% from their baseline values in the direction that leads to a reduction in the minimum infection size. All values are rounded to two significant figures and all unaltered parameters take their baseline values. The + and - symbols in the table headers indicate whether the respective parameter is increased or decreased by 25%.

3-parameter analysis

Finally, we considered a triple function treatment by taking the three most sensitive parameters r_b, r_0 and q_α and reducing them by a percentage of their baseline values. We are interested in seeing if altering three parameters by a smaller amount can produce the same magnitude effect as altering two parameters by a larger amount. Figure 4.27 shows the numerical simulation for the total density of bacteria when r_b, r_0 and q_α are at 90% of their baseline values (red line) compared with that for the baseline parameter regime (blue line). The minimum infection size when r_b, r_0 and q_α are at 90% of their baseline values is approximately 7.9×10^{-5} , implying that the infection is eradicated by a treatment that performs this parameter reduction. The minimum infection size when r_b and r_0 are at 75% of their baseline values is over 2000 times smaller than that when r_b, r_0 and q_α are at 90% of their baseline values, suggesting that it is more effective to strongly target the net growth rate of bacteria and the production rate of bacterial mRNA than to weakly target the aforementioned and the bacterial susceptibility to aminoglycosides. Targeting all three strongly will of course give an even better response than only targeting two; the minimum infection size when r_b, r_0 and q_α are at 75% of their baseline values is approximately 1.4×10^{-9} . This might be an unnecessarily strong treatment for typical cases and therefore an inefficient use of time and resources, but it might be necessary for particularly virulent strains of EPEC or for patients with weak or compromised immune responses.

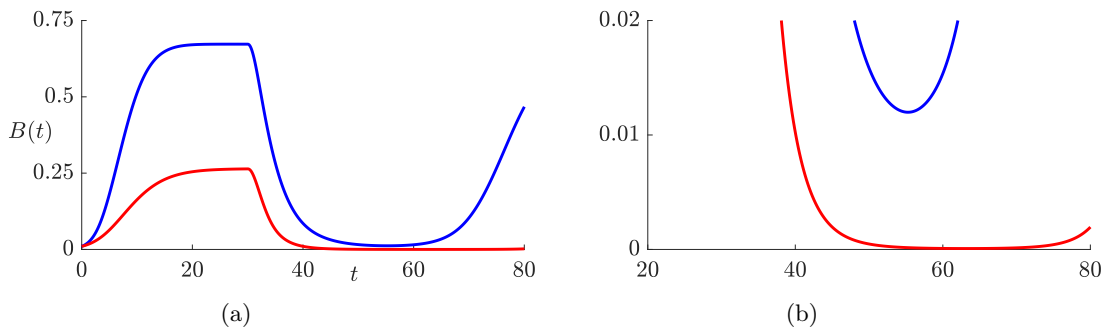


Figure 4.27: (a) Numerical simulations of system (4.36) for the total density of bacteria for the baseline parameter set (blue) and the baseline parameter set but with r_b, r_0, q_m and q_γ at 90% of their baseline values (red). (b) The same graphs as in (a), but zoomed in on $\min(B(t))$.

4.6.5 Coupled model summary

We combined the population-level basic model and the sub-cellular GRN to form a coupled multi-scale model that gives a more physiologically realistic representation of the dynamics of an EPEC infection than the basic model. Due to the transience of the application of the antibiotic, we considered the minimum density of bacteria (rather than the steady state density). We made the assumption that the infection is eradicated if the density of bacteria becomes sufficiently small.

We first analysed the effect on the minimum density of bacteria, $\min(B(t))$, as we varied each parameter of a selected group. We considered the percentage change in each parameter value required to give a 90% reduction in $\min(B)$, from the value that yields $\min(B(t > 0)) = B(t = 0)$. We found that reducing the net growth rate of bacteria ($r_b - d_b$), reducing the production rate of bacterial mRNA (r_0) and increasing the susceptibility of bacteria to the antibiotic (q_α) are the three most effective methods for reducing the minimum infection size. The model therefore suggests that a treatment that executes any of these functions is useful in eradicating the infection. Altering parameters such as the injection delay time (t_{inj}) and the susceptibility of the antibiotic to pro-inflammatory mediators (q_p) reduces the minimum infection size, but not significantly for changes that are not large. Altering parameters such as the stimulated production rate of pro-inflammatory mediators (c_b), and the rate of effector protein production (c_{ij} for $i = 1, 2, 3$ and $j = 1, 2$), makes little difference to the minimum infection size for changes that are not large. Both the basic and coupled models agree that (1) by sufficiently reducing the growth rate of bacteria (r_b) or raising the inflammation-stimulated attraction rate of leukocytes (r_l), the infection can be eradicated, (2) the steady state infection size is not particularly sensitive to c_b , and (3) the steady state infection size cannot be reduced to a negligible size by solely reducing the effector protein production rates.

Once we found the parameters that the minimum infection size is most sensitive to, we altered pairs of these parameters by 25% from their baseline values in the direction favouring the host, to see if any combinations give a significant reduction in the minimum infection size. We found that by (1) decreasing both the net growth rate of the bacteria and the production rate of bacterial mRNA or (2) decreasing the net growth rate of the bacteria and increasing the susceptibility of the bacteria to the antibiotic, the infection is eradicated. Reducing the net growth rate of bacteria can be achieved

by a bactericidal (increased death) or a bacteriostatic (decreased growth) treatment. If it is possible for a treatment to sequester free nutrients, in particular sugars, nitrogen and phosphates, within bacteria or within the local surroundings of the bacteria, then this treatment effectively decreases the production rate of bacterial mRNA. If it is possible for a treatment to make a greater affinity for binding between aminoglycosides and bacterial ribosomes, then this treatment effectively increases the susceptibility of the bacteria to aminoglycosides.

Finally, we considered a triple function treatment in which the three most sensitive parameters were altered by a percentage of their baseline values in favour of the host. The model predicts that strongly targeting the net growth rate of bacteria and the production rate of bacterial mRNA will be a more effective treatment than weakly targeting the aforementioned and the bacterial susceptibility to aminoglycosides, implying that treatments should not be spread too thin. Strongly targeting the three most sensitive parameters reduces the minimum infection size even further and is regarded as an expensive overkill for typical infections; however, it may be necessary in patients with particularly virulent strains of EPEC or weak or compromised immune responses.

4.7 Summary

In the previous chapter, we developed a mathematical model to represent the dynamics of a general infection. In this chapter, we adapted this model by adding relevant dynamics to make two models for an EPEC-specific infection. We are interested in studying EPEC due to its contribution to the deaths of many humans around the world, particularly the young in countries without adequate healthcare. The goals of this work are to make predictions regarding the feasibility and safety of potential anti-bacterial therapies, both current and hypothetical.

We first developed a basic population-level ODE model, based on the literature and on recent unexplained experimental findings, which governs the dynamics between EPEC and the immune response. In particular, the model includes the injection and action of effector proteins, host cell death and pro-inflammatory mediator internalization. Under the assumption that $\phi \ll 1$, $r_b, d_b, r_l, d_l = \mathcal{O}(1)$, the disease-free state is stable when :

$$r_b - d_b < \frac{\phi r_l}{d_l}. \quad (4.37)$$

Physiologically, for the infection to be eradicated, the net strength of the inflammation-stimulated immune response must out-compete the net growth of the bacteria. We did not observe the existence of multiple diseased states for positive parameter values. It is interesting to note that the EPEC-specific dynamics have a negligible effect on the stability of the disease-free state; they only affect the temporal dynamics of the infection.

We then formulated a sub-cellular GRN to govern the dynamics of bacterial protein synthesis and its inhibition by aminoglycosides. We explored the effects of both constant and time-dependent concentrations of pro-inflammatory mediators and aminoglycosides on the model dynamics. We found

that the model only has one steady state. We then combined the basic model and the GRN to provide a more physiologically accurate representation of an EPEC infection, considering dynamics on both the population-level and the sub-cellular level. We coupled the two models by making the production rates of bacteria and effector proteins proportional to the density of correctly translated proteins produced in the GRN. We adopted the interpretation that the infection is eradicated if the density of bacteria becomes sufficiently low, as the continuous ODE representation of the infection breaks down when the discreteness of the number of bacteria becomes significant.

We predict that treatments that (1) reduce the net growth rate of bacteria, (2) boost the inflammation- or apoptosis-stimulated recruitment rate of leukocytes, or (3) boost the longevity of leukocytes, can eradicate the infection. Bactericidal and bacteriostatic antibiotics can increase the death rate and decrease the growth rate of bacteria respectively. Treatments that reduce the rate of internalization of pro-inflammatory mediators by bacteria, or reduce the net production rates of effector proteins, can reduce the infection size, although not enough to eradicate the infection. These styles of treatment can be combined with other more direct treatment strategies to successfully eradicate the infection. We predict that treatment combinations such as these will be (1) more effective than individual treatments, (2) effective long-term as they are less likely to promote the spread of resistance, due to the reduced emphasis on directly attacking the bacteria and (3) safer for the host, as the reduced emphasis on boosting the immune response will reduce the risk of auto-immune diseases such as chronic inflammation and leukytosis. Treatment circumstances depend on individual host factors, such as the immune response strength and the virulence of the infecting strain. Therefore, in clinical practice, an informed decision may need to be taken based on what can be predicted.

We found that bacterial protein synthesis can be effectively shut down, and the infection eradicated, if (1) the susceptibility of bacteria to aminoglycosides is sufficiently high, (2) the concentration of internalized pro-inflammatory mediators is sufficiently low and (3) the ability of internalized pro-inflammatory mediators to inhibit the action of aminoglycosides is sufficiently weak. Therefore, a treatment that inhibits the ability of EPEC to internalize pro-inflammatory mediators, increases the susceptibility of EPEC to aminoglycosides and/or reduces the susceptibility of aminoglycosides to inhibition by internalized pro-inflammatory mediators will be effective. The susceptibility of the bacteria to aminoglycosides can be increased by a treatment that creates a greater affinity for binding between aminoglycosides and bacterial ribosomes. Conversely, the susceptibility of aminoglycosides to pro-inflammatory mediators can be decreased by a treatment that reduces the affinity for binding between the two. Sufficiently reducing the delay before antibiotic administration significantly reduces the infection size; therefore, correctly diagnosing the infection and commencing antibiotic treatment as soon as possible increases the potential for eradication.

The minimum infection size is significantly reduced for decreased bacterial proliferation and bacterial mRNA synthesis, and is noticeably reduced for increased bacterial membrane permeability and slower aminoglycoside molecule degradation. A treatment that sequesters free nutrients (in particular, sugars, nitrogen and phosphates; the main components of mRNA) within bacteria, or the local surroundings of the bacteria, will decrease the synthesis rate of bacterial mRNA.

The two most effective treatment combinations are (1) decreasing both the net growth rate of the bacteria and the synthesis rate of bacterial mRNA and (2) decreasing the net growth rate of the bacteria and increasing the susceptibility of the bacteria to aminoglycosides. We predict that it is more effective to strongly target two aspects of the infection than to weakly target three. Strongly targeting more than two aspects of the infection may be superfluous and expensive for most patients, but may be necessary for patients who have a particularly virulent strain of EPEC or have weak or compromised immune responses.

The analysis of our EPEC specific models has revealed several potential treatment strategies for eradicating EPEC infections, some of which are hypothetical. The predictions relating to protein synthesis and antibiotic action cannot be obtained through the basic model, demonstrating that the coupled model, through its more detailed formulation, is able to predict a wider variety of effective treatment strategies.

Chapter 5

A hybrid model of an EPEC-specific infection

5.1 Introduction

In the previous chapter, we developed and analyzed a deterministic, population-level ODE model to explore the dynamics of an EPEC infection. However, this continuous model breaks down for small numbers of cells, due to the discreteness of the number of cells becoming significant. Furthermore, the modelling of leukocyte chemotaxis requires knowledge of spatial chemoattractant gradients; a feature that the ODE model does not accommodate. Therefore to more realistically model an EPEC infection, we need to consider spatial dependence and stochasticity. To address this we present, in this chapter, a model that combines an individual-based cell model, accounting for stochastic bacterial protein synthesis and cell-level behaviour, with continuum descriptions of inflammatory mediator and antibiotic dynamics.

The model, termed the hybrid model, acts over three spatial scales: the macro-, cell- and sub-cellular scales. We implement macro-scale processes (for inflammatory mediators and antibiotics) using PDEs that are space- and time-dependent, the relevant quantities assumed to be continuous in space and time. We model cell-scale processes using an agent-based representation, in which cells respond individually to environmental cues such as local chemical gradients and the presence of other cells. A sub-cellular stochastic gene regulatory network (GRN) within each bacterium governs its individual protein synthesis and replication, the involved processes implemented using the Gillespie algorithm [120]. The model is only valid in the early stages of infection and we assume the model breaks down after sufficient time for an infection that is not eradicated. This is due to us not implementing significant infection mechanisms occurring at large time, the main two being (1) the formation of extra-cellular matrix around bacteria and (2) damage-induced diarrhoea.

In section 5.2, we formulate the hybrid model and in section 5.3, we describe the numerical implementation of the macro-, cell- and sub-cellular scale processes. The model is flexible to the inclusion of additional mechanisms including novel antibiotic treatment strategies; however, we only consider

the class of antibiotics considered in the ODE model — aminoglycosides. The model is used to explore different treatment conditions with the aim of furthering our knowledge on which components of the infection strategy of EPEC contribute the most to its virulence, and determining which model parameters most effectively aid/hinder the return to health when altered. We are primarily interested in the peak and long-term infection sizes and the spatial profile of the bacteria over the course of the infection. We use the spatial profile of bacteria to consider the presence or absence of bacterial biofilms (clusters), how they evolve over time and whether there is a correlation between biofilms and infection efficacy. The results from this model can be used to inform future experimental work (see Chapter 6 for model analysis).

Table 5.1 describes how all processes are modelled and numerically implemented.

Process	Modelling	Implementation
Inflammatory mediator and antibiotic processes (production/administration, degradation, transport)	Continuum PDE	Finite difference scheme
Chemotaxis	Continuum PDE	Finite difference scheme
IEC damage and repair	Continuum PDE	Finite difference scheme
Bacterial and leukocyte death	Agent-based	Individually chosen from a distribution
Bacterial and leukocyte movement	Agent-based	Off-lattice biased/unbiased random walks and volume exclusion algorithms
Phagocytosis	Agent-based	Probabilistic time step-dependent success
Effector protein actions	Agent-based	Probabilistic density-dependent success
Bacterial protein synthesis and replication	Stochastic gene network	Gillespie algorithm

Table 5.1: Descriptions of how all processes are modelled and numerically implemented

5.2 Model formulation

5.2.1 Overview

The model is termed hybrid as it combines continuous, discrete, deterministic and stochastic implementations of the relevant infection mechanisms. Macro-scale processes comprise the production/administration, degradation, transport and internalisation of inflammatory mediators and antibiotic. Cell-scale processes consist of the movement of bacteria and leukocytes (where both Brownian and chemotactic motion are accommodated through a biased random walk), phagocytosis, volume exclusion and the replication and degradation of leukocytes. Sub-cellular scale processes include the transcription and translation of bacterial proteins (both structural and effector), the

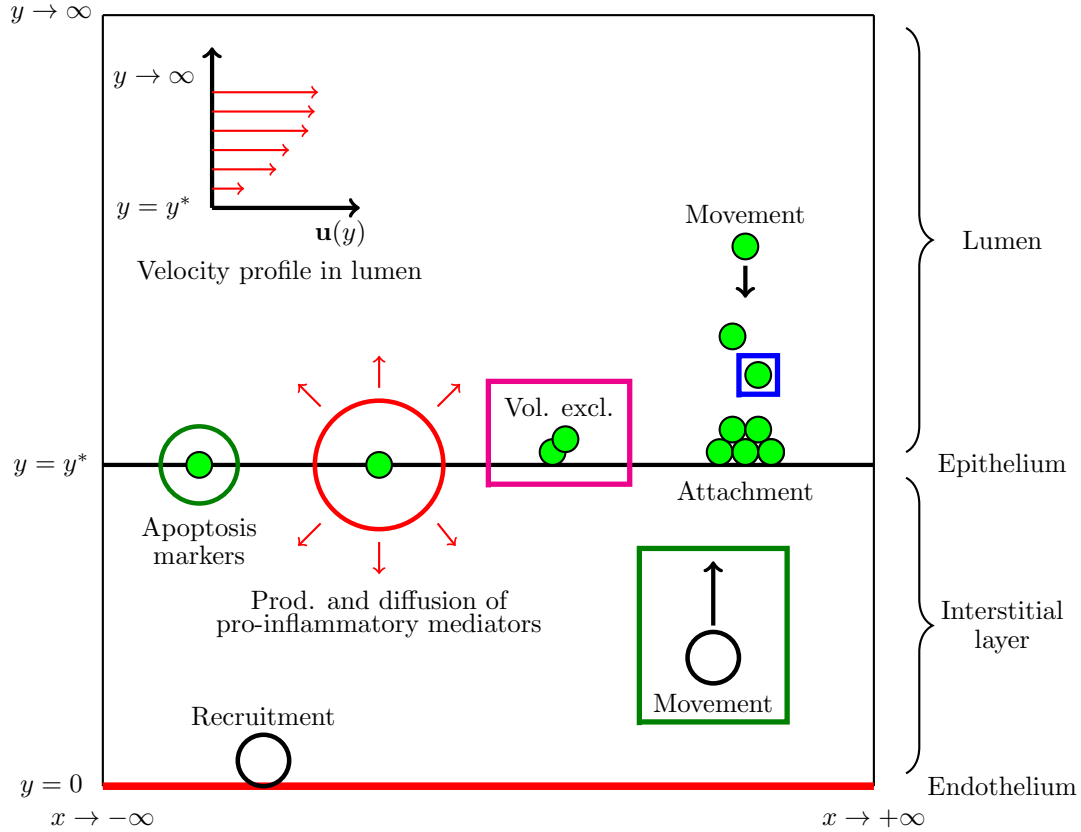


Figure 5.1: A global schematic diagram of the computational hybrid model. Green and white circles represent bacteria and leukocytes respectively. Bacteria move towards the epithelium where they bind, triggering the production and diffusion of pro-inflammatory mediators. Leukocytes are recruited into the region of interest when the presence of pro-inflammatory mediators at the endothelium is sufficiently high. IECs display apoptosis markers when they are sufficiently damaged, these stationary markers chemotactically attracting leukocytes. There is a shear flow in the lumen along the positive x -axis that transports diffusible substances along and out of the region of interest, the functional form of the flow given by (5.9).

replication of bacteria from structural proteins, the inhibitory actions of aminoglycosides and pro-inflammatory mediators and the degradation of mRNA, proteins and bacteria. Figures 5.1 and 5.2 give schematic diagrams of the hybrid model. The region of interest is a two dimensional transverse section, extending from the lumen of the intestines to the underlying blood supply. The endothelium and the epithelium-lumen interface are set at $y = 0$ and $y = y^*$ respectively and are orientated parallel to the x -axis. We assume that the epithelium is infinitesimally thin and that the cell layers and the lumen extend indefinitely into the respective far field(s).

5.2.2 Macro-scale model equations

Inflammatory mediators

We define pro- and anti-inflammatory mediator presences as $P(x, y, t) \in [0, 1]$ and $A(x, y, t) \in [0, 1]$ respectively, where $P(x, y, t) = 1$ and $A(x, y, t) = 1$ represent maximum presences of pro- and anti-inflammatory mediators respectively. $P(x, y, t)$ and $A(x, y, t)$ are governed by the following

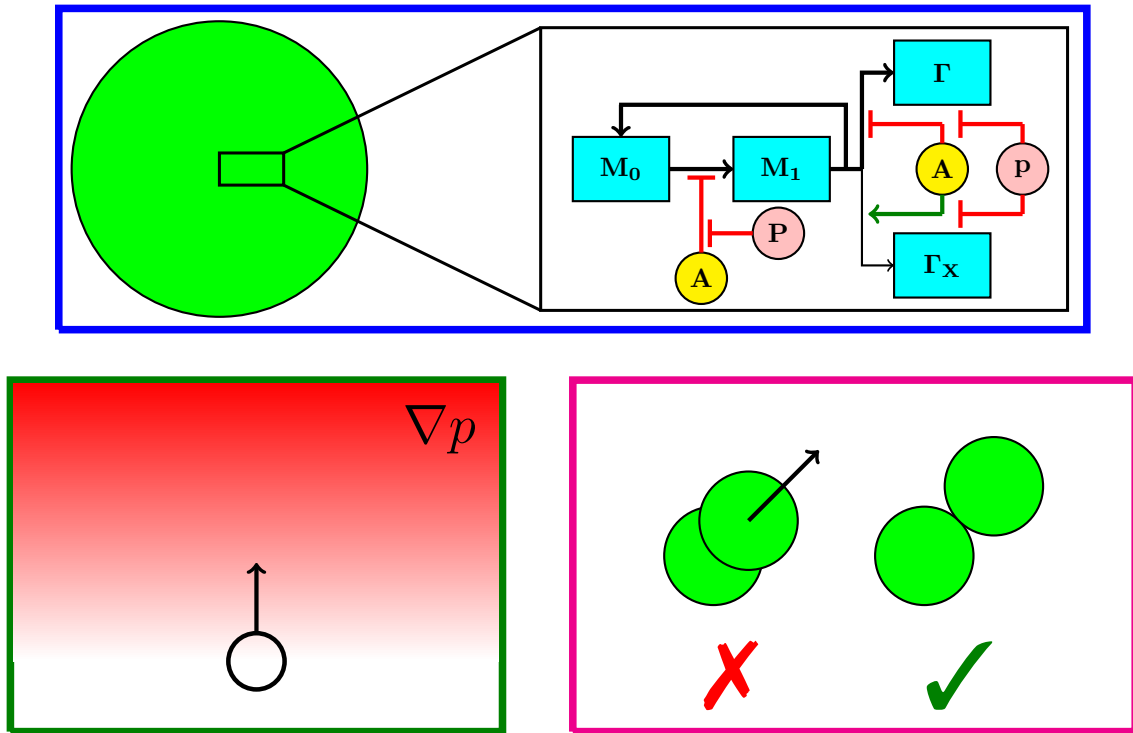


Figure 5.2: Schematic diagrams of the specific dynamics highlighted by the blue, green and pink boxes in Figure 5.1. Green and hollow black circles represent bacteria and leukocytes respectively. Blue box: There is a GRN within each bacterium governing its individual protein synthesis and replication. Green box: Leukocytes are attracted chemotactically up gradients in pro-inflammatory mediator presence. Pink box: Bacteria and leukocytes obey volume excluding algorithms.

PDEs:

$$\frac{\partial P}{\partial t} + \mathbf{u} \cdot \nabla P = D_I \nabla^2 P + (f_1 + f_2)f_3f_4 - f_5 - d_I P + H^*, \quad (5.1)$$

$$\frac{\partial A}{\partial t} + \mathbf{u} \cdot \nabla A = D_I \nabla^2 A + g_1 - d_I A, \quad (5.2)$$

where D_I and d_I are the (constant) diffusion coefficient and natural degradation rate of all inflammatory mediators respectively (assumed equal for each of P and A , for simplicity). The functions $f_1, f_2, f_3, f_4, f_5, g_1$ and H^* govern particular dynamics, the form of each function given in the main text of this subsection. Equations (5.1) and (5.2) are subject to the following boundary conditions:

$$\frac{\partial \mathbf{P}}{\partial x}(x = \pm\infty) = 0, \quad \frac{\partial \mathbf{P}}{\partial y}(y = \infty) = 0, \quad \frac{\partial \mathbf{P}}{\partial y}(y = 0) = \frac{R_I}{D_I} \mathbf{P}(y = 0), \quad (5.3)$$

where $\mathbf{P} = [P(x, y, t), A(x, y, t)]^T$ and R_I is the inflammatory mediator endothelial flux coefficient. The function f_1 governs the production of pro-inflammatory mediators by IECs and is given by:

$$f_1 = \left(\Phi \begin{cases} 1, & \text{for } y = y^*, \\ 0, & \text{otherwise,} \end{cases} + (1 - \Phi) \begin{cases} 1, & \text{for } \mathbf{x} = \mathbf{x}_B, \\ 0, & \text{otherwise,} \end{cases} \right) (1 - P), \quad (5.4)$$

where $\mathbf{x} = (x, y, t)$ and $\mathbf{x}_B$ describes the positions of the bound bacteria. All IECs produce pro-inflammatory mediators at a low rate (Φ) in the absence of pathogenic stimulation, while the IECs with bacteria attached to them produce at a higher rate constantly.

The function f_2 governs the production of pro-inflammatory mediators by leukocytes and is given by:

$$f_2 = r_{PL} |\nabla P| \begin{cases} 1, & \text{for } \mathbf{x} = \mathbf{x}_L, \\ 0, & \text{otherwise,} \end{cases} \quad (5.5)$$

where $\mathbf{x}_L$ gives the positions of the leukocytes and r_{PL} denotes the global production rate of pro-inflammatory mediators by leukocytes. We assume leukocytes produce pro-inflammatory mediators at a rate proportional to the magnitude of the chemotactic gradient at their respective locations, so that production only occurs during a stimulated inflammatory response.

The function f_3 governs the inhibition of pro-inflammatory mediator production by anti-inflammatory mediators and has the form $f_3 = (1 - A)$. The production of pro-inflammatory mediators is uninhibited in the local absence of anti-inflammatory mediators and decreases to zero as the local presence of anti-inflammatory mediators tends to the maximum value of 1.

The function f_4 governs the inhibition of pro-inflammatory mediator production by effector proteins and has the form:

$$f_4 = \frac{1}{1 + \phi_1 E_1}, \quad (5.6)$$

where $E_1 = E_1(x, t)$ gives the density of the effector proteins that inhibit pro-inflammatory signalling and ϕ_1 gives the efficacy of E_1 . Since E_1 are secreted into IECs, they are fixed at $y = y^*$. The production of pro-inflammatory mediators by an IEC is uninhibited when there are no internal E_1 , and decreases to zero as the number of internal E_1 increases.

The function f_5 governs the internalisation of pro-inflammatory mediators by bacteria and has the form:

$$f_5 = r_{\text{int},P}(P - P_{\text{int}}) \begin{cases} 1, & \text{for } \mathbf{x} = \mathbf{x}_B, \\ 0, & \text{otherwise,} \end{cases} \quad (5.7)$$

where $P_{\text{int}} = P_{\text{int}}(x, y, t)$ represents the profile of internalised pro-inflammatory mediators and $r_{\text{int},P}$ denotes the global internalisation rate of pro-inflammatory mediators. The rate of internalisation is proportional to the difference in pro-inflammatory mediator presence across the membrane of the bacterium, the internal and external presences tending to equilibrium over time. The evolution of P_{int} is governed by:

$$\frac{\partial P_{\text{int}}}{\partial t} = f_5 - d_I P_{\text{int}}. \quad (5.8)$$

$P_{\text{int}}(\mathbf{x}_B) \in [0, 1]$ and $P_{\text{int}} = 0$ elsewhere. We assume that pro-inflammatory mediators naturally degrade at the same rate internally as externally.

We assume no-slip shear flow in the lumen along the positive x -axis that advects inflammatory mediators (and antibiotic), the flow rate tending to a constant in the far-field. The flow profile, denoted $\mathbf{u}$, is given by:

$$\mathbf{u}(y) = \theta(y - y^*) \frac{(y - y^*)}{\phi_u + (y - y^*)} \hat{\mathbf{x}}, \quad (5.9)$$

where the epithelium-lumen interface is $y = y^*$, $\hat{\mathbf{x}}$ is the unit vector along the positive x -axis, θ is the Heaviside step function and ϕ_u is a constant.

The function H^* in (5.1) represents the source of pro-inflammatory mediators due to necrosis of IECs. To model this, we introduce a new variable, denoted $H(x, t)$, that represents the health of the continuous IEC layer. The necrosis-driven production of P by an IEC occurs when the health of the IEC reaches zero, therefore:

$$H^*(H) = r_N \begin{cases} 1, & \text{for } H = 0, \\ 0, & \text{otherwise,} \end{cases}$$

where r_N is the necrosis-driven production rate of pro-inflammatory mediators. The evolution of $H(x, t)$ is governed by:

$$\frac{\partial H}{\partial t} = -d_H C \begin{cases} 1, & \text{for } \mathbf{x} = \mathbf{x}_B, \\ 0, & \text{otherwise,} \end{cases} + \left(r_{HN}(1 - C) + r_{HF}C \right) \theta(1 - H). \quad (5.10)$$

While bacteria are attached to a functioning IEC, they damage it at constant rate d_H . When functioning IECs undergo apoptosis or necrosis, a new IEC forms in its place. Newly forming IECs are described as non-functioning and do not act as sources for pro-inflammatory mediators. We assume that non-functioning and functioning IECs are repaired at rates r_{HN} and r_{HF} respectively, where $r_{HN} > r_{HF}$. This results in the repair of non-functioning IECs being faster than that of functioning IECs. $C = C(x, t)$ is a Boolean function, where $C(a, t)$ takes a value of 0 or 1 when the IEC at $x = a$ is non-functioning or functioning respectively. The inclusion of C in (5.10) ensures that non-functioning IECs are not damaged by bacteria, due to the bacteria being unable to bind to them. Functioning IECs are fully repaired, and therefore cease being repaired, when $H = 1$. We model this using a Heaviside function, denoted θ , with argument $(1 - H)$.

When the damage to a functioning IEC becomes pronounced, apoptosis markers are displayed by the IEC (unless inhibited by effector proteins). These markers are chemoattractants for leukocytes and if a leukocyte comes into sufficiently close proximity with an IEC, then the IEC undergoes apoptosis. The chemoattractive strength of apoptosis markers on the IEC located at $x = a$, denoted $\beta_a(x, y, t)$, is given by:

$$\beta_a(x, y, t) = r_\beta F_{\text{apop}}(x, y, t) \theta \left(H_{\text{apop}} - H(a, t) \right) \left(\frac{1}{1 + \phi_2 E_2(a, t)} \right), \quad (5.11)$$

where $E_2 = E_2(x, t)$ gives the density of the effector proteins that inhibit apoptosis, ϕ_2 gives the efficacy of E_2 , H_{apop} denotes the health value below which IECs display apoptosis markers, r_β denotes the global chemoattractive strength of apoptosis markers and θ denotes the Heaviside function. $F_{\text{apop}}(x, y, t)$ is a decreasing function of the distance from the markers, and the form and implementation of this function are given by Figure 5.9 in section 5.3. IECs act as point sources of apoptosis markers provided that they are functioning with health $0 < H < H_{\text{apop}}$, the markers acting as sources of propagating chemotactic signals for convenience. The effector proteins that inhibit apoptosis (E_2) act by inhibiting the display of apoptosis markers. The strength of the signal from an IEC is uninhibited when there are no internal E_2 and decreases to zero as the number of internal E_2 increases. The global chemoattractive strength of apoptosis markers, denoted $\beta(x, y, t)$, is the sum of the strengths of all markers.

The function g_1 governs the stimulated production of anti-inflammatory mediators and is given by:

$$g_1 = r_A \frac{P^2}{\phi_A^2 + P^2}, \quad (5.12)$$

where r_A denotes the production rate and ϕ_A denotes the pro-inflammatory mediator presence at which production is half-maximal. The production rate of anti-inflammatory mediators increases as the local presence of pro-inflammatory mediators increases, plateauing at a maximum production rate.

Antibiotic

The concentration of antibiotic, $\alpha(x, y, t)$, is governed by the following PDE:

$$\frac{\partial \alpha}{\partial t} + \mathbf{u} \cdot \nabla \alpha = D_\alpha \nabla^2 \alpha - f_5^* - d_\alpha \alpha, \quad (5.13)$$

where D_α and d_α are the (constant) diffusion coefficient and natural degradation rate of the antibiotic respectively. Equation (5.13) is subject to the following boundary conditions:

$$\frac{\partial \alpha}{\partial y}(x = \pm\infty) = 0, \quad \frac{\partial \alpha}{\partial y}(y = \infty) = 0, \quad D_\alpha \frac{\partial \alpha}{\partial y}(y = 0) = R_\alpha \left(\alpha(y = 0) - \alpha^*(t) \right), \quad (5.14)$$

where R_α is the antibiotic endothelial flux coefficient and $\alpha^*(t)$ denotes the concentration of antibiotic in the circulatory system as a result of an external treatment. The far field boundary conditions are the same for the inflammatory mediators and the antibiotic, but the boundary conditions at the endothelium are different. External antibiotic is administered intravenously and enters the region of interest via the endothelium, via the Robin boundary condition given in (5.14). Since the antibiotic acts at the sub-cellular level, the various treatment styles governing the form of $\alpha^*(t)$ are considered in section 5.5.

The internalisation of antibiotic by bacteria is represented by f_5^* in (5.13) and takes the form:

$$f_5^* = r_{\text{int},\alpha}(\alpha - \alpha_{\text{int}}) \left(z_0 + (1 - z_0) \frac{\Lambda}{\phi_\Lambda + \Lambda} \right) \begin{cases} 1, & \text{for } \mathbf{x} = \mathbf{x}_B, \\ 0, & \text{otherwise,} \end{cases} \quad (5.15)$$

where $\alpha_{\text{int}} = \alpha_{\text{int}}(x, y, t)$, analogous to P_{int} , represents the profile of internalised antibiotic. The rate of internalisation is proportional to the difference in concentration across the membrane of the bacterium. The measure of bacterial membrane permeability, denoted $\Lambda = \Lambda(\mathbf{x}_B)$, is given by:

$$\Lambda = \frac{\Gamma_X}{w_1 + \Gamma_X}, \quad (5.16)$$

where $\Gamma_X = \Gamma_X(\mathbf{x}_B)$ represents the number of mistranslated proteins in all bacteria. The definitions of $r_{\text{int},\alpha}$, z_0 , ϕ_Λ and w_1 are given in Table 5.2. There are basal ($z_0 r_{\text{int},\alpha}$) and maximal ($r_{\text{int},\alpha}$) rates of internalisation, where $0 \leq z_0 < 1$. More antibiotic is internalised by a bacterium when the number of mistranslated proteins in its membrane, and therefore its permeability, is higher. Since internalised antibiotic results in bacteria producing more mistranslated proteins on average (see section 5.2.4), a positive feedback loop for antibiotic internalisation is created.

Parameter	Definition
$r_{\text{int},\alpha}$	The maximum internalisation rate of antibiotic
z_0	The proportion of antibiotic that can enter a fully functioning bacterial membrane
ϕ_Λ	The permeability measure at which internalisation is half-maximal
w_1	The number of mistranslated proteins at which permeability is half-maximal

Table 5.2: The definitions of the parameters present in (5.15) and (5.16)

The evolution of α_{int} is governed by:

$$\frac{\partial \alpha_{\text{int}}}{\partial t} = f_5^* - d_\alpha \alpha_{\text{int}}. \quad (5.17)$$

5.2.3 Cell-scale dynamics

For simplicity, we assume that bacterial and leukocyte motion is via a biased random walk, neglecting the sub-cellular mechanisms involved in motion. As a simple model for the time taken during the binding process of EPEC to IECs, we choose the speed of bacterial motion to be proportional to their distance from the epithelium; binding defined to occur when the distance to the epithelium is below a small critical value.

To model chemotaxis, we construct a scalar field by summing the pro-inflammatory mediator presence and the signal strength of apoptosis markers (we assume for simplicity that apoptosis markers behave identically to pro-inflammatory mediators in the context of chemotaxis). This scalar field defines the chemotactic gradient, and therefore the direction in which leukocytes move. Similarly to [135] (in the context of neural crest cell migration), we define a sensing accuracy below which leukocytes do not respond to chemotactic gradients, and exhibit an unbiased random walk (Brownian motion). All leukocytes move with constant velocity; chemotaxis only changes the direction of motion.

We assume that mobile cells (bacteria and leukocytes) are identically circular, with each cell type associated with a fixed radius. As a result of the model's two-dimensional representation, volume exclusion is represented as area exclusion. When two like-cells overlap, they follow a volume exclusion algorithm that results in the cells no longer overlapping (see Algorithm 5.46), the volume exclusion algorithm used depending on the cell type. When there is an overlap between a bacterium and a leukocyte, we assume the leukocyte instantaneously removes the bacterium through phagocytosis (unless inhibited by effector proteins) for simplicity.

Leukocytes enter the region of interest through the endothelium, with recruitment activated once the presence of pro-inflammatory mediators at the endothelium exceeds a critical threshold. While activated, leukocyte recruitment occurs at a deterministic frequency with entry occurring at the point on the endothelium with the highest presence of pro-inflammatory mediators. When a leuko-

cyte is recruited, it is given a degradation time chosen from a normal distribution.

5.2.4 Sub-cellular scale dynamics

Bacterial protein synthesis and replication

A GRN is used to govern protein synthesis and replication within each bacterium. This GRN is based on that presented in Chapter 4 (equations (4.34)); however, we also include the dynamics of polymerases and ribosomes. We make distinctions between the polymerases and ribosomes that are and are not bound to DNA and mRNA strands respectively. Each bacterium has an independent GRN inside of it and therefore has its own sub-population of the GRN variables. Table 5.3 gives a description and the initial condition (within each bacterium) of each GRN variable. We refer the reader to the blue box in Figure 5.2 for a schematic of the GRN.

We make the following assumptions for simplicity:

- There is an unlimited supply of the nutrients required for DNA replication to occur,
- Polymerases and ribosomes are all initially unbound,
- The numbers of polymerases and ribosomes remain constant throughout the infection (no creation or destruction),
- DNA and mRNA strands can only support one polymerase and one ribosome at a time respectively,
- mRNA strands that are unbound can degrade, but those being actively translated cannot.

The proteins synthesised in the bacterium either become effector proteins or the structural proteins to be used for bacterial replication. We model bacterial replication by assuming, for simplicity, that structural proteins combine to form a new bacterium once there are sufficiently many to do so, the number required denoted Γ^* . Bacteria have varying numbers of structural proteins present initially, the number chosen randomly from $\mathcal{U}(0, \Gamma^* - 1)$, as they are at varying stages of their life cycle at any given point in time. We assume all structural proteins are identical for simplicity.

The effector protein groups that inhibit pro-inflammatory signalling (E_1) and host cell apoptosis (E_2) are secreted into IECs and so are not found within bacteria, but the effector proteins that inhibit phagocytosis (E_3) remain inside the bacterium that created them and become effective when the bacterium is challenged by phagocytosis. We assume that the production of effector proteins by a bacterium is triggered when the bacterium is bound to an IEC, and that E_1 and E_2 are secreted into that IEC. When ribosomal proof-reading is impaired by aminoglycosides, the GRN produces mistranslated proteins, denoted Γ_X , instead of correctly translated structural proteins. In the absence of aminoglycosides, there is no ribosomal impairment, therefore all produced structural proteins are correctly translated ($\Gamma_X = 0$ for all t). We implement the dynamics of the GRN in numerical simulations using a Gillespie algorithm (see section 5.5 for details on the implementation).

Variable	Symbol	Initial quantity within each bacterium
Unbound RNAP	θ_0	$\theta_{\max}$
Bound RNAP	θ_1	0
Unbound mRNA	M_0	0
Bound mRNA	M_1	0
Free ribosomes	R	$R_{\max}$
Structural proteins	Γ	$\mathcal{U}(0, \Gamma^* - 1)$
No of E_1	E_1	0
No of E_2	E_2	0
No of E_3	E_3	0
Mistranslated proteins	Γ_X	0

Table 5.3: A description and the initial condition (within each bacterium) of each GRN variable, $\mathcal{U}$ denoting a uniform distribution. The initial quantities for E_1 and E_2 are always 0 as they are not located inside bacteria.

Antibiotic delivery strategies

Inspired by the antibiotic treatment styles carried out by Ternent et al. [85], similar treatment styles are explored in this hybrid model. These treatment styles are:

- No antibiotic (control),
- One decaying dose,
- Repeated decaying doses,
- Constant concentration, e.g. via an intravenous drip.

This allows a variety of treatment styles to be considered and the comparison of our results to those obtained in [85]. As in the ODE model (Chapter 4), the class of antibiotics to be considered is aminoglycosides, the concentration of an aminoglycoside dose denoted $\alpha_{\text{dos}}(t)$ and the concentration of a constant delivery denoted $\alpha_{\text{con}}(t)$. We assume for simplicity that the antibiotic concentration is uniform throughout the circulatory system. For a dose style treatment, we assume that the antibiotic concentration in the circulatory system increases quickly after administration, peaks and then slowly decays to zero as the antibiotic degrades. The antibiotic concentration over time, of dose n , is governed by:

$$\alpha_{\text{dos}}^n(t) = H(t - t_n) z_n \frac{2q(t - t_n)}{q^2 + (t - t_n)^2}, \quad (5.18)$$

where t_n is the administration time of the dose, z_n is the peak antibiotic concentration, q is the time taken from administration for the antibiotic concentration to peak and H is the Heaviside step function (see the blue curve in Figure 5.3). In the case of one dose, the concentration of antibiotic in the circulatory system $\alpha^*(t) = \alpha_{\text{dos}}^1(t)$ and in the case of repeated doses, $\alpha^*(t)$ is the sum of the individual doses with each dose having its own administration time and strength (we remind the

reader that $\alpha^*(t)$ appears in (5.14)).

The antibiotic concentration over time for a constant delivery is governed by:

$$\alpha_{\text{con}}(t) = H(t - t_1)z_1, \quad (5.19)$$

where t_1 is the administration time and z_1 is the antibiotic concentration being delivered (see the red curve in Figure 5.3).

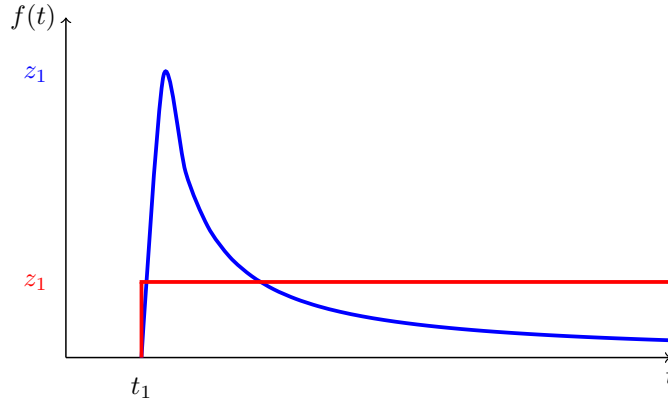


Figure 5.3: The shapes of the antibiotic administration functions $\alpha_{\text{dos}}^1(t)$ (blue; given by (5.18) when $n = 1$) and $\alpha_{\text{con}}(t)$ (red; given by (5.19)).

The antibiotic concentrations in the circulatory system in the four treatment strategies are given by:

None:	$\alpha^*(t) = 0,$
One dose:	$\alpha^*(t) = \alpha_{\text{dos}}^1(t),$
Repeated doses:	$\alpha^*(t) = \sum_{n=1}^N \alpha_{\text{dos}}^n(t),$
Constant:	$\alpha^*(t) = \alpha_{\text{con}}(t),$

where N is the number of doses in the repeated dose case. We are interested in finding which styles of delivery are the most effective at eradicating an infection given a fixed amount of antibiotic. The total amount of antibiotic delivered over a fixed period of time, denoted α_{tot} , is therefore kept equal across the various (nonzero) treatment strategies, giving:

$$\int_{x=-X}^{x=X} \int_{t=0}^{t=t_{\text{max}}} \alpha^*(t) dt dx = \alpha_{\text{tot}}, \quad (5.20)$$

where t_{max} is the time at which all treatment is ceased. Figure 5.4 shows an example of each

treatment style when the spatial average antibiotic concentration is 1, i.e. $\alpha_{\text{tot}}/(2X) = 1$. By asserting that the total antibiotic over a fixed period of time is the same for all delivery styles, fair comparisons between the delivery styles can be made. The specific treatment styles to be explored will be discussed in Chapter 6, along with the addressing of the question of which delivery style is the most effective.

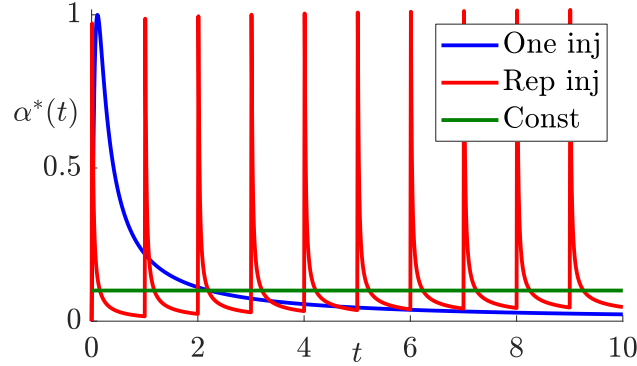


Figure 5.4: Example graphs showing the antibiotic concentration in the circulatory system for the various treatment styles. For all graphs, $t_1 = 0, t_{\text{max}} = 10$ and $\alpha_{\text{tot}}/(2X) = 1$. In the one dose case, $z_1 = 1$. In the repeated doses case, the doses are spaced with unit time intervals and all have strength $z_i = 1$. In the constant delivery case, $z_1 = 0.1$.

In both the hybrid model and the ODE model, the internalised antibiotic is an increasing function of the number of mistranslated proteins; however, in the hybrid model distinctions between internal and external pro-inflammatory mediators and antibiotic are made. Each bacterium has an individual internal (1) number of mistranslated proteins, (2) presence of pro-inflammatory mediators and (3) concentration of antibiotic, allowing us to model the local spatial dynamics, giving richer and more realistic infection dynamics. The hybrid model can therefore highlight effective treatment strategies that the well-mixed ODE model cannot.

5.3 Numerical implementation

We refer the reader back to Table 5.1 for a summary of how all processes are modelled and numerically implemented.

5.3.1 Macro-scale

Inflammatory mediators

We apply a finite difference discretisation of the PDEs for pro- and anti-inflammatory mediator presences (equations (5.1) and (5.2) respectively), where $P_{i,j}^k$ denotes the approximation to $P(x, y, t)$ at the i th horizontal mesh point (x_i), j th vertical mesh point (y_j) and k th time point (t_k). The mesh has N_x and N_y mesh points along the x and y axes respectively and we denote the step between discrete time points as Δt , and the discrete steps in the horizontal and vertical directions as Δx and Δy respectively. We assume that bacteria do not typically leave $|x| \leq 1$; therefore in order to reduce computation time, we make the mesh non-uniform in x such that it is fine for $|x| \leq 1$, but

coarse for $x \gg 1$ (see Figure 5.5). On the mesh, Δx_i gives the length between x_i and x_{i+1} . The IEC layer is discretised onto the same mesh, the individual IECs defined by the regions between horizontal mesh points at $y = y^*$, the cell centres being the mid-points of each adjacent pair of mesh points. We use first order forward difference schemes for time derivatives and second order central difference schemes for spatial derivatives.

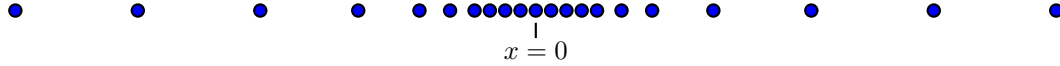


Figure 5.5: Caricature of the (horizontally) non-uniform mesh.

The PDE for P (equation (5.1)) is discretised according to:

$$\begin{aligned} \frac{P_{i,j}^{k+1} - P_{i,j}^k}{\Delta t} = & D_I \left(\frac{2}{\Delta x_{i-1}(\Delta x_{i-1} + \Delta x_i)} P_{i-1,j}^k - \frac{2}{\Delta x_{i-1}\Delta x_i} P_{i,j}^k + \frac{2}{\Delta x_i(\Delta x_{i-1} + \Delta x_i)} P_{i+1,j}^k \right) \\ & + \frac{D_I}{\Delta y^2} \left(P_{i,j-1}^k - 2P_{i,j}^k + P_{i,j+1}^k \right) + \Phi \left(1 - P_{i,j}^k \right) \left(1 - A_{i,j}^k \right) \frac{1}{1 + \phi_1 \bar{E}_{1,j}} + (H^*)_i^k \\ & - d_I P_{i,j}^k - \frac{\bar{P}_{\mathbf{x}_B}^k}{\Delta t} + \begin{cases} u_j \left(P_{i-1,j}^k - P_{i,j}^k \right), & \text{for } i = 2, \dots, N_x, \\ -u_j P_{i,j}^k, & \text{for } i = 1. \end{cases} \end{aligned} \quad (5.21)$$

In (5.21), $\bar{P}_{\mathbf{x}_B}^k$ represents the amount of internalised P in the bacteria (at positions $\mathbf{x}_B$). We now explain the discretised form of each term, bar diffusion, in (5.21). At all unbound mesh points along the IEC layer, background production of P obeys the following scheme:

$$P_{i,j}^{k+1} = P_{i,j}^k + \Delta t \Phi \left(1 - P_{i,j}^k \right) \left(1 - A_{i,j}^k \right), \quad (5.22)$$

where $\Phi < 1$. Since P is discretised it must be created at a mesh point, i.e. one of the IEC walls, the left wall chosen arbitrarily. For each bacterium, the distance to the IEC layer is calculated and if it is less than or equal to the radius of the bacterium (rad_B), the bacterium is defined as being bound to the IEC layer, triggering stimulated production of pro-inflammatory mediators. We approximate P at the nearest mesh point to the left of each bound bacterium, the mesh point denoted (x_B, y_B) . Stimulated production of P obeys the following scheme:

$$P_{x_B, y_B}^{k+1} = P_{x_B, y_B}^k + \Delta t (1 - \Phi) \left(1 - P_{x_B, y_B}^k \right) \left(1 - A_{x_B, y_B}^k \right). \quad (5.23)$$

We implement background and stimulated production of P (term $f_1 f_3$ in (5.1)) using equations (5.22) and (5.23). Figure 5.6 shows a graphical caricature of the horizontal discretisation and the stimulated production of pro-inflammatory mediators by IECs.

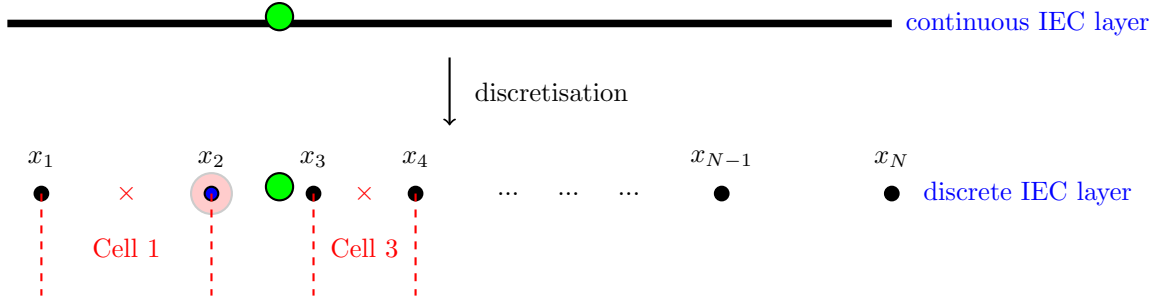


Figure 5.6: Caricature showing the discretisation of the IEC layer and the stimulated production of pro-inflammatory mediators. The green circle represents a bacterium and the red transparent circle represents the mesh point acting as the pro-inflammatory mediator source point as a result of the bacterium binding to the IEC layer, in this case to cell 2.

If N bacteria are attached to an IEC, then (5.23) is executed N times, giving a larger increase in the presence of P at the relevant mesh point. The amount increased has a linear negative relationship with the presence of A . Newly created pro-inflammatory mediators will be transported away, degrade or be internalised by bacteria, creating space for the further production of pro-inflammatory mediators at each IEC. Combining (5.22) and (5.23) approximates $f_1 f_3$ in (5.1).

We found that including leukocyte production of pro-inflammatory mediators (f_2 in (5.1)) rendered chemotaxis ineffective (see Appendix section A.4), therefore we omit the process in this chapter. We refer the reader to Chapter 7 for an investigation of this in a continuum model.

We now consider the inhibition of pro-inflammatory signalling by E_1 ¹ (function f_4 in (5.1)). We implement the success of inhibition as a probabilistic process, with the probability of inhibition increasing as the number of E_1 increases. At each time point, the following quantities are calculated for the j th bound bacterium:

$$H_{E_1}^j = \frac{1}{1 + \phi_1 \bar{E}_{1,j}}, \quad R_j \sim \mathcal{U}(0, 1), \quad (5.24)$$

where $\bar{E}_{1,j}$ denotes the number of E_1 contained within the IEC that the j th bacterium is bound to. It can be seen that $H_{E_1}^j \in (0, 1]$. Algorithm 2 is executed at each time step. We implement function f_4 in (5.1) using (5.24).

Algorithm 2 Algorithm for successful stimulated pro-inflammatory mediator production

1. **for** $j = 1, \dots, N_B$,
 - (a) **if** Bacterium j is bound to epithelium,
 - i. **if** $H_{E_1}^j < R_j$,
 - no change,
 - ii. **else**, execute (5.23).

¹The production and placement of E_1 are discussed later, but for now only the quantity of E_1 is important.

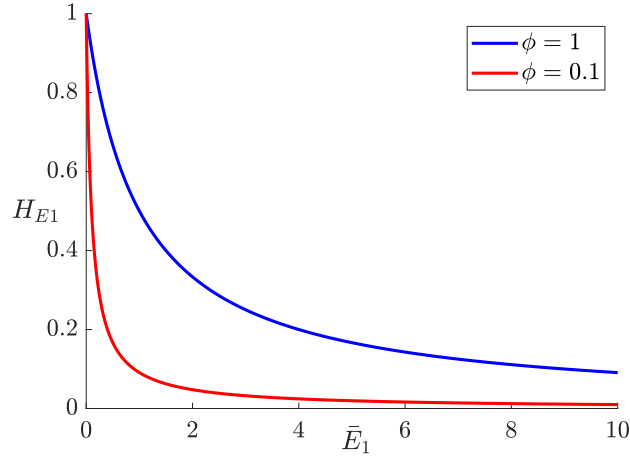


Figure 5.7: Graphs showing how H_{E1} varies with $\bar{E}_1$ and ϕ_1 .

Background and stimulated production of P , described by (5.22) and (5.23), will always be executed in the absence of internal E_1 , but the probability of successful pro-inflammatory mediator production decreases to zero as $\bar{E}_{1,j}$ increases. If there are multiple bacteria attached to an IEC, there is a higher chance of successful pro-inflammatory mediator production by the IEC, since Algorithm 2 is executed for each individual bacterium. Figure 5.7 shows example graphs for H_{E1} .

To implement the internalisation of pro-inflammatory mediators by bacteria (f_5 in (5.1)), let bacterium z (B_z) have position (x_z, y_z) , $P_{\text{ext},z}^k$ be the current presence of external P at the closest mesh point to bacterium z and $P_{\text{int},z}^k$ be the presence of P currently internalised by bacterium z . The amount of P internalised by B_z over time step Δt is denoted $\tilde{P}_z^k$ and is given by:

$$\tilde{P}_z^k = \Delta t r_{\text{int},P} (P_{\text{ext},z}^k - P_{\text{int},z}^k). \quad (5.25)$$

To apply the internalisation, we execute:

$$P_{\text{int},z}^{k+1} = P_{\text{int},z}^k + \tilde{P}_z^k - \Delta t d_I P_{\text{int},z}^k, \quad (5.26a)$$

$$P_{\text{ext},z}^{k+1} = P_{\text{ext},z}^k - \tilde{P}_z^k. \quad (5.26b)$$

Equations (5.26a) and (5.26b) are approximations of (5.8) and (5.7) respectively. The amount of P is conserved during internalisation and it can be shown that $P_{\text{int},z}^k \leq 1$ provided that $\Delta t \leq r_{\text{int},P}^{-1}$. The term $-\Delta t d_I P_{\text{int},z}$ represents the degradation of internalised pro-inflammatory mediators and (5.26) is applied independently for all bacteria. Bacterium z is defined as being of phenotype B_2 if $P_{\text{int},z} \geq P_{\text{crit}}$, where P_{crit} is some critical value. Since internalised pro-inflammatory mediators can degrade, reversal back to phenotype B_1 occurs if $P_{\text{int},z}$ returns below P_{crit} . Phenotypes B_1 and B_2 produce effector proteins at different rates (discussed in section 5.5).

We now consider advection of P due to lumen flow. We calculate u_j by substituting $y = y_j$ into (5.9). Since advection is along the positive x -axis, there is no source term due to advection at the left-most mesh point (x_1). To implement the boundary conditions (given by (5.3)), we use ghost points that lead to the following substitutions in (5.21):

$$\text{for } i = 1, \quad P_{0,j} = P_{2,j}, \quad (5.27a)$$

$$\text{for } i = N_x, \quad P_{N_x+1,j} = P_{N_x-1,j}, \quad (5.27b)$$

$$\text{for } j = N_y, \quad P_{i,N_y+1} = P_{i,N_y-1}, \quad (5.27c)$$

$$\text{for } j = 1, \quad P_{i,0} = P_{i,2} - 2\Delta y(R_I/D_I)P_{i,1}. \quad (5.27d)$$

The implementation of H^* in (5.1), given by $(H^*)_i^k$ in (5.21), is discussed separately, in section 5.3.1, due to the amount of detail.

The PDE for A (equation (5.2)) is discretised according to:

$$\begin{aligned} \frac{A_{i,j}^{k+1} - A_{i,j}^k}{\Delta t} = & D_I \left(\frac{2}{\Delta x_{i-1}(\Delta x_{i-1} + \Delta x_i)} A_{i-1,j}^k - \frac{2}{\Delta x_{i-1}\Delta x_i} A_{i,j}^k + \frac{2}{\Delta x_i(\Delta x_{i-1} + \Delta x_i)} A_{i+1,j}^k \right) \\ & + \frac{D_I}{\Delta y^2} \left(A_{i,j-1}^k - 2A_{i,j}^k + A_{i,j+1}^k \right) + \left(r_A \frac{P_{3 \times 3}^2}{\phi_A^2 + P_{3 \times 3}^2} - \frac{A_{i,j}^k}{\Delta t} \right) \\ & - d_I A_{i,j}^k + \begin{cases} u_j \left(A_{i-1,j}^k - A_{i,j}^k \right), & \text{for } i = 2, \dots, N_x, \\ -u_j A_{i,j}^k, & \text{for } i = 1. \end{cases} \end{aligned} \quad (5.28)$$

In (5.28), the terms for diffusion, advection and degradation are identical to those in (5.21), but in terms of $A_{i,j}^k$. The production of $A(x, y, t)$, given by $g_1(P)$ in (5.2), at each mesh point is approximated by:

$$A_{i,j}^{k+1} = \Delta t r_A \frac{P_{3 \times 3}^2}{\phi_A^2 + P_{3 \times 3}^2}, \quad (5.29)$$

where

$$P_{3 \times 3} = \sum_{n=-1}^1 \sum_{m=-1}^1 P_{i+m,j+n}^k. \quad (5.30)$$

The production of A at a specific mesh point depends on $P_{3 \times 3}$, the amount of P in an enveloping 3 by 3 square mesh around the mesh point. This function is then passed into an increasing Hill function with Hill parameter 2; the same function as the equivalent function used in the ODE model. $P_{3 \times 3}$ uses a wider local region than P , giving more reliable results that are less sensitive to numerical artefacts in pro-inflammatory mediator presence. We implement the boundary conditions for (5.2) using (5.27), but in terms of $A_{i,j}^k$ rather than $P_{i,j}^k$.

Antibiotic

The PDE for α (equation (5.13)) is discretised according to:

$$\begin{aligned} \frac{\alpha_{i,j}^{k+1} - \alpha_{i,j}^k}{\Delta t} = & D_\alpha \left(\frac{2}{\Delta x_{i-1}(\Delta x_{i-1} + \Delta x_i)} \alpha_{i-1,j}^k - \frac{2}{\Delta x_{i-1} \Delta x_i} \alpha_{i,j}^k + \frac{2}{\Delta x_i(\Delta x_{i-1} + \Delta x_i)} \alpha_{i+1,j}^k \right) \\ & + \frac{D_\alpha}{\Delta y^2} \left(\alpha_{i,j-1}^k - 2\alpha_{i,j}^k + \alpha_{i,j+1}^k \right) \\ & - d_\alpha \alpha_{i,j}^k - \frac{\bar{\alpha}_{\mathbf{x}_B}^k}{\Delta t} + \begin{cases} u_j \left(\alpha_{i-1,j}^k - \alpha_{i,j}^k \right), & \text{for } i = 2, \dots, N_x, \\ -u_j \alpha_{i,j}^k, & \text{for } i = 1. \end{cases} \end{aligned} \quad (5.31)$$

In (5.31), the terms for diffusion, advection and degradation are identical to those in (5.21), but with $P_{i,j}^k$, D_I and d_I replaced with $\alpha_{i,j}^k$, D_α and d_α respectively. To implement the antibiotic boundary conditions (given by (5.14)), we use ghost points that lead to the following substitutions in (5.31):

$$\text{for } i = 1, \quad \alpha_{0,j} = \alpha_{2,j}, \quad (5.32a)$$

$$\text{for } i = N_x, \quad \alpha_{N_x+1,j} = \alpha_{N_x-1,j}, \quad (5.32b)$$

$$\text{for } j = N_y, \quad \alpha_{i,N_y+1} = \alpha_{i,N_y-1}, \quad (5.32c)$$

$$\text{for } j = 1, \quad \alpha_{i,0} = \alpha_{i,2} - 2\Delta y(R_\alpha/D_\alpha) \left(\alpha_{i,1} - \alpha^*(t_k) \right). \quad (5.32d)$$

To implement the internalisation of antibiotic by bacteria (f_5^* in (5.13)), let bacterium z have position (x_z, y_z) , $\alpha_{\text{ext},z}^k$ be the current concentration of external antibiotic at the closest mesh point to bacterium z and $\alpha_{\text{int},z}^k$ be the concentration of antibiotic currently internalised by bacterium z . The amount of antibiotic internalised by B_z over a time step Δt is denoted $\tilde{\alpha}_z^k$ and is given by:

$$\tilde{\alpha}_z^k = \Delta t r_{\text{int},\alpha} (\alpha_{\text{ext},z}^k - \alpha_{\text{int},z}^k) \left(z_0 + (1 - z_0) \frac{\Lambda_z}{\phi_\Lambda + \Lambda_z} \right), \quad (5.33)$$

where:

$$\Lambda_z = \frac{\Gamma_{X,z}}{w_1 + \Gamma_{X,z}},$$

$\Gamma_{X,z}$ denoting the density of mistranslated proteins in bacterium z . To apply the internalisation, we execute:

$$\alpha_{\text{int},z}^{k+1} = \alpha_{\text{int},z}^k + \tilde{\alpha}_z^k - \Delta t d_\alpha \alpha_{\text{int},z}^k, \quad (5.34a)$$

$$\alpha_{\text{ext},z}^{k+1} = \alpha_{\text{ext},z}^k - \tilde{\alpha}_z^k. \quad (5.34b)$$

Equations (5.34a) and (5.34b) are approximations of (5.17) and (5.15) respectively. The mass of antibiotic is conserved during internalisation and it can be shown that $\alpha_{\text{int},z}^k \leq 1$ provided that $\Delta t \leq r_{\text{int},\alpha}^{-1}$. The term $-\Delta t d_\alpha \alpha_{\text{int},z}$ represents the degradation of internalised antibiotic and (5.34) is applied independently for all bacteria.

Health and death of IECs

We remind the reader that the IEC layer is continuous in the x direction. Figure 5.8 gives a schematic diagram of the implementation of IEC health and death. We assume all IECs are functioning and have $H(t = 0) = 1$. We implement IEC function and death with Algorithm 3, the definitions of the parameters and functions used in Algorithm 3 given in Table 5.4.

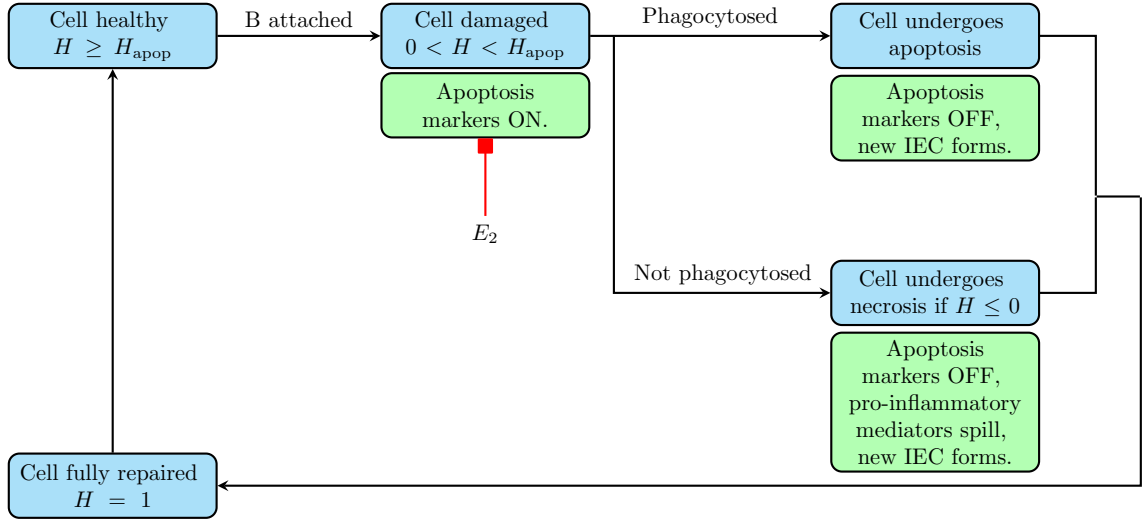


Figure 5.8: Flow diagram depicting the implementation of IEC health and death. The red square-headed arrow represents inhibition.

Algorithm 3 Algorithm for health and death of IECs

1. **For** $j = 1 - N_B$: Define bacterium j as bound to IEC layer if $|y_{Bj} - y^*| \leq \text{rad}_B$. Bacterium j is bound to IEC i if $x_i \leq x_{Bj} < x_{i+1}$.
2. **For** $i = 1 - N_I$: **If** IEC i is functioning, denote the number of bacteria bound to IEC i as N_{bnd}^i and apply:

$$H_i^{k+1} = H_i^k - \Delta t r_{\text{dam}} N_{\text{bnd}}^i \mathcal{U}(0.75, 1.25). \quad (5.35)$$

- (a) **If** $H_i^k < H_{\text{apop}}$, calculate:

$$I_{E2}^i = \frac{1}{1 + \phi_2 \bar{E}_{2,i}}, \quad R_i \sim \mathcal{U}(0, 1), \quad (5.36)$$

- (b) **If** $I_{E2}^i < R_i$, **then** do not display apoptosis markers on IEC i ,
else apply:

$$\beta_{m,n}^k = \beta_{m,n}^k + r_\beta \begin{cases} 1, & m = i, n = y^*, \\ 0.5, & 1 \leq |m - i| + |n - y^*| \leq 2, |m - i| \leq 1, |n - y^*| \leq 1, \\ 0.25, & 2 \leq |m - i| + |n - y^*| \leq 4, |m - i| \leq 2, |n - y^*| \leq 2, \\ 0, & \text{otherwise.} \end{cases} \quad (5.37)$$

- (c) **For** $m = 1 - N_L$: **If** $x_i \leq x_{Lm} < x_{i+1}$ and $|y_{Lm} - y^*| \leq \text{rad}_L$:
- i. Set $H_i^k = 0$,
 - ii. Define IEC i as newly formed and non-functioning ($C(x, t_k) = 0$ for $x_i \leq x < x_{i+1}$).
- (d) **If** IEC i is functioning and $H_i^k \leq 0$:
- i. set $H_i^k = 0$ and define IEC i as newly formed and non-functioning,
 - ii. remove apoptosis markers if present (reverse (5.37)),
 - iii. apply:

$$P_{m,n}^k = \begin{cases} 1, & |m - i| \leq 1, |n - y^*| \leq 1, \\ 0, & \text{otherwise,} \end{cases} \quad (5.38)$$

- iv. unbind any bacteria bound to IEC i and destroy any internal E_1 and E_2 .

- (e) Repair IEC i according to:

$$H_i^k = H_i^k + \begin{cases} (\Delta t / t_a), & \text{if died via apoptosis,} \\ (\Delta t / t_n), & \text{if died via necrosis,} \\ (\Delta t / t_f), & \text{if functioning with } H_i^k < 1, \\ 0, & \text{if functioning with } H_i^k \geq 1. \end{cases} \quad (5.39)$$

Par / fun	Definition
N_B, N_L, N_I	The number of bacteria, leukocytes and IECs respectively
(x_{Bj}, y_{Bj})	The position of bacterium j
(x_{Lm}, y_{Lm})	The position of leukocyte m
$(x_i \leq x < x_{i+1}, y^*)$	The position of IEC i
H_i^k	The health of IEC i at the k th time point
r_{dam}	The average rate at which a bacterium damages an IEC
H_{apop}	The maximum value of H at which apoptosis markers are presented
$\bar{E}_{2,i}$	The number of E_2 within IEC i
$\text{rad}_B, \text{rad}_L$	The radii of bacteria and leukocytes respectively
ϕ_2	The efficacy of E_2 to inhibit the display of apoptosis markers
$\beta_{m,n}^k$	The presence of apoptosis markers at mesh point (x_m, y_n) at the k th time point
t_a	The time for an IEC that died via apoptosis to return to full health
t_n	The time for an IEC that died via necrosis to return to full health
t_f	The time for a functioning IEC that is infinitesimally close to necrosis to return to full health
$\mathcal{U}(a, b)$	A number chosen uniformly randomly from (a, b)

Table 5.4: The definitions of the parameters and functions used in Algorithm 3.

We assume that an IEC that is non-functioning:

- does not get damaged by bacteria, as there are no bacteria bound,
- does not secrete pro-inflammatory mediators or display apoptosis markers,
- cannot have effector proteins injected into it,
- becomes fully mature and functioning once its health value reaches 1.

Equation (5.37) is a computationally inexpensive approximation to the equation for the chemoattractive strength of apoptosis markers (equation (5.11)) and Figure 5.9 shows the effects of (5.37) graphically. When an IEC displays apoptosis markers it does so on a 3 by 3 grid of mesh points with the grid centre on the left wall of the IEC, creating a gradient in apoptosis markers that enables chemotactic attraction of leukocytes. When an IEC dies via necrosis, all neighbouring mesh points around the left wall of the IEC have their pro-inflammatory mediator presences set to the maximum value, i.e. 1 (see equation (5.38)). The production and placement of effector proteins will be discussed in section 5.5. Finally, we impose that (1) $t_a < t_n$ as we assume it takes less time to replace an IEC that died via apoptosis than via necrosis, due to the lesser damage and (2) $t_n < t_f$ as we assume repair of functioning cells takes longer due to the higher consumption of resources in other metabolic pathways, such as secretion of inflammatory mediators. The implementation of host cell death and its related mechanisms is comparable to the ODE model in that necrosis acts as a source of pro-inflammatory mediators due to damage by bacteria and that apoptosis markers

chemotactically attract leukocytes.

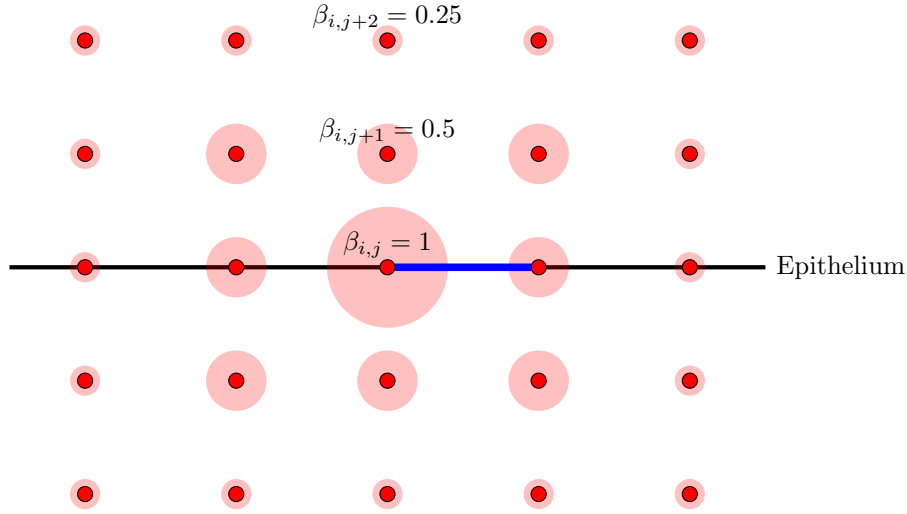


Figure 5.9: Diagram showing the strength of the apoptosis markers displayed by an individual IEC at neighbouring mesh points. The filled red circles represent mesh points, the size of each partially transparent red circle represents the strength of the apoptosis markers at the respective mesh point and the blue line represents the IEC that is displaying the apoptosis markers.

5.4 Cell level mechanisms

5.4.1 Overview

Now that we have considered the processes that occur at the macro-scale, we now consider the implementation of the phenomena that occur at the cell-scale. These include cell movement, phagocytosis, volume exclusion, recruitment and death of leukocytes and the production of pro-inflammatory mediators by leukocytes. The implementation of the cell-scale processes is agent-based and involves probability, stochasticity and logical arguments, giving realistic dynamics both locally and globally. The two scales are coupled, as the discretised presence of pro-inflammatory mediators and concentration of antibiotic inform many cell-scale actions, including chemotaxis, internalisation of pro-inflammatory mediators and antibiotic, and recruitment of leukocytes. In this section, we give the mathematical implementation of each cell-scale process.

5.4.2 Movement of bacteria

To simply model the binding of EPEC to IECs, bacteria move via a biased random walk towards the epithelium. We denote the position of bacterium j at the k th time point as (x_{Bj}^k, y_{Bj}^k) . We implement the movement of bacterium j with the following scheme:

$$\begin{bmatrix} x_{Bj}^{k+1} \\ y_{Bj}^{k+1} \end{bmatrix} = \begin{bmatrix} x_{Bj}^k \\ y_{Bj}^k \end{bmatrix} + \Delta t \frac{B_{\text{mot}}}{r_B^*} \left(\frac{|y_{Bj}^k - y^*|}{\phi_B + |y_{Bj}^k - y^*|} \right) \begin{bmatrix} \cos \theta_B \\ \sin \theta_B \end{bmatrix} - \Delta t B_{\text{mot}} (y_{Bj}^k - y^*) \begin{bmatrix} 0 \\ 1 \end{bmatrix}, \quad (5.40)$$

with the parameters involved in (5.40) defined in Table 5.5. The second and third terms on the RHS of (5.40) represent Brownian motion and directed movement towards the epithelium respectively, the magnitude of each decreasing as the distance to the epithelium decreases. There is negligible movement when the bacterium is sufficiently close to the epithelium since for small $|y_{Bj} - y^*|$, the speed of movement is $\mathcal{O}(|y_j - y^*|)$. Once (5.40) has been applied to a bacterium, we check whether the bacterium has travelled past each of the four boundaries of the region of interest, and if it has, we move the bacterium so that it is within the boundary. We apply (5.40) and the above inclusion algorithm for all bacteria independently and although the inclusion algorithm is crude, it is computationally inexpensive.

Parameter	Definition
θ_B	a random number computed from $\mathcal{U}(0, 2\pi)$.
y^*	the y -coordinate of the epithelium.
ϕ_B	the $ y_j - y^* $ value at which random movement is half-maximal.
B_{mot}	the directed motility rate of bacteria.
r_B^*	the scale factor of how strong directed motion is compared to Brownian motion.

Table 5.5: The definitions of the parameters involved in (5.40).

5.4.3 Movement of leukocytes

Leukocytes move via a biased random walk when the chemotactic gradient is sufficiently strong, and via an unbiased walk otherwise (Brownian motion). We implement the motion of leukocyte j at time-point k via:

$$(x_{Lj}^k, y_{Lj}^{k+1}) = (x_{Lj}^k, y_{Lj}^k) + \Delta t L_{\text{mot}} (\cos \Theta, \sin \Theta), \quad (5.41)$$

where (x_{Lj}^k, y_{Lj}^k) represents the position of the leukocyte, L_{mot} represents the motility rate of leukocytes and Θ represents the angle of movement. Θ is calculated by:

$$\Theta = \begin{cases} \mathcal{N}(\theta^*, \sigma_{\text{mot},L}^2), & G \geq G_{\min}, \\ \mathcal{U}(0, 2\pi), & G < G_{\min}, \end{cases} \quad (5.42)$$

where G denotes the chemotactic gradient across the leukocyte, θ^* denotes the angle of the gradient, $\sigma_{\text{mot},L}$ denotes the standard deviation of the biased random walk angle and $G_{\min}$ denotes the sensing accuracy of the leukocytes. Figure 5.10 shows the probability distributions in (5.42) graphically.

To calculate the chemotactic gradient, the steepest gradient across the surface of the leukocyte is calculated. The discretised surface of leukocyte j , denoted L_S^j , is given by:

$$L_S^j = (x_{Lj}^k + \text{rad}_L \cos \theta, y_{Lj}^k + \text{rad}_L \sin \theta), \quad \text{for } \theta = 2\pi i/N, \quad (5.43)$$

where rad_L denotes the radius of leukocytes and $i = 1, \dots, N$. We take N uniformly spaced points on the surface to allow $N/2$ gradients across the centre of the leukocyte to be computed. At the closest mesh point to each surface point, we compute the chemoattractive strength, given by the sum of pro-inflammatory mediator presence and apoptosis marker strength. We then define the chemotactic gradient, G , to be the maximum difference in chemoattractive strengths across each pair of opposing surface points. Figure 5.11 shows this method graphically. If there is more than one pair of opposing surface points that share the highest chemoattractive strength, we select one of the pairs uniformly randomly.

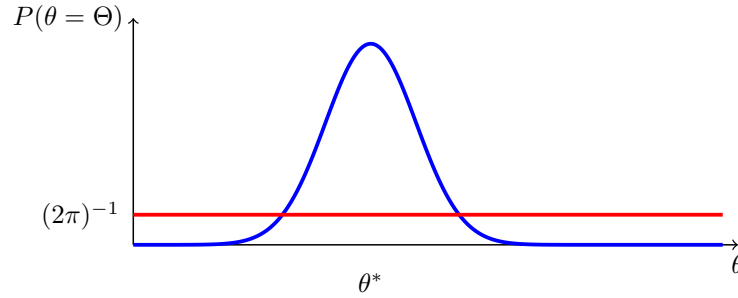


Figure 5.10: The shapes of the probability distributions in (5.42), the biased and unbiased random walks given in blue and red respectively.

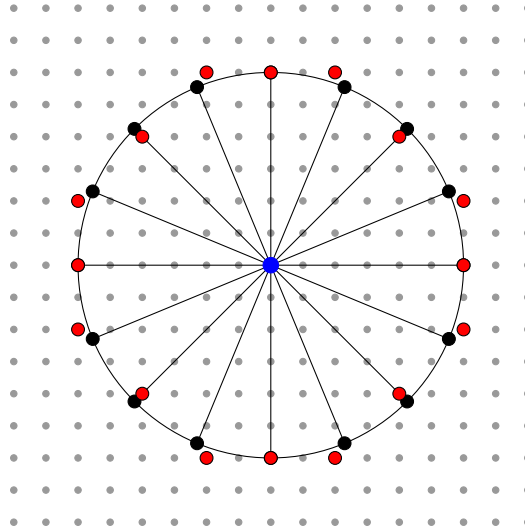


Figure 5.11: Diagram showing how the chemotactic gradient over the leukocyte is approximated. The blue central circle represents the centre of the leukocyte, light grey circles represent mesh points, black circles represent the N evenly spaced points on the surface of the leukocyte and red circles represent the mesh points that approximate the leukocyte surface points.

As with bacteria, we check whether the leukocyte has travelled past each of the four boundaries of the region of interest, and if it has, we move the leukocyte so that it is within the boundary. We apply (5.41)–(5.43) for all leukocytes independently.

5.4.4 Phagocytosis

We then compute whether there are any bacteria that can be removed through phagocytosis, under the assumption that only one bacterium can be removed by each leukocyte at each time step. We consider the closest bacterium for simplicity. Successful phagocytosis occurs if the distance between the leukocyte and the closest bacterium is less than the radius of the leukocyte, and the E_3 within the bacterium fail at inhibition. If phagocytosis is successful, we assume that (1) the bacterium is (instantaneously) removed and (2) anything within the bacterium, including pro-inflammatory mediators, effector proteins and antibiotic, is destroyed. We define the probability that bacterium i is removed through phagocytosis in time step Δt to be:

$$\mathcal{P}(\text{phag in } t \leq \Delta t) = 1 - (1 - Q)^k. \quad (5.44)$$

Q represents the the probability that bacterium i is removed through phagocytosis in an infinitesimally small time step $t_\epsilon = (\Delta t/k)$, where constant $k \in \mathbb{N}$ and $k \gg 1$. Q is given by:

$$Q(\bar{E}_{3,i}) = \frac{1}{1 + \phi_3 \bar{E}_{3,i}}.$$

$\bar{E}_{3,i}$ is the density of E_3 contained within bacterium i and ϕ_3 denotes the efficacy of E_3 at preventing phagocytosis. For a derivation of (5.44), we refer the reader to Appendix section A.7. The probability of phagocytosis is 1 when $\bar{E}_{3,i} = 0$ and decreases to zero as $\bar{E}_{3,i}$ increases. Figure 5.12 gives a contour plot of $\log_{10}(\mathcal{P})$ for varying Δt and $\bar{E}_{3,i}$, the logarithm of $\mathcal{P}$ being used as it shows the changes due to Δt more clearly than the linear plot. $\mathcal{P}$ decreases rapidly as $\bar{E}_{3,i}$ increases and increases as Δt increases. $\mathcal{P}$ is compared to a random number R chosen from $\mathcal{U}(0, 1)$ and if $\mathcal{P} < R$, then the phagocytosis of bacterium i is inhibited in the time window $(t, t + \Delta t]$ by the E_3 within the bacterium. The movement and phagocytosis algorithms presented in this subsection are executed independently for each leukocyte at each time step.

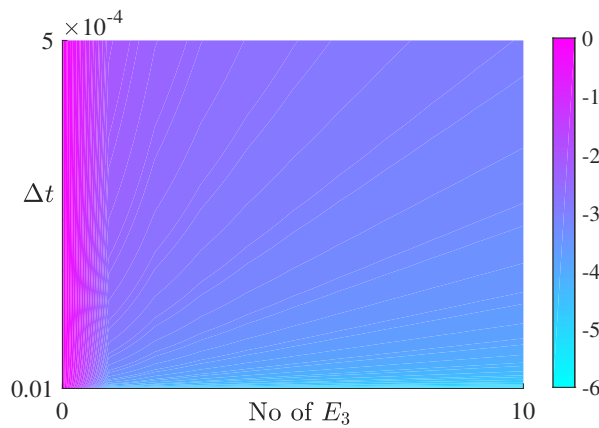


Figure 5.12: A contour plot of $\log_{10}(\mathcal{P})$ for varying Δt and $\bar{E}_{3,i}$, $\mathcal{P}$ denoting the probability that phagocytosis occurs in time step Δt .

5.4.5 Volume exclusion

We use a simple volume exclusion algorithm, based on the Lennard-Jones and Buckingham potentials discussed in the literature review (see section 2.9), to ensure that cells do not overlap. There are two variations of the algorithm; one for bacterium-bacterium volume exclusion and the other for leukocyte-leukocyte volume exclusion. For bacterium-bacterium volume exclusion, we apply movement to the bacterium with the highest y -coordinate to balance the downward movement of bacteria towards the epithelium and to ensure that bacteria can bind to, but not cross, the epithelium. For leukocyte-leukocyte volume exclusion, we apply movement equally to both cells, since the upward bias is not required. We do not consider bacterium-leukocyte exclusion as it represents phagocytosis, and effector protein-effector protein exclusion for simplicity. We first show how the algorithm works for a pair of overlapping cells and then extend the algorithm to work for any number of simultaneously overlapping cells.

Bacterium-bacterium volume exclusion

For a pair of bacteria that occupy mutual volume, movement takes place along the vector that connects their centres; however, care must be taken for bacteria that have identical positions. To circumvent the possibility of having a zero vector connect cells, we apply for all pairs of bacteria with identical centres:

$$(x_{Bi}^k, y_{Bi}^k) = (x_{Bi}^k, y_{Bi}^k) + (\epsilon^*, \epsilon^*), \quad (5.45a)$$

$$(x_{Bj}^k, y_{Bj}^k) = (x_{Bj}^k, y_{Bj}^k) - (\epsilon^*, \epsilon^*), \quad (5.45b)$$

where $0 < \epsilon^* \ll \text{rad}_B$. We then find the indices of the pairs of bacteria that are within 2rad_B of each other, and therefore overlapping. We calculate the minimum distance that one of the bacteria needs to move so that they are no longer overlapping. We also add to this distance a small positive amount ϵ , to ensure that the boundaries of the two cells are not touching after the movement has been applied. The distance to move is given by $2\text{rad}_B - d + \epsilon^*$, where d is the distance between the centres of the bacteria. Figure 5.13 shows how this distance is calculated.

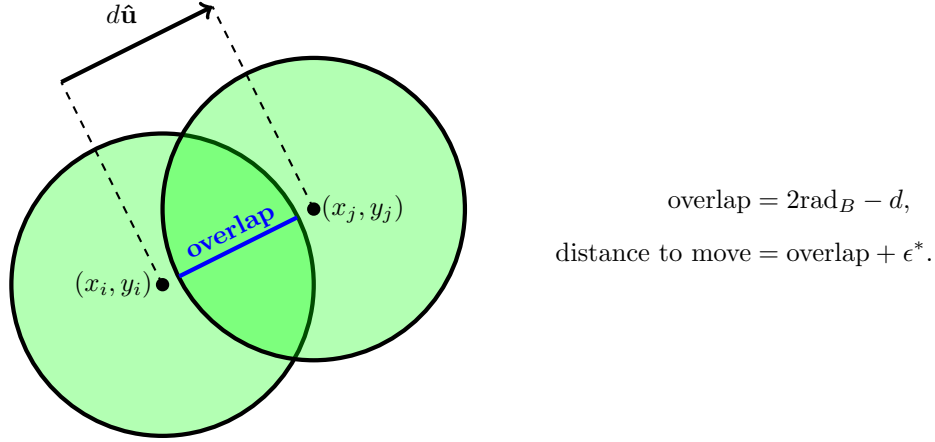


Figure 5.13: Diagram showing how the minimum distance to move a bacterium in a pair of overlapping bacteria so that they occupy mutually exclusive space is calculated.

The unit vector connecting the cell centres is given by:

$$\hat{\mathbf{u}} = \frac{(x_{Bj}^k - x_{Bi}^k, y_{Bj}^k - y_{Bi}^k)}{d}.$$

We then move the bacterium with the highest y -coordinate by an amount $(2\text{rad}_B - d + \epsilon^*)$ along $\hat{\mathbf{u}}$, such that movement is upwards.

We generalise this algorithm to work for any number of simultaneously overlapping bacteria, the generalised algorithm given by Algorithm 4.

Algorithm 4 Algorithm for general bacteria-bacteria volume exclusion

1. Compute the matrix containing the distances between the centres of all pairs of bacteria, denoted $\mathbf{B}$, and only consider the strictly lower triangular part, denoted $\mathbf{B}_L$.
2. **while** $\min(\mathbf{B}_L) < 2\text{rad}_B$, **then**:

- (a) Find the indices i, j such that $(B_L)_{ij} = 0$. Put these pairs of indices into two lists, denoted $\mathbf{p}$ and $\mathbf{q}$, the elements of which are i and j respectively. Apply the scheme:

$$(\mathbf{x}_{B\mathbf{p}}^k, \mathbf{y}_{B\mathbf{p}}^k) = (\mathbf{x}_{B\mathbf{p}}^k, \mathbf{y}_{B\mathbf{p}}^k) + (\epsilon^*, \epsilon^*), \quad (5.46a)$$

$$(\mathbf{x}_{B\mathbf{q}}^k, \mathbf{y}_{B\mathbf{q}}^k) = (\mathbf{x}_{B\mathbf{q}}^k, \mathbf{y}_{B\mathbf{q}}^k) - (\epsilon^*, \epsilon^*), \quad (5.46b)$$

where $0 < \epsilon^* \ll \text{rad}_B$ and we are considering the k th time point.

- (b) Find the indices i, j such that $(B_L)_{ij} < 2\text{rad}_B$. Put these pairs of indices into two lists, denoted $\mathbf{m}$ and $\mathbf{n}$, the elements of which are i and j respectively.
- (c) Calculate the distance to move all pairs of overlapping bacteria $\mathbf{D}_{mn} = (2\text{rad}_B - (\mathbf{B}_L)_{mn} + \epsilon)$, where $0 < \epsilon \ll \text{rad}_B$.
- (d) Calculate the unit vectors connecting the centres of bacteria $\mathbf{m}$ and $\mathbf{n}$, pointing towards bacteria $\mathbf{m}$:

$$\hat{\mathbf{u}}_{mn} = \frac{(\mathbf{x}_{B\mathbf{m}}^k - \mathbf{x}_{B\mathbf{n}}^k, \mathbf{y}_{B\mathbf{m}}^k - \mathbf{y}_{B\mathbf{n}}^k)}{(\mathbf{B}_L)_{mn}}. \quad (5.47)$$

- (e) **If** $\mathbf{y}_{B\mathbf{m}}^k \geq \mathbf{y}_{B\mathbf{n}}^k$:

$$(\mathbf{x}_{B\mathbf{m}}^{k+1}, \mathbf{y}_{B\mathbf{m}}^{k+1}) = (\mathbf{x}_{B\mathbf{m}}^k, \mathbf{y}_{B\mathbf{m}}^k) + \mathbf{D}_{mn} \hat{\mathbf{u}}_{mn}, \quad (5.48a)$$

$$(\mathbf{x}_{B\mathbf{n}}^{k+1}, \mathbf{y}_{B\mathbf{n}}^{k+1}) = (\mathbf{x}_{B\mathbf{n}}^k, \mathbf{y}_{B\mathbf{n}}^k), \quad (5.48b)$$

- (f) **else if** $\mathbf{y}_{B\mathbf{m}}^k < \mathbf{y}_{B\mathbf{n}}^k$:

$$(\mathbf{x}_{B\mathbf{m}}^{k+1}, \mathbf{y}_{B\mathbf{m}}^{k+1}) = (\mathbf{x}_{B\mathbf{m}}^k, \mathbf{y}_{B\mathbf{m}}^k), \quad (5.49a)$$

$$(\mathbf{x}_{B\mathbf{n}}^{k+1}, \mathbf{y}_{B\mathbf{n}}^{k+1}) = (\mathbf{x}_{B\mathbf{n}}^k, \mathbf{y}_{B\mathbf{n}}^k) - \mathbf{D}_{mn} \hat{\mathbf{u}}_{mn}. \quad (5.49b)$$

- (g) Recalculate $\mathbf{B}$.

In Algorithm 4, one of the cases is arbitrarily chosen to include the case $\mathbf{y}_{B\mathbf{m}}^k = \mathbf{y}_{B\mathbf{n}}^k$ for simplicity. Figure 5.14 shows a simple one pair example of (5.48) and (5.49) graphically.

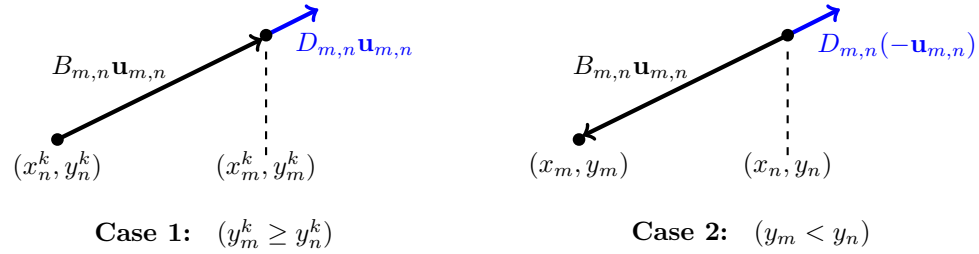


Figure 5.14: Diagram showing (5.48) and (5.49) for one pair of bacteria, demonstrating that the bacterium with the highest y -coordinate moves upwards. The black arrows represent the unit vector $\mathbf{u}_{m,n}$ and the blue arrows represent the directions of movement of the relevant bacteria.

Leukocyte-leukocyte volume exclusion

Leukocyte-leukocyte volume exclusion is the same as that for bacteria, except that leukocytes $\mathbf{m}$ and $\mathbf{n}$ both move along $\mathbf{u}_{m,n}$ half the required distance in opposite directions. The leukocyte-leukocyte volume exclusion algorithm is therefore the same as Algorithm 4, but with (5.48) and (5.49) replaced with:

$$(\mathbf{x}_{L\mathbf{m}}^{k+1}, \mathbf{y}_{L\mathbf{m}}^{k+1}) = (\mathbf{x}_{L\mathbf{m}}^k, \mathbf{y}_{L\mathbf{m}}^k) + 0.5\mathbf{D}_{\mathbf{m},\mathbf{n}}\hat{\mathbf{u}}_{\mathbf{m},\mathbf{n}}, \quad (5.50a)$$

$$(\mathbf{x}_{L\mathbf{n}}^{k+1}, \mathbf{y}_{L\mathbf{n}}^{k+1}) = (\mathbf{x}_{L\mathbf{n}}^k, \mathbf{y}_{L\mathbf{n}}^k) - 0.5\mathbf{D}_{\mathbf{m},\mathbf{n}}\hat{\mathbf{u}}_{\mathbf{m},\mathbf{n}}. \quad (5.50b)$$

5.4.6 Recruitment and death of leukocytes

The activation of leukocyte recruitment is dependent upon the presence of pro-inflammatory mediators at the endothelium ($y = 0$). Let P_{endo} be the average pro-inflammatory mediator presence across $x = [x_l, x_r]$ at the k th time point such that $x_l = -x_r$:

$$P_{\text{endo}}^k = \frac{\sum_{i=l}^r P_{i,1}^k}{x_r - x_l}. \quad (5.51)$$

Once P_{endo} reaches a critical threshold, denoted P_{endo}^* , leukocyte recruitment is activated, with a leukocyte recruited into the region of interest every $t_{L\text{rep}}$ units of time, at the endothelial mesh point in $[x_l, x_r]$ with the highest pro-inflammatory mediator presence. Leukocytes are assigned a lifetime, sampled from a normal distribution with mean $\mu_{\text{deg},L}$ and standard deviation $\sigma_{\text{deg},L}$, allowing variability in the lifetimes of leukocytes. If P_{endo} returns below P_{endo}^* , then leukocyte recruitment is deactivated. The leukocyte recruitment algorithm is given by Algorithm 5, N_L^k denoting the number of leukocytes at time-point k and (x_{LN_L}, y_{LN_L}) denoting the position of leukocyte N_L . A deterministic recruitment rate is chosen (1) for simplicity, (2) to make changes in the leukocyte population due to the actions of effector proteins easier to observe and (3) to prevent the number of leukocytes becoming too large. Algorithm 5 is applied to all leukocytes independently.

Algorithm 5 Algorithm for leukocyte recruitment

-
1. At initialization, define the values of P_{endo}^* and $t_{L\text{rep}}$, and set $t_{\text{next}L} = 0$.
 2. **while** $P_{\text{endo}}^k \geq P_{\text{endo}}^*$,
 - (a) **if** $t_{\text{next}L} = 0$,

$$x^* = \max_i \{P_{i,1}^k\} \text{ for } i = l, \dots, r,$$

$$N_L^k = N_L^k + 1,$$

$$(x_{LN_L}, y_{LN_L}) = (x^*, 0),$$

$$L_{\text{deg}, N_L} = t + \mathcal{N}(\mu_{\text{deg}, L}, \sigma_{\text{deg}, L}^2),$$

$$t_{\text{next}L} = t_{L\text{rep}},$$
 - (b) **else** $t_{\text{next}L} = t_{\text{next}L} - \Delta t$.
-

If $t \geq L_{\text{deg}, i}$ then leukocyte i dies, and we apply this check for all leukocytes (for $i = 1, \dots, N_L$).

5.4.7 Production of pro-inflammatory mediators by leukocytes

We have found the production of pro-inflammatory mediators by leukocytes to be incompatible with the chemotactic dynamics already present in the model; the pro-inflammatory mediators that the leukocytes produce dampen the chemotactic gradients they are following, leading to them getting stuck in local maxima of pro-inflammatory mediators, even for low production rates. Pro-inflammatory mediator production by leukocytes is therefore omitted from the model. A toy model exploring the observed antagonistic dynamics in more detail in one spatial dimension for one leukocyte is given in Appendix section A.4. A more thorough mathematical investigation into the antagonistic dynamics is carried out in Chapter 7, where we model the concentrations of both pro-inflammatory mediators and leukocytes as continuous quantities in space and time.

5.5 Sub-cellular scale processes

Overview

Now that we have considered the processes that occur at the macro- and cell-scales, we now consider the implementation of the processes that occur at the sub-cellular scale. These include (1) bacterial protein synthesis, (2) the inhibitory effect of aminoglycosides on bacterial protein synthesis and (3) the inhibitory effect of internalised pro-inflammatory mediators on aminoglycoside action. We implement bacterial protein synthesis stochastically via a Gillespie algorithm.

Bacteria gene network for protein synthesis and replication

We implement the dynamics of the GRN for bacterial protein synthesis using the Gillespie algorithm (see [121] for a detailed explanation of the algorithm). The GRN includes:

- the transcription of DNA into mRNA by polymerase enzymes,
- the translation of mRNA into proteins by ribosomes,
- the replication of bacteria,

- the degradation of mRNA, proteins and bacteria.

The 16 reactions present in the Gillespie algorithm, along with the corresponding reaction rates, are given in Table (5.6). We implement the reactions using the Law of Mass Action.

θ_0	$\xrightarrow{b_E}$	θ_1	Initiation of transcription.
θ_1	$\xrightarrow{r_0}$	$M_0 + \theta_0$	Transcription.
$M_0 + R$	$\xrightarrow{b_1(1-f_{P\alpha})}$	M_1	Initiation of translation.
M_1	$\xrightarrow{r_1(1-f_{P\alpha})}$	$E_1 + M_0 + R$	Translation of an E_1 .
M_1	$\xrightarrow{r_2(1-f_{P\alpha})}$	$E_2 + M_0 + R$	Translation of an E_2 .
M_1	$\xrightarrow{r_3(1-f_{P\alpha})}$	$E_3 + M_0 + R$	Translation of an E_3 .
M_1	$\xrightarrow{r_\Gamma(1-f_{P\alpha})}$	$\Gamma + M_0 + R$	Translation of a structural protein.
$\Gamma^*\Gamma$	$\xrightarrow{r_B}$	B	Fusing of structural proteins into a new bacterium.
M_0	$\xrightarrow{d_0}$	$\emptyset$	Degradation of an (unbound) mRNA strand.
E_1	$\xrightarrow{d_1}$	$\emptyset$	Degradation of an E_1 .
E_2	$\xrightarrow{d_2}$	$\emptyset$	Degradation of an E_2 .
E_3	$\xrightarrow{d_3}$	$\emptyset$	Degradation of an E_3 .
Γ	$\xrightarrow{d_\Gamma}$	$\emptyset$	Degradation of a structural protein.
B	$\xrightarrow{d_B}$	$\emptyset$	Degradation of a bacterium.
M_1	$\xrightarrow{r_\Gamma f_{P\alpha}}$	$\Gamma_X + M_0 + R$	Translation of a mistranslated protein.
Γ_X	$\xrightarrow{d_X}$	$\emptyset$	Degradation of a mistranslated protein.

Table 5.6: The reactions involved in the Gillespie algorithm. We implement the reactions using the Law of Mass Action.

The production rates of E_1, E_2 and E_3 are r_1, r_2 and r_3 respectively and are set to 0 for a bacterium if it is not bound to an IEC. All effector protein production is linear in M_1 , which is very different to the corresponding production used in the ODE model (see system (4.36)). We assume for simplicity that the difference in effector protein production by the different bacterial phenotypes is given by:

$$r_i = \begin{cases} r_{i,\text{base}}, & \text{if produced by } B_1, \\ r^* r_{i,\text{base}}, & \text{if produced by } B_2, \end{cases}$$

where $r_{i,\text{base}}$ is the baseline production rate of E_i for $i = 1, 2, 3$ and $r^* > 1$ is a constant, ensuring that B_2 is the more virulent phenotype. Figure 5.15 shows numerical simulations of the Gillespie algorithm, demonstrating that the transcription variables (θ_0, θ_1) reach a steady state much faster than the translation variables (M_0, M_1, R) .

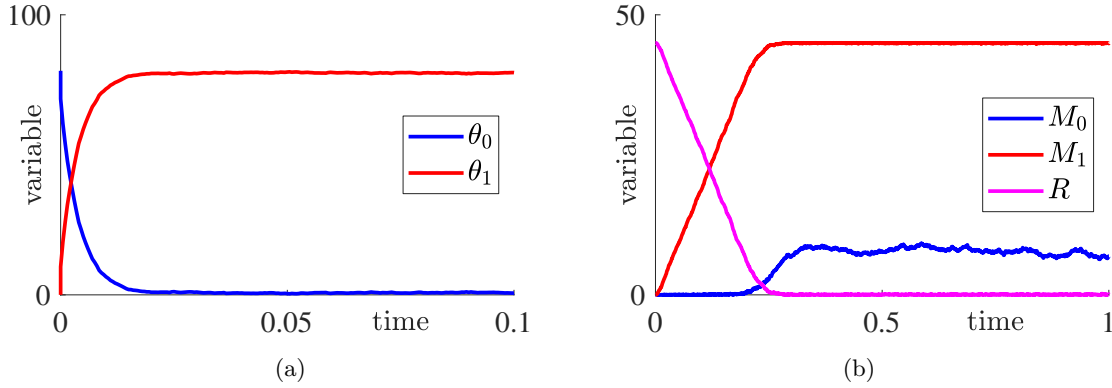


Figure 5.15: Numerical simulations of the Gillespie algorithm showing the mean quantities of the (a) transcription and (b) translation variables for 30 bacteria in the absence of bacterial growth and death. Non-zero parameter values are $R_{\max} = 45$, $\theta_{\max} = 80$, $b_E = 250$, $b_1 = 250$, $r_0 = 2.5$, r_Γ , $d_0 = 25$. All other parameter values are set to 0.

The propensity functions of the 16 reactions are:

$$\begin{aligned}
 \eta_1 &= b_E \theta_0, & \eta_2 &= r_0 \theta_1, \\
 \eta_3 &= b_1(1 - f_{P\alpha})M_0R, & \eta_4 &= r_1(1 - f_{P\alpha})M_1, \\
 \eta_5 &= r_2(1 - f_{P\alpha})M_1, & \eta_6 &= r_3(1 - f_{P\alpha})M_1, \\
 \eta_7 &= r_\Gamma(1 - f_{P\alpha})M_1, & \eta_8 &= r_B \Gamma^*, \\
 \eta_9 &= d_0 M_0, & \eta_{10} &= d_1 E_1, \\
 \eta_{11} &= d_2 E_2, & \eta_{12} &= d_3 E_3, \\
 \eta_{13} &= d_\Gamma \Gamma, & \eta_{14} &= d_B, \\
 \eta_{15} &= r_\Gamma f_{P\alpha}, & \eta_{16} &= d_X \Gamma_X.
 \end{aligned}$$

Table 5.7: The propensity functions of the 16 reactions given in Table 5.6

Note that for the propensity of the degradation of bacteria (reaction 14), the number of bacteria is set equal to 1, since the Gillespie algorithm is applied within each individual bacterium. From Tables 5.6 and 5.7, the type and time of the next reaction for each bacterium can be computed. The algorithm is made more efficient by executing all Gillespie algorithms simultaneously, each bacterium having an independent type and time of reaction. The time of the next reaction in bacterium j is denoted τ_j and the bacterium with the smallest τ executes the next reaction. This algorithm repeats until $\tau_j \geq t_0 + \Delta t$ for $j = 1, \dots, N_B$, where N_B is the number of bacteria and t_0 is the time at which the Gillespie algorithm is initiated. When the algorithm is terminated, the rest of the model is updated, the value of Δt chosen to ensure sufficient accuracy of the model.

We implement the inhibitory actions of aminoglycosides and pro-inflammatory mediators in the function $f_{P\alpha}$, where, for bacterium j :

$$f_{P\alpha} = \left[\alpha_0 + (1 - \alpha_0) \frac{q_P}{q_P + P_{\text{int},j}} \right] \left(\frac{\alpha_{\text{int},j}}{q_\alpha + \alpha_{\text{int},j}} \right). \quad (5.52)$$

In (5.52), $\alpha_0 \leq 1$ is the proportion of internalised aminoglycosides that is unaffected by the inhibitory action of internalised pro-inflammatory mediators and q_P and q_α are the presence of internalised pro-inflammatory mediators and the concentration of aminoglycosides at which inhibition is half-maximal respectively. As $f_{P\alpha}$ increases, the strength of inhibition increases, with $f_{P\alpha}$ monotonically increasing from 0 to 1 as $\alpha_{\text{int},j}$ increases and decreasing as $P_{\text{int},j}$ increases. It can be shown that production rate of structural proteins is reduced due to ribosomal blockage and conserved due to ribosomal proof-reading inhibition. The inhibitory function f_{PA} resembles the equivalent functions given by f_M and f_Γ in the ODE model when the sensitivity of bacteria to ribosomal blockage (q_m) and proof-reading inhibition (q_γ) are varied at the same rate (see equations (4.30)).

5.6 Summary

In this chapter, we have presented the formulation and implementation of a multi-scale computational model that governs the dynamics of an EPEC infection both spatially and temporally. The model acts over three spatial scales: the macro-, cell- and sub-cellular scales and is termed a hybrid model as it combines continuum, agent-based and stochastic GRN representations of the infection dynamics.

We implement macro-scale processes (the production/administration, degradation, transport and internalisation of inflammatory mediators and antibiotic) using a PDE representation. We implement cell-scale processes (cell movement, chemotaxis, volume exclusion, phagocytosis and leukocyte recruitment and death) using an agent-based representation. A sub-cellular stochastic GRN within each bacterium, governs its individual protein synthesis and replication via the Gillespie algorithm. We include in the model the inhibitory effects of aminoglycosides on bacterial protein synthesis and the hypothesized inhibitory effects of internalised pro-inflammatory mediators on aminoglycoside action. The region of interest of the model is a two-dimensional transverse section of the intestines, extending from the lumen to the underlying blood supply. The region of interest expands indefinitely into the lumen and along the cell layers, allowing us to implement no-flux boundary conditions along those boundaries. We omit the production of pro-inflammatory mediators by leukocytes from the model, due to its incompatibility with the chemotactic movement of leukocytes. We explore this incompatibility in detail in a toy model in Appendix section A.4 and in a continuous spatio-temporal PDE model in Chapter 7.

In the previous chapter, we developed and analysed a deterministic, population-level ODE model to explore the temporal dynamics of an EPEC infection, the analysis highlighting several effective treatment strategies, both direct and indirect. While these findings are beneficial, the ODE model is incapable of finding treatments relating to the spatial dynamics of the infection or the stochastic variability between individual infections, motivating us to develop the hybrid model. The stochastic implementation of many mechanisms in the hybrid model, in particular protein synthesis and cell movement, is more physiologically realistic than the equivalent deterministic implementations in the ODE model. The probabilistic implementation of effector protein actions further increases the variation between individual infections, more realistically capturing the variance in infection virulence observed clinically. We consider the death of IECs in the hybrid model by introducing an

artificial health metric for each IEC, allowing apoptosis and necrosis to occur independently for each IEC once the cell becomes sufficiently damaged. Leukocyte recruitment is dependent on the pro-inflammatory presence at the endothelium, the time needed for the transport of pro-inflammatory mediators from the epithelium to the endothelium leading to a delay in leukocyte recruitment. These delays allow a distinction between the inflammatory response and the leukocyte response to be observed. Similarly, the antibiotic enters the region of interest via the endothelium, the time taken to travel to the bacteria leading to a realistic delay between administration and action.

The main drawback of the hybrid model is its computational expense, largely due to the implementation of a stochastically-simulated GRN within each bacterium. Conversely, the ODE model takes seconds to solve numerically allowing repetitive analyses, such as a parameter sensitivity analysis, to be executed much faster.

In the next chapter, we present results obtained through simulation of the hybrid model, focusing on the peak and long-term infection sizes both in the absence and presence of an antibiotic treatment, with the aim of finding parameter changes and antibiotic delivery strategies that lead to eradication or a significant reduction in the infection size. We also consider the spatial spread of bacteria to look for the presence or absence of bacterial clusters and a relationship between clustering and infection success.

Chapter 6

Results of the hybrid model

6.1 Overview

In this Chapter, we present numerical results obtained from the hybrid model (presented in Chapter 5) of EPEC infection dynamics in both untreated and treated regimes. From our results, we make various predictions of effective treatment strategies regarding bacterial protein synthesis and replication, the immune response, antibiotic therapy and the spatial positioning of bacteria. We compare these predictions with the corresponding predictions made using the ODE model (see Chapter 4) and highlight predictions that cannot be made by the ODE model, such as those involving spatial dynamics and variations between individual infections, demonstrating the benefits of modelling EPEC infection dynamics using the hybrid model. The ODE model can be viewed as a population-level description of the infection dynamics, allowing the ODE model trajectories and the mean trajectories from the hybrid model to be compared. As noted in section 5.1, we reiterate that the hybrid model is only valid in the early stages of infection and we assume the model breaks down after sufficient time for an infection that is not eradicated.

6.2 Numerical experiments and parameters

The model is implemented (see section 5.3) in MATLAB. While in reality there are very high numbers of cells (bacteria, leukocytes and IECs), we scale these down to $\mathcal{O}(100)$ to reduce computational cost. We show in Appendix section A.5 that the hybrid model converges sufficiently well as the time-step decreases. Due to the absence of experimental data, model parameters are estimated, with several ‘baseline’ parameter sets used to highlight different types of behaviour. All baseline parameter sets are given in Appendix section A.8.

We define a biofilm (cluster) as a region with a non-zero density of bacteria everywhere. We remark that the mechanisms involved in the formation of the extra-cellular matrix of a biofilm, and the resulting bacterial properties of being in a biofilm, have not been considered. An extension to the hybrid model, incorporating biofilm mechanisms and properties in a simple way, is considered in Chapter 8.

We record over time the numbers of bacteria, leukocytes and effector proteins, the mean spatial presence of external pro-inflammatory mediators, the total concentration of internalized antibiotic and the horizontal positions of the bacteria. Data is recorded until either all bacteria have died or time reaches a cut-off time, denoted $t_{\max}$. If $t_{\max}$ is reached, we assume that the infection is not eradicated and that the model becomes invalid at this point. For each experiment, the model is simulated numerous times (typically 100), allowing us to obtain averages and measures of spread. This allows us to show typical dynamics of an infection as well as dynamics of individual infections. We are more interested in whether an infection has been eradicated, as opposed to the exact infection size in steady state or at the cut-off time. However, considering variables over time is still important as this allows the dynamics between different scenarios to be compared. The percentage of infections that are eradicated in the hybrid model will be considered in later analysis as a way of measuring treatment success. Throughout the analysis of this model, we interpret time as number of days and the bacterial population as a sub-population within the whole infection.

6.3 Results in the absence of antibiotic

Varying effector protein production rates individually

The effector proteins that inhibit pro-inflammatory signalling (E_1) and phagocytosis (E_3) improve the survivability of the infection, particularly E_1 : Figure 6.1 shows the number of bacteria over time in each of 100 simulations and the mean number of bacteria over time for each combination of effector protein groups. Figure 6.2(a) shows a comparison of the mean population in each case. If an effector protein group is present, then its production rate (r_i) is set to its baseline value and if a group is absent, then its production rate is set to 0.

In the case with no effector proteins present (Figure 6.1(a)), the infections are all eradicated by $t = 6$, whereas in the case with all effector proteins present (Figure 6.1(h)), most infections survive past the cut-off time ($t_{\max}$). Many more infections peak at higher population values, and persist for longer when E_1 is present compared to when either E_2 or E_3 are present. Figure 6.1(d) highlights that while some infections survive when E_3 is the only effector protein group present, the average infection size at the cut-off time is very low. The presence of both E_1 and E_3 further increases the number and survival times of infections, showing the synergy between the two effector protein groups. The model therefore predicts that inhibiting the production of the effector proteins that inhibit pro-inflammatory signalling or phagocytosis could be an effective treatment strategy.

Figure 6.4(a) shows the mean number of bacteria over time as the production rate of E_1 (r_1) is varied. The mean infection size over time and the mean peak infection size both increase monotonically with r_1 ; a greater number of E_1 grants the infection more time to grow until it is discovered by the leukocyte response. The mean infection size follows the same increasing trend initially for all r_1 . For an r_1 value below approximately 40% of the baseline value, the mean infection is expected to be eradicated. By comparing this result to that from the ODE model (see Figure 4.9(a)), we see the models are in agreement for varying r_1 .

We find that the ODE and hybrid models do not always agree on the effectiveness of each effector

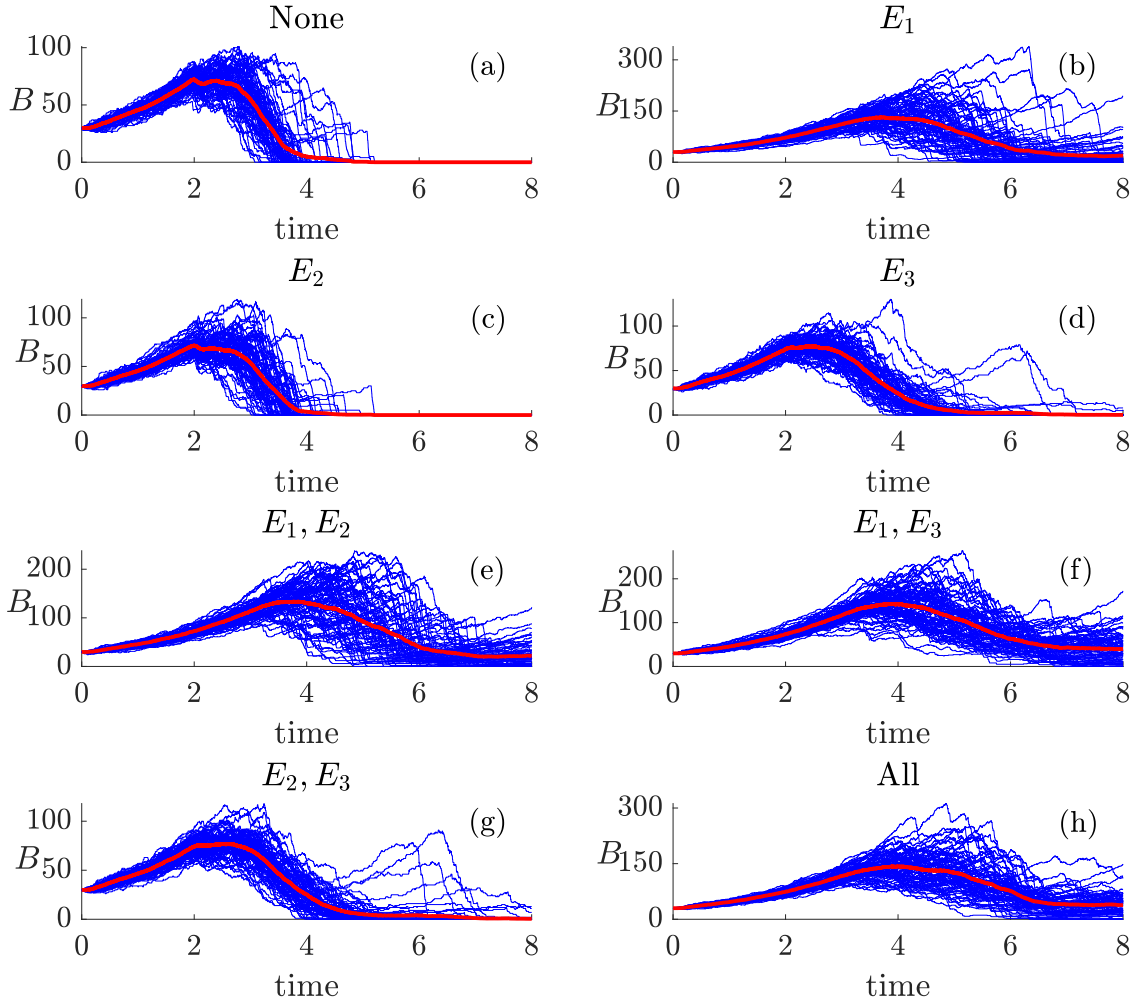


Figure 6.1: The number of bacteria over time in each simulation (blue) and the mean number of bacteria over time (red) for each combination of effector protein groups. The effector protein groups present in each case are given in the graph titles. Each case has 100 simulations and all other parameters take their baseline values (see set 1 in Table A.2).

protein group relative to that of the other groups; the ODE model predicts that secretion of E_1 is less beneficial to the infection than E_3 (see Figure 4.9). We speculate this difference to be due to the difference in how leukocyte recruitment and attraction are modelled. In the hybrid model they are dependent on the spatial profile of pro-inflammatory mediators; whereas in the ODE model, they depend on the concentration of pro-inflammatory mediators and the density of bacteria.

The effector proteins that inhibit pro-inflammatory signalling increase the delay in the leukocyte response, making eradication more difficult: Figure 6.2(b,c) shows the mean (external) presence of pro-inflammatory mediators and the mean number of leukocytes respectively over time for each combination of effector protein groups. The delay in leukocyte recruitment is longer in the presence of E_1 than in the absence and although more leukocytes are recruited, Figure 6.2(a) shows the leukocyte response is unable to eradicate the infection in the presence of E_1 . These results further suggest that the effector proteins that inhibit pro-inflammatory signalling make the infection significantly more virulent and that inhibiting them is an effective treatment strategy.

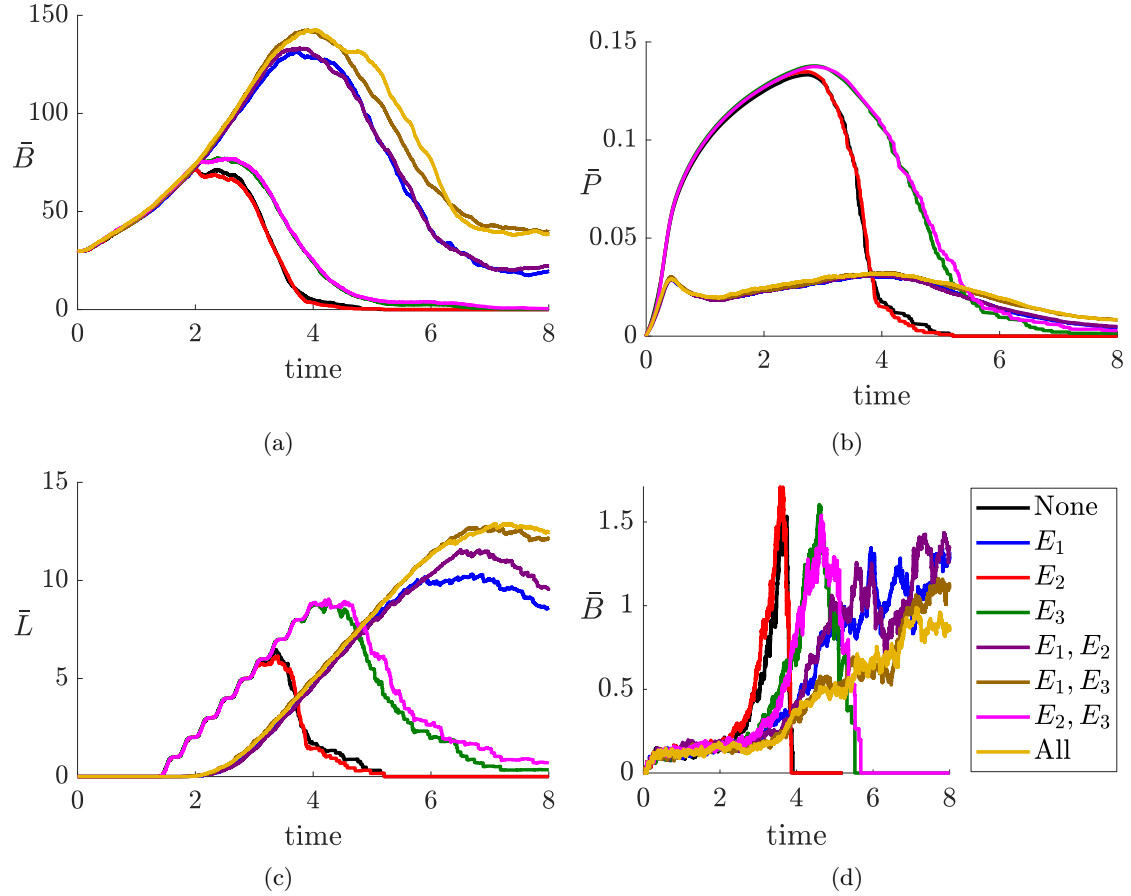


Figure 6.2: The mean (a) number of bacteria, (b) external presence of pro-inflammatory mediators and (c) number of leukocytes and (d) the interquartile range of the number of bacteria over time for each combination of effector protein groups. Each graph is the mean of 100 simulations and all other parameters take their baseline values (see set 1 in Table A.2).

The presence of E_1 causes a three-four fold reduction in the pro-inflammatory mediator presence over the course of the infection. By contrast, E_1 causes only a two-fold reduction in the ODE model (see Figure 4.15(a)), the difference due to the differing implementations of inhibition in both models. The two models are also qualitatively different, suggesting that the spatial dynamics of the hybrid model and the stochastic implementation of pro-inflammatory mediator production play a significant role in the evolution of the inflammatory conditions within the host. The pro-inflammatory mediator production reduction by E_1 in the hybrid model occurs early in the infection ($t \approx 0.5$) and the knock-on effect of delayed leukocyte recruitment is evident. This delay allows the bacteria more time to grow before the leukocytes reach them, as shown by comparing the peak infection times and sizes in the presence and absence of E_1 in Figure 6.2(a). The delayed leukocyte response makes it much harder for the infection to be eradicated. Due to the infection being given more time to grow, the damage done to the host increases, eventually leading to an inflammatory response that is sufficiently strong to attract leukocytes.

The ODE model also predicts that the delay in leukocyte recruitment is longer in the presence of

E_1 ; but by contrast, the number of leukocytes decreases as E_1 increases (see Figures 4.15 and 4.16). This difference is due to the differing implementations of leukocyte recruitment activation; in the ODE model, the leukocyte recruitment rate is proportional to the concentration of pro-inflammatory mediators, whereas in the hybrid model, (1) activation is dependent on the pro-inflammatory mediator presence at the endothelium and (2) the large infection size causes recruitment to be activated for longer, leading to more recruited leukocytes.

Inhibiting the effector proteins that inhibit pro-inflammatory signalling or phagocytosis increases the efficacy of phagocytosis: Figure 6.2(d) shows the interquartile range (IQR) of the number of bacteria, relative to the number of bacteria over time for each combination of effector protein groups. Early in the infection ($t < 2$), before the bacteria are attacked by leukocytes, the relative spread in the infection sizes is low. Moreover, in all cases, the IQR increases after the time of the peak infection size, which is the time at which the leukocytes reach the bacteria and begin to attack them. This suggests that the efficacy of phagocytosis has a greater influence on the spread in the efficacy of individual infections than the other aspects of the immune response. The peak IQR is lower when E_1 and/or E_3 are present. This suggests that the effector proteins that inhibit pro-inflammatory signalling and phagocytosis reduce the relative spread in individual infection sizes. The reduced spread is because (1) E_1 gives the infection more time to grow, the larger infection size making the IQR less sensitive to sudden changes and (2) E_3 inhibits phagocytosis, leading to the reduction in the infection size being less rapid. The model therefore predicts that inhibiting these effector proteins increases the efficacy of phagocytosis, potentially leading to more reliable return to health.

The effector proteins that inhibit host cell apoptosis make negligible contribution to the success of the infection: Figures 6.1 and 6.2, show that E_2 makes negligible difference to both the infection size and the immune response and this is in agreement with ODE model (see Figure 4.9(b)). Although both models demonstrate this redundancy, it is a peculiar finding; we believe it is unlikely that EPEC would use resources to inject proteins that do not aid the infection. We acknowledge that this finding may be due to our chosen parameter regime being incorrect.

The numbers of the effector proteins that inhibit pro-inflammatory signalling and phagocytosis peak at approximately the same time and sooner than the number of bacteria: Figure 6.3(a) shows that the mean of E_1 increases steadily before peaking at a time sooner than the peak infection size. The number then steadily decreases to a smaller population. We infer from this that the effector proteins that inhibit pro-inflammatory signalling act early on to allow the bacteria to remain undetected and then once this becomes difficult due to the size of the infection, the production of E_1 is reduced allowing resources to be used more effectively. Figure 6.3(b) shows the mean of E_2 peaks twice — early on and soon before the infection peaks; however, since the results presented in Figure 6.2 indicate that the presence of E_2 makes negligible difference to the success of the infection, we do not pursue this finding any further.

Figure 6.3(c) shows that after a rapid initial production, the mean of E_3 increases, peaks and decreases in a similar manner to E_1 , peaking at approximately the same time. By contrast, in the

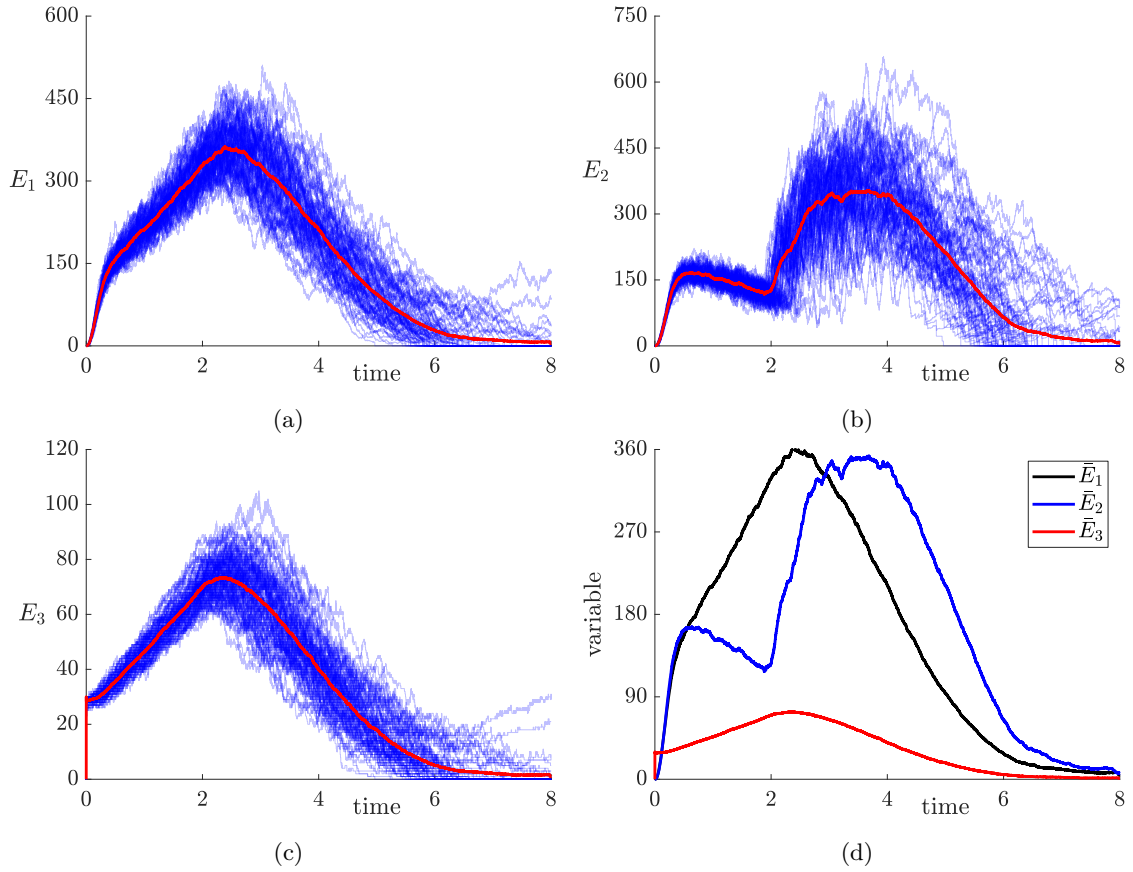


Figure 6.3: The number of (a) E_1 , (b) E_2 and (c) E_3 over time in each simulation (blue) and the corresponding mean number over all simulations (red) when all effector protein groups are present. (d) The mean curves from the previous graphs plotted on the same axes. The mean graphs correspond to the light gold lines in Figure 6.2. Each case has 100 simulations and all other parameters take their baseline values (see set 1 in Table A.2).

ODE model, E_1 and E_3 increase steadily before plateauing (see Figure 4.11). The steady decreases observed in the hybrid model are not observed in the ODE model due to the differences in how effector protein production are modelled. In the ODE model, the production rates depend on the densities of bacteria and correctly translated proteins, and the concentration of pro-inflammatory mediators; whereas in the hybrid model, the production of all effector proteins is only linear in the number of mRNA. The discrepancy between model behaviours at large time is not important, due to the hybrid model not being valid at large time (see section 6.1).

There appears to be an optimum production rate for the effector proteins that inhibit phagocytosis: Figure 6.4(b) shows the mean number of bacteria over time as the production rate of E_3 (r_3) is varied. The trend for increasing r_3 is not as distinguished as that for increasing r_1 . For the values of r_3 chosen, 80 – 100% of the baseline value marginally give the highest mean peak infection size. As expected, the mean peak infection size is smallest when E_3 is absent; however, this case does not give the lowest mean infection size post-peak and strikingly, the highest rates of r_3 give the lowest mean infection size at later times. The difference in the mean post-peak infection size between 80-100% and 120-140% of the baseline value is also striking. These results suggest the

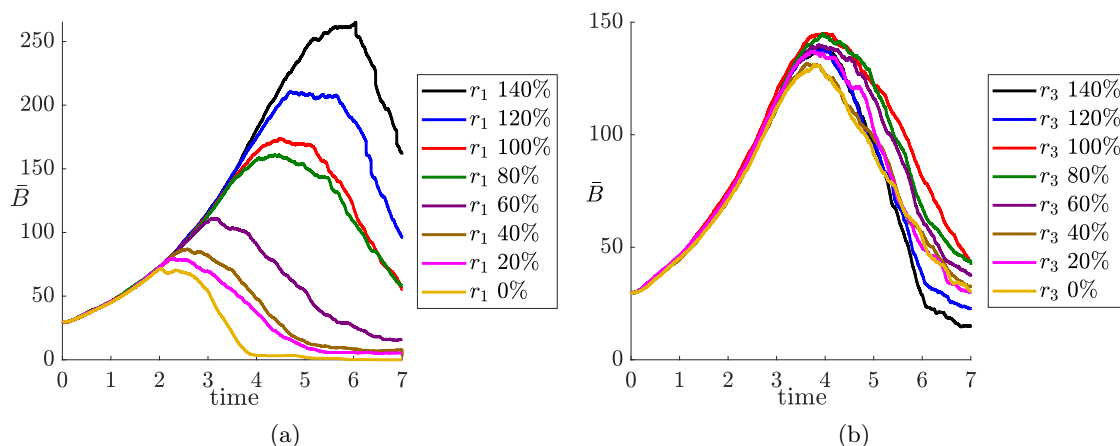


Figure 6.4: The mean number of bacteria over time as (a) r_1 and (b) r_3 are varied. The value for each effector protein production rate is given as a percentage of the baseline rate. Each graph is the mean of 100 simulations and all other parameters take their baseline values (see set 1 in Table A.2).

possibility of an optimum r_3 . This is an unexpected prediction given our ODE model simulation results — that a higher value of r_3 results in a stronger infection (see Figures 4.9 and 4.16(a)). This implies that therapeutically decreasing the production rate of the effector proteins that inhibit phagocytosis can be counter-productive, leading to a worse resolution of the infection and that care would therefore need to be taken if a treatment used such a strategy. By comparing this result to that from the ODE model (see Figure 4.9(a)), we see the models are not in agreement for varying r_3 — the ODE model predicts a monotonically increasing relationship similar to that for increasing r_1 . We explore model behaviour for varying r_1 and r_3 in more detail later in this chapter.

Varying effector protein production rates simultaneously

Alongside the infection size and duration, we are interested in elucidating whether E_1 and E_3 affect the spatial profile of the infection (we consider the relationship between the spatial profile and the success of the infection later in this chapter). As discussed in section 6.2, we define a biofilm as a region with a non-zero density of bacteria everywhere. To implement this, we measure the bacterial density across $-1 \leq x \leq 1$, define the maximum biofilm height as the peak bacterial density and define the maximum biofilm width as the longest length across $-1 \leq x \leq 1$ with a non-zero density (see Figure 6.5). We now present results showing how biofilm structures and the infection size and duration vary when r_1 and r_3 are varied, both individually and simultaneously.

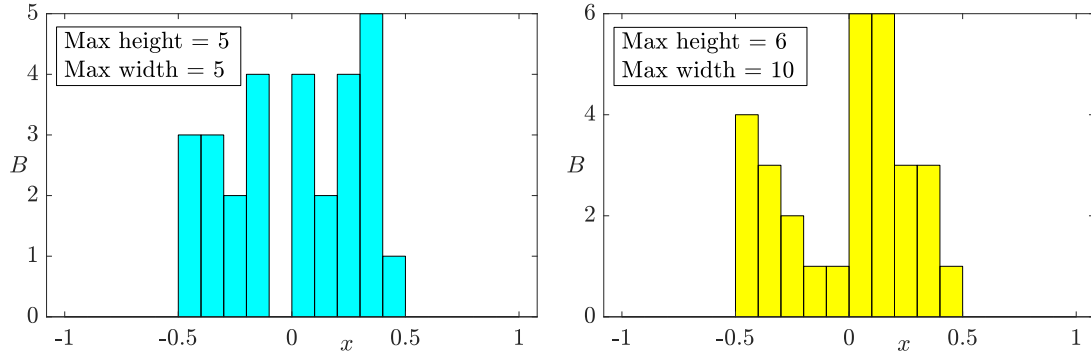


Figure 6.5: Example simulation results showing how the (artificial) maximum biofilm heights and widths are calculated.

The infection duration is sensitive to both the effector proteins that inhibit pro-inflammatory signalling and phagocytosis, but the infection size is only sensitive to the former: Figure 6.6(a) shows that the peak infection size noticeably increases as r_1 increases, but does not noticeably change when r_3 is altered. Figures 6.6 and 6.4 are in agreement that increasing r_1 increases the peak infection size, but Figure 6.2 predicts that increasing r_3 does increase the peak infection size slightly. All results suggest that E_1 have a stronger effect on the peak infection size than E_3 . Figure 6.6(b) shows that the mean infection end time increases as both r_1 and r_3 increase, but is more sensitive to changes in r_1 . For a typical strain of bacteria that has sufficiently high r_1 and r_3 , the infection survives (see section 6.1 for clarification). A reduction in pro-inflammatory signalling leads to a longer delay in leukocyte recruitment activation and a reduction in phagocytosis results in slower infection eradication. Figure 6.6(c) shows that the maximum biofilm height noticeably increases as r_1 increases; E_1 allow the infection more time to grow, leading to a higher peak infection size and therefore a larger maximum biofilm height. E_3 have little effect on the maximum biofilm height and the peak infection size since their effect comes into play when phagocytosis commences (first occurring at the peak infection size). Figure 6.6(d) shows that there is negligible effect on the maximum biofilm width when either r_1 or r_3 are varied. The maximum biofilm width occurs early in the infection, before the effects of E_1 and E_3 make a significant difference to the evolution of the infection.

Together, these results suggest that the infection duration is sensitive to both the effector proteins that inhibit pro-inflammatory signalling and phagocytosis, but the mean peak infection size and maximum biofilm height are only sensitive to the the effector proteins that inhibit pro-inflammatory signalling. We infer from these results that targeting both the effector proteins that inhibit pro-inflammatory signalling and those that inhibit phagocytosis is more effective than targeting either individual group. An adaptation of the hybrid model that includes a more detailed and biologically realistic model of a biofilm is considered in Chapter 8, where the effects of the biofilm on the success of the infection are modelled in more detail.

Comparing different treatment strategies

Infections are less likely to survive if the production of bacterial structural proteins is inhibited: Figure 6.7(a,b) shows that increasing the structural protein production rate, r_Γ , significantly increases

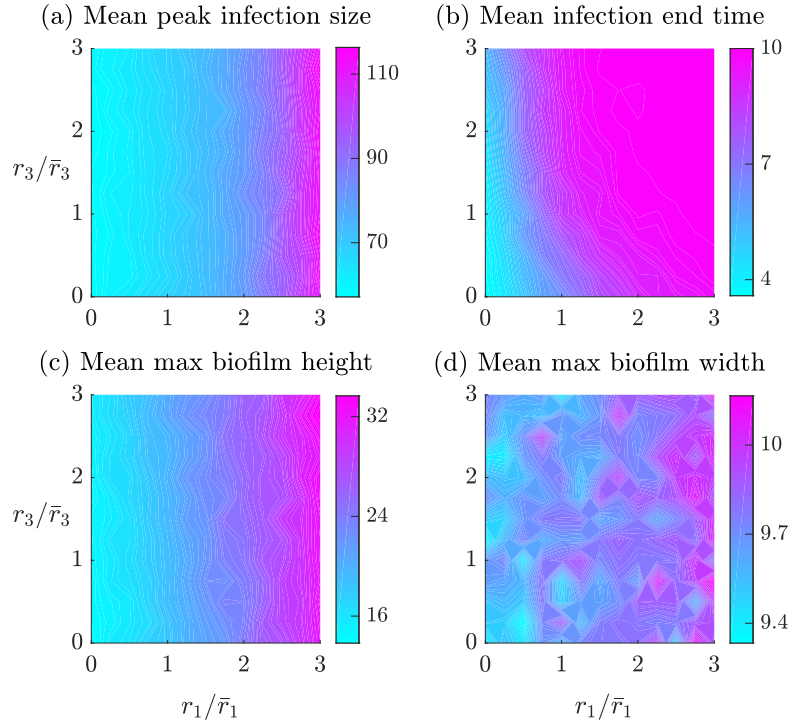


Figure 6.6: The mean (a) peak infection size, (b) infection end time, (c) maximum biofilm height and (d) maximum biofilm width, as r_1 and r_3 are both varied. The value of each metric for a given parameter value combination is given by the colour bar adjacent to the respective plot. $\bar{r}_1$ and $\bar{r}_3$ denote the baseline r_1 and r_3 values used respectively. Each combination of $r_i/\bar{r}_i$ ranges from 0 to 3 in increments of 0.25. The metrics at each pair of r_1 and r_3 are the means of 100 simulations and all other parameters take their baseline values (see set 1 in Table A.2).

the mean infection size over time and the percentage of infections that survive. The leukocyte response often cannot handle the infection for sufficiently high r_Γ , leading to the infection continuing to grow in size. A treatment that inhibits the production of bacterial structural proteins will be effective, since the bacteria become less efficient at replicating themselves. Figure 6.7(c) shows that for r_Γ sufficiently small, the infection is very likely to be eradicated.

Inhibiting the production of bacterial structural proteins is a more effective treatment strategy than increasing the frequency of leukocyte recruitment: Figure 6.7(d,e) shows that increasing the time period of leukocyte recruitment, r_L , increases the infection size over time for the eradicated infections, but has a less significant effect on surviving infections. Figure 6.7(f) shows that for r_L sufficiently small, the infection is very likely to be eradicated. Together, these findings suggest that increasing the recruitment rate of leukocytes is an effective treatment strategy, but not as effective as inhibiting the production of bacterial structural proteins. This result is in agreement with that obtained from the ODE model (see Figures 4.5(b) and 4.12(b)¹).

Inhibiting the effector proteins that inhibit pro-inflammatory signalling is a more effective treatment

¹In the ODE model, r_l is the rate of leukocyte recruitment; whereas in the hybrid model, r_L is the time period of leukocyte recruitment. Therefore, statements regarding r_l must be inverted to obtain the equivalent statements regarding r_L (and vice versa).

strategy than inhibiting the production of bacterial structural proteins: Figure 6.7(g,h) shows that infection becomes significantly more virulent as r_1 increases, with almost half of the infections surviving when $r_1 = 1$. Moreover, the peak infection size in both surviving and eradicated infections is higher for large r_1 than for large r_Γ . Figure 6.7(i) shows that for r_1 sufficiently small, the infection is very likely to be eradicated. These results further demonstrate that a treatment that inhibits the production or action of the effector proteins that inhibit pro-inflammatory signalling could be effective.

Inhibiting the effector proteins that inhibit phagocytosis is less effective than inhibiting the production of bacterial structural proteins or the effector proteins that inhibit pro-inflammatory signalling: Figure 6.7(j,k) shows that increasing r_3 increases the infection size less significantly than increasing either r_Γ or r_1 . This result is in qualitative agreement with the ODE model (see Figures 4.9(a) and 4.26(f)); however, the ODE model predicts that increasing r_3 gives a larger increase in the infection size than increasing r_1 by the same proportion. Figure 6.7(l) shows that for r_3 sufficiently small, the infection is very likely to be eradicated. Together, these findings suggest that inhibiting the production of the effector proteins that inhibit phagocytosis is an effective treatment strategy, but not as effective as inhibiting the production of bacterial structural proteins or the effector proteins that inhibit pro-inflammatory signalling.

Comparing Figures 6.7(f) and 4.5(b), we see that the hybrid and ODE models are in agreement that attracting leukocytes sufficiently quickly leads to infection eradication. In contrast, comparing Figures 6.7(i,l) and 4.9(a) shows that the ODE model does not agree with the hybrid model that the infection can be eradicated by sufficiently reducing r_1 or r_3 . We infer that in the hybrid model, the spatial- and inflammation-dependent modelling of leukocyte recruitment, chemotactic attraction and phagocytosis has a significant effect on the dynamics of the system.

A healthy resolution is possible in many infections using a non-extreme strength treatment: Figure 6.7(c,i) shows that the trend in the percentage of infections that survive as r_Γ or r_1 is varied is switch-like, implying that if the natural value for each parameter is close to the switch value, then many infections that would survive without intervention can be eradicated with a small decrease in the respective parameter. Moreover, the compounded effects of a treatment that inhibits the production of all bacterial protein synthesis (reduces r_Γ, r_1 and r_3) will further increase the potential for infection eradication.

Exploring the relationship between the spatial positioning of bacteria and the success of the infection

Having explored the effects of parameters on solution behaviour, we now investigate how spatial effects contribute to infection success. In particular, we test whether the spatial positioning of bacteria affects the strength of the stimulated production of pro-inflammatory mediators by considering biofilms. We hypothesize that bacteria that spread out across the IEC layer in a sparse biofilm trigger a strong inflammatory response, whereas bacteria that clump in a denser biofilm trigger a milder inflammatory response (see Figure 6.8). A milder inflammatory response will then lead to a delayed leukocyte response, reducing the capacity of the immune response to eradicate

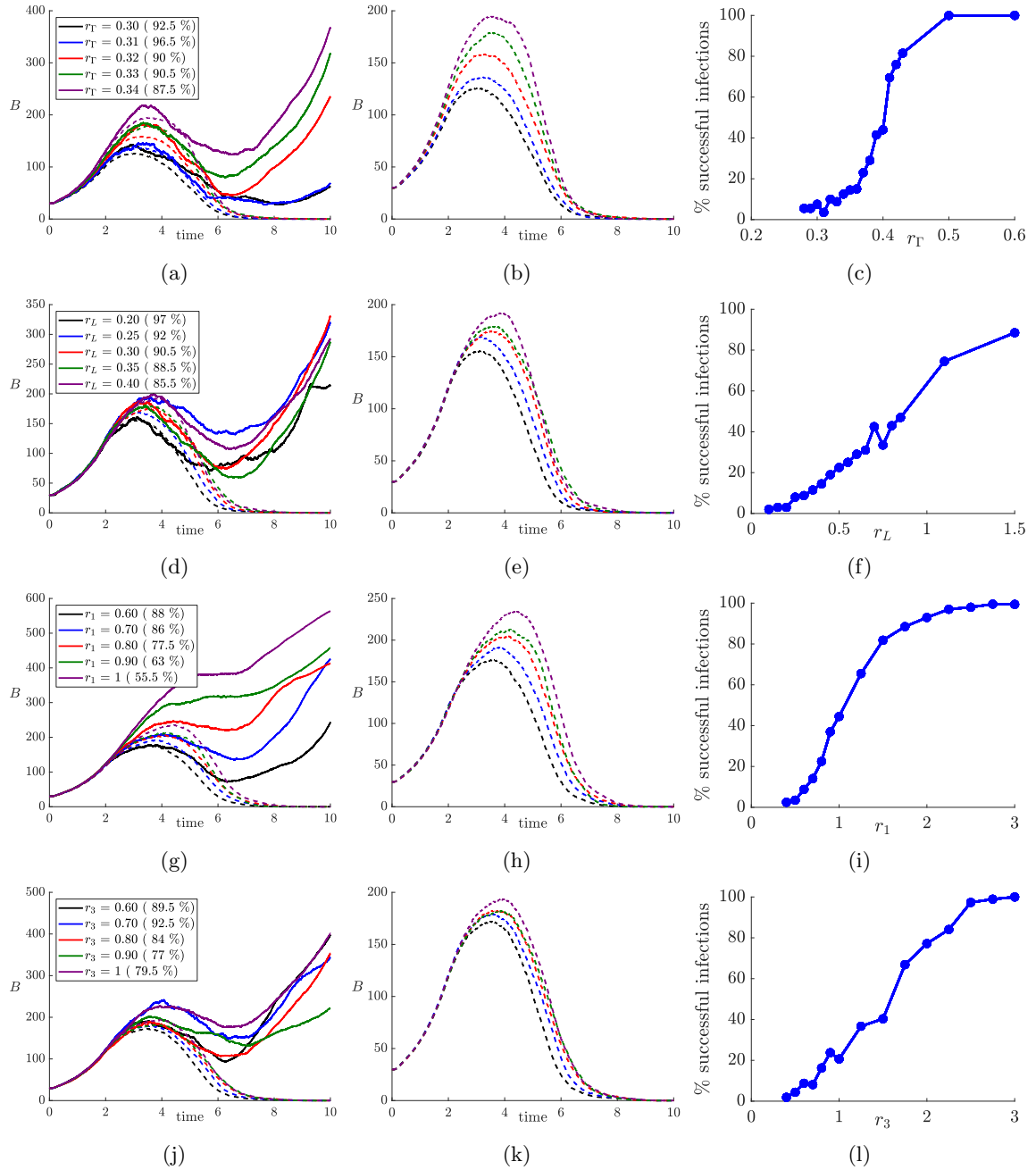


Figure 6.7: The mean number of bacteria over time and the percentage of successful infections as (a,b,c) r_T , (d,e,f) r_L , (g,h,i) r_1 and (j,k,l) r_3 are varied. The means of only the surviving infections (full lines) and only the eradicated infections (dashed lines) are given separately in (a,d,g,j) and only the means of the eradicated infections are given in (b,e,h,k). For the plots of the mean number of bacteria, the parameter being altered in each row and its value in each graph are given in the row's legend, along with the percentage of infections that are eradicated (bracketed quantity). Each graph/marker is the mean of 200 simulations and all other parameters take their baseline values (see set 3 in Table A.2).

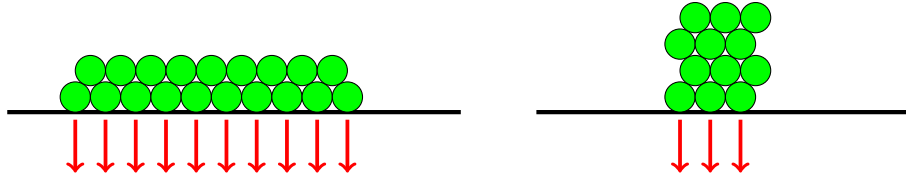


Figure 6.8: A caricature showing the hypothesis that sparse biofilms trigger a milder inflammatory response than dense biofilms.

the infection. It is possible that the height of the biofilm will be significant, since a more populous biofilm will be more difficult to eradicate. We therefore further hypothesize that the shape of biofilms relates to the success of the infection.

We now define two measures that will be used to correlate the success of the infection with the spatial profile of the bacteria. Based on the discussion above:

$$\text{Biofilm score 1} = \frac{H}{W}, \quad \text{Biofilm score 2} = \frac{HB}{W}, \quad (6.1)$$

where H , W and B denote the height of, width of and number of bacteria in the biofilm at a given time point respectively. If there is more than one biofilm for an individual infection, we only consider the biofilm that gives the highest value for each metric. Below, we consider the values of these measures at the time at which successful and unsuccessful infections diverge on average. Figure 6.7(a,d,g,j) showing this time to be $t \approx 2.5$, therefore we take $t = 2.5$ to be the average time of divergence.

Figure 6.9 shows that there are no strong trends between the measures given in (6.1) and r_Γ, r_L, r_1 or r_3 . While there are weak correlations between each measure and parameter, in particular r_L and Biofilm score 2 (Figure 6.9(d)), and r_3 and both measures (Figure 6.9(g,h)), Figure 6.10 shows that there is a large IQR for each measure, for most chosen parameter values. We infer from these large spreads in measures that any correlations between the measures given in (6.1) and r_Γ, r_L, r_1 or r_3 are weak. Figure 6.7(c,f,i,l) shows how the percentage of infections that survive varies as r_Γ, r_L, r_1 and r_3 are varied (analogous to bifurcation diagrams) and shows that all four variables are strongly correlated with the success of the infection. Since the measures given in (6.1) are not significantly affected by r_Γ, r_L, r_1 or r_3 , we infer that the measures do not significantly affect the success of the infection and, as a result, are not worth exploring in more detail.

Infections that survive are typically reduced to one small biofilm. These infections recur, exploiting the diminished immune response: We now consider trends between the infection size and the spatial (horizontal) positioning of the bacteria over time. Figure 6.11 shows contour plots of the number of bacteria over horizontal space and time for a random sample of surviving and eradicated infections. For the eradicated infections, the bacteria are typically well spread out early in the infection, thus eliciting a stronger immune response. There is usually only one biofilm shortly before eradication, which suggests that leukocytes are typically drawn to one biofilm which they remove through phagocytosis before attacking other biofilms. The biofilms on the right tend to get attacked by the

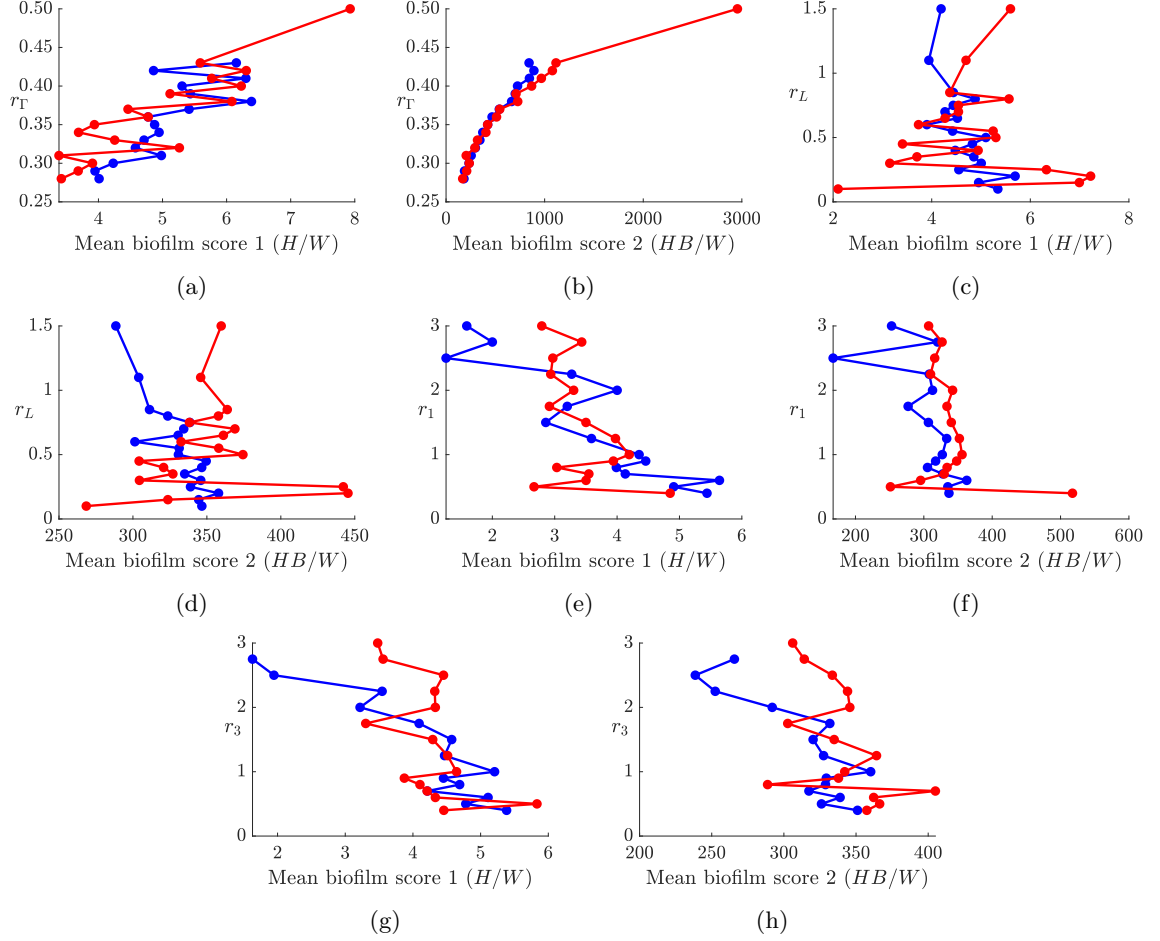


Figure 6.9: The means of the measures given by (6.1) as (a,b) r_Γ , (c,d) r_L , (e,f) r_1 and (g,h) r_3 are varied. The blue and red lines represent the mean biofilm scores for only the eradicated infections and only the surviving infections respectively. H , W and B denote the maximum height of any biofilms present, the maximum width of any biofilms present and the bacterial population respectively. The parameter values used are denoted by the markers on the graphs and each marker represents 200 simulations. All other parameters take their baseline values (see set 3 in Table A.2).

leukocytes before those on the left, due to the advective effect of the lumen flow. This flow causes changes in the chemotactic gradient that each leukocyte is following, leading to them travelling rightward as well as upward. For infections that do not survive, the peak population is typically 80-100, with some reaching lower or higher than this (top left and bottom right panels of Figure 6.11(a) respectively). The peak infection sizes vary considerably more for the infections that survive, with most being approximately 100-130 but with some reaching much higher sizes (e.g. 400 in the bottom right panel of Figure 6.11(b)). The similarity between the typical peak infection sizes of surviving and eradicated infections suggests that they have similar dynamics during the early stages of the infection. From the sample of surviving infections taken, the infections that give the largest peak infection sizes have their peaks occur at the cut-off time, showing that the infections were continuing to grow when the simulations were stopped. For the surviving infections sampled, the infection is always reduced to one biofilm, the bacterial density often reduced close to zero. From this small population, the infection recurs and in some cases, the infection returns stronger than

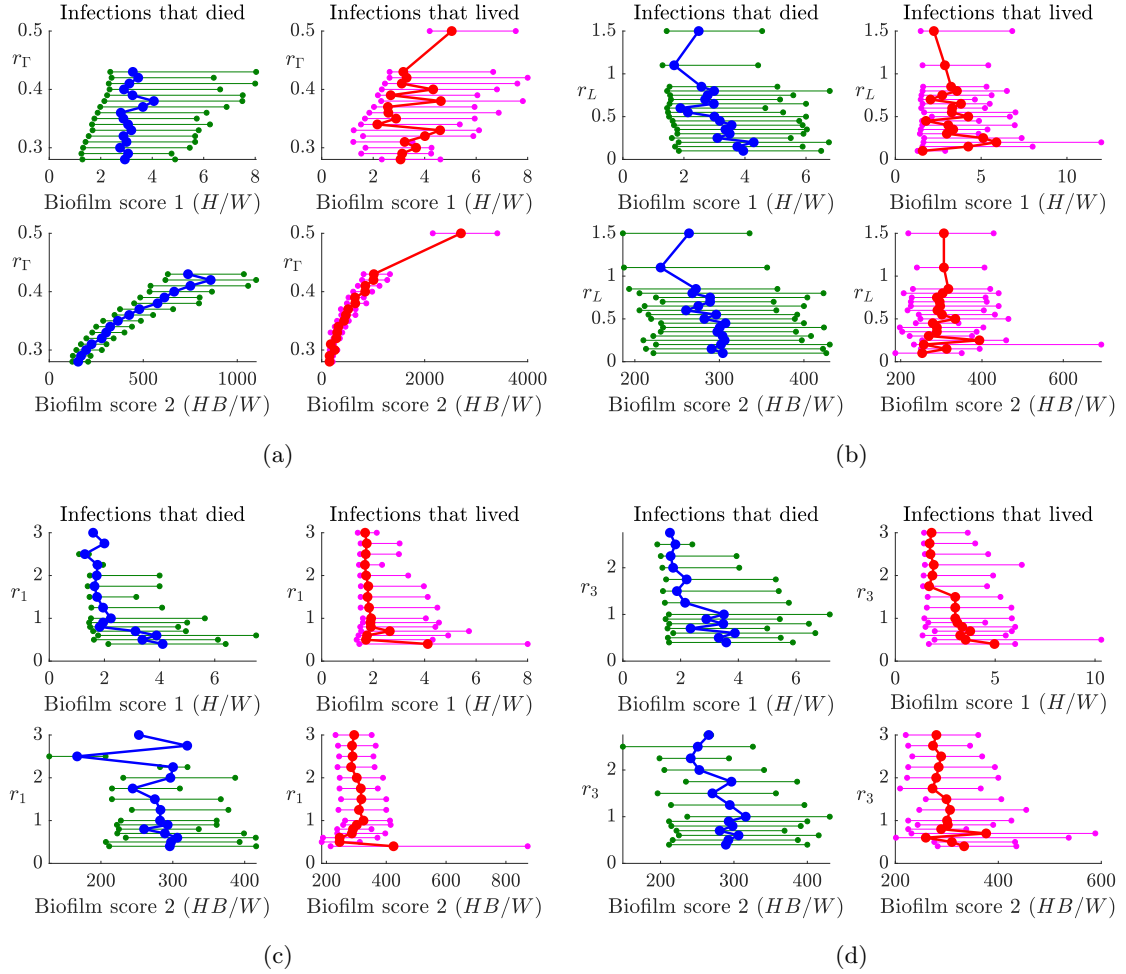


Figure 6.10: The median and lower and upper quartiles of the measures given in (6.1) as (a) r_Γ , (b) r_L , (c) r_1 and (d) r_3 are varied. The blue and red lines denote the medians of the eradicated and surviving infections respectively and the green and magenta lines denote the upper and lower quartiles of the eradicated and surviving infections respectively at the respective parameter value. H , W and B denote the maximum height of any biofilms present, the maximum width of any biofilms present and the bacterial population respectively. The parameter values used are denoted by the markers on the graphs and each marker represents 200 simulations. All other parameters take their baseline values (see set 3 in Table A.2).

the initial infection, implying that the leukocyte response has failed to eradicate the infection. This occurs because the number of leukocytes decreases to a very low value and in some simulations even decreases to zero. This occurs for the following reasons; (1) the leukocytes take time to remove a large number of bacteria, (2) leukocytes have a finite life span and (3) when the number of bacteria decreases to a very low value, the inflammatory response is allayed, leading to the termination of further leukocyte recruitment. The lull in the host's immune response following the initial infection gives the surviving bacterial population time to regrow and therefore overcome the secondary immune response. This explains the large difference in infection sizes over time between individual infections from the same parameter set. The dependence of eradication on maintaining the immune response above a critical level is in keeping with other studies; in particular, Ternent et al. [85] and Gjini et al. [86]. We showed that the mean infection size over time increases as any of r_T , r_L , r_1 and r_3 are increased (see Figure 6.7). These findings are in agreement with the above interpretation — (1) increasing the production rate of bacterial structural proteins (r_T) leads to bacteria replicating faster, making it more difficult for the leukocyte response to eradicate the initial infection, (2) increasing the time period of leukocyte recruitment (r_L) leads to fewer leukocytes being recruited to help eradicate the initial infection, (3) increasing the production rate of the effector proteins that inhibit pro-inflammatory signalling (r_1) leads to a reduction in the host's inflammatory response, leading to reduced leukocyte recruitment particularly at the tail end of the initial infection, allowing a recurring infection to grow with insufficient challenge and (4) increasing the production rate of the effector proteins that inhibit phagocytosis (r_3) allows a sub-population of bacteria to survive sufficiently long to outlast the leukocyte response and then regrow the infection. These results suggest that infections can survive, and therefore recur, if they are sufficiently virulent to outlast the leukocyte response while diminishing the inflammatory response. These results also suggest that reducing the production rate of bacterial proteins, both structural and effector, or increasing the recruitment rate of leukocytes, will contribute towards the eradication of the infection.

Wishing to find a relationship between biofilm width and infection success, we now explore how the widths of biofilms vary over time — a feature that the measures given in (6.1) do not possess due to being measured at a specific time point ($t = 2.5$).

Surviving infections typically have narrower biofilms: Figure 6.12 shows that early in the infection (top row), there is large variation in maximum biofilm widths and in all cases the modal maximum width is the same (10 bins of length 0.1); however, there is less spread in biofilm width for the infections that survive. As the infection progresses, both the variation in the maximum biofilm widths and the mean maximum biofilm width decrease for both surviving and eradicated infections. At $t = 4$ (bottom row), we see that the infections that survive typically have narrower biofilms; an important finding given that no infections have been eradicated by this time. This finding is in agreement with our hypothesis that denser biofilms elicit a weaker inflammatory response, and therefore a delayed leukocyte response.

We have found a significant relationship between the spatial positioning of bacteria (biofilm shape) and infection success; denser biofilms tend to be more successful. We now explore this relationship in more detail via a controlled investigation of biofilm phagocytosis.

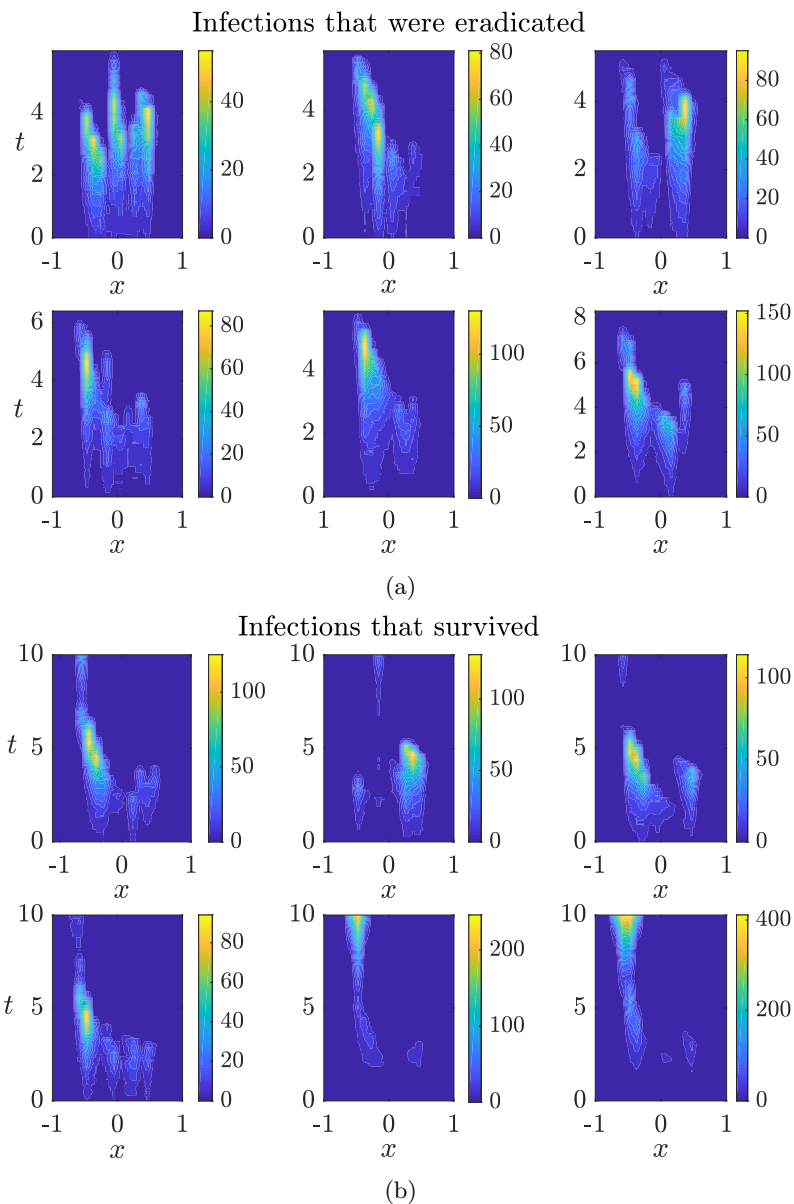


Figure 6.11: Contour plots of the horizontal positions of bacteria over time from a random sample of six infections that (a) are eradicated and (b) survive. The number of bacteria in each plot is represented by the colour bar adjacent to the plot. All other parameters take their baseline values (see set 3 in Table A.2).

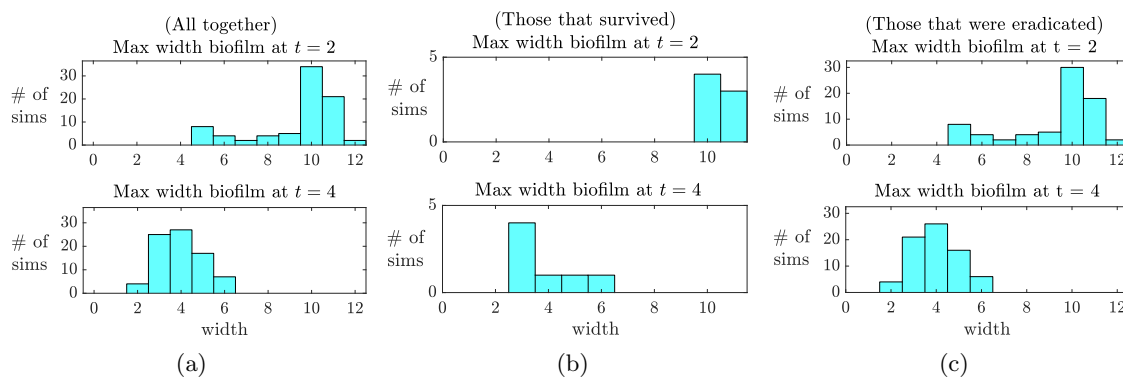


Figure 6.12: Histograms of the maximum width biofilm in each infection at times $t = 2, 4$ for (a) all infections, (b) surviving infections and (c) infections that are eradicated. The width of a biofilm is measured by the number of consecutive histogram bins that the bacteria cover. 80 individual infections are considered and all other parameters take their baseline values (see set 3 in Table A.2).

A controlled investigation of biofilm phagocytosis

We are interested to see how leukocytes attack biofilms and whether biofilm shape affects the time taken to eradicate the infection. To investigate this, we prescribe five different rectangular formations, each with a set number of bacteria present. We also turn off (1) bacterial growth and death so that phagocytosis is the only mechanism for changing the number of bacteria, (2) the production of the effector proteins that inhibit pro-inflammatory signalling to allow us to observe the effects of bacterial positioning on the immune response more clearly and (3) the movement of bacteria to allow the shapes of the biofilms to be preserved throughout the infection. We vary the height, width and number of biofilms, as well as the total bacterial population. Figure 6.13 shows histograms of the spatial positions of the bacteria in each of the five prescribed formations. Four of the five formations have the same number of bacteria (300; Figure 6.13(a,b,d,e)) and the other formation has 600 (Figure 6.13(c)), allowing us to consider both the shape and the size of biofilms. Three of the five formations are one biofilm (Figure 6.13(a,b,c)) and the other two are three separate, uniform, smaller biofilms (Figure 6.13(d,e)), allowing us to explore whether having one large biofilm or multiple smaller biofilms is more effective.

Infections that occupy a smaller area on the IEC layer trigger a milder inflammatory response and a delayed leukocyte response: Figure 6.14 shows the number of bacteria over time for 150 individual simulations as well as the mean number of bacteria over time for each of the five formations considered. For each of the formations with one biofilm (Figure 6.13(a-c)), the number of bacteria decreases steadily to zero, with relatively small variance between individual infections. The formations with three biofilms (Figure 6.13(d,e)) decrease in three separate waves, due to leukocytes requiring time to travel between clusters. Of the two formations with three biofilms, the one with taller, narrower clusters has a much greater variation in the time taken to eradicate each cluster. This is because the milder inflammatory response leads to a shallower chemotactic gradient, making it more difficult for leukocytes to locate other clusters once they have finished eradicating one. Figure 6.14(f) shows the mean number of bacteria over time for all formations plotted on the same

axes for comparison. The formation with three short and wide biofilms is found by the leukocytes first and the formation with one tall and narrow biofilm is found last, implying that infections that occupy a smaller area on the IEC layer trigger a milder inflammatory response, leading to a longer delay in the leukocyte response. However, once the formation with one tall and narrow cluster has been found by the leukocytes, it is eradicated rapidly. This demonstrates that, while the shape of a biofilm affects the delay in the leukocyte response, it does not significantly affect the efficacy of phagocytosis of the biofilm.

Having separate biofilms makes it more difficult for the leukocyte response to fully eradicate the infection: Figure 6.14(f) shows that both formations with three separate biofilms take the longest time on average to be eradicated despite being found earlier than the formation with one tall and narrow biofilm. This suggests that having separate biofilms makes it more difficult for the leukocyte response to fully eradicate the infection.

Infections with larger biofilms are more successful: The formation with double the number of bacteria (red line in Figure 6.14)(f) is the same width and twice the height as the formation with one short and wide biofilm (black line). Both biofilms are found by the leukocytes at approximately the same time, further demonstrating that it is the width of the biofilm(s) that determines the efficacy of the leukocyte response at finding the bacteria. The biofilm with double the number of bacteria takes longer to be eradicated, demonstrating that infections with larger biofilms are more successful.

We remind the reader that bacterial growth, death and movement, as well as the production of the effector proteins that inhibit pro-inflammatory signalling, have been turned off and so the results do not translate to the full model. However, the results have helped us to understand how the spatial aspects of the infection contribute to the success of the infection. A treatment that makes the pro-inflammatory signalling of host IECs more sensitive to foreign invaders will subvert the delay in leukocyte recruitment caused by the tactical positioning of clustering bacteria. A treatment that either increases the recruitment rate of leukocytes, the longevity of leukocytes or the susceptibility of bacteria to phagocytosis will increase the efficacy of the leukocyte response at eradicating the initial infection, preventing the recurrence of a potentially even stronger infection. A treatment that renders leukocytes more sensitive to chemotactic gradients will allow leukocytes to traverse to and between biofilms faster, giving the infection less time to grow.

Results in the absence of antibiotic summary

Analyzing the hybrid model in the absence of the antibiotic has illuminated numerous possible treatment strategies. These treatment strategies work by ultimately reducing the ability of the infection to grow, rendering the infection more susceptible to the leukocyte response or both. Both of these effects make it more probable that the leukocyte response will eradicate the initial infection, thereby preventing the recurrence of a potentially stronger infection. Table 6.1 gives a list of the treatment strategies predicted by this analysis to be effective, along with their effects and the Figures that demonstrate them.

The model predicts that there is an optimum, intermediate production rate for the effector proteins

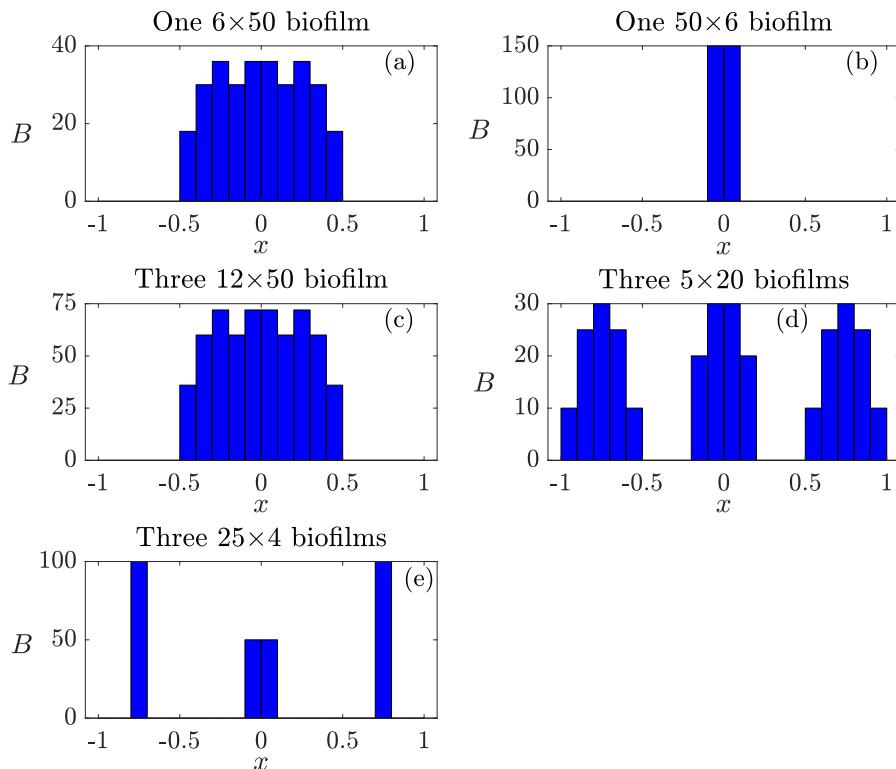


Figure 6.13: Horizontal spatial profiles of the bacteria at time $t = 0$ for each of the artificial rectangular biofilm types (some biofilms appear uneven due to the way the histogram bins are defined, but are indeed rectangular). The biofilm types are given in the figure titles and each biofilm type has 300 bacteria except for the third biofilm type (panel (c)) which has 600 bacteria. Each case has 150 simulations and all other parameters take their baseline values (see set 4 in Table A.2).

that inhibit phagocytosis — a finding that cannot be explained with the current model or literature. We believe that this prediction should be explored in more biological detail, as doing so may uncover vital information regarding the infection strategy of EPEC and the effectiveness of this treatment strategy. While applying any of the above treatment strategies sufficiently strongly leads to confidence that the infection will be eradicated, the clinical safety of such strong treatments has not been considered; adverse side effects or infection complications may occur. Treatment combinations can be made, allowing individual treatments to be used at non-extreme doses clinically. This will be safer for the patient and is expected to be more cost-effective than using one strong dose of one treatment strategy. Harsh immune-boosting treatment strategies, such as increasing the longevity of leukocytes, must be used carefully to avoid auto-immune diseases such as leukocytosis and inflammatory diseases. Treatments of this kind need to have individual patient factors taken into account, such as natural immune strength and delay in diagnosis. We showed that denser biofilms tend to be more successful than sparser biofilms; therefore, preventing bacteria from clustering close together will improve the ability of the immune response to clear the infection. We remark here that it is not known whether the highlighted treatment strategies are feasible in reality.

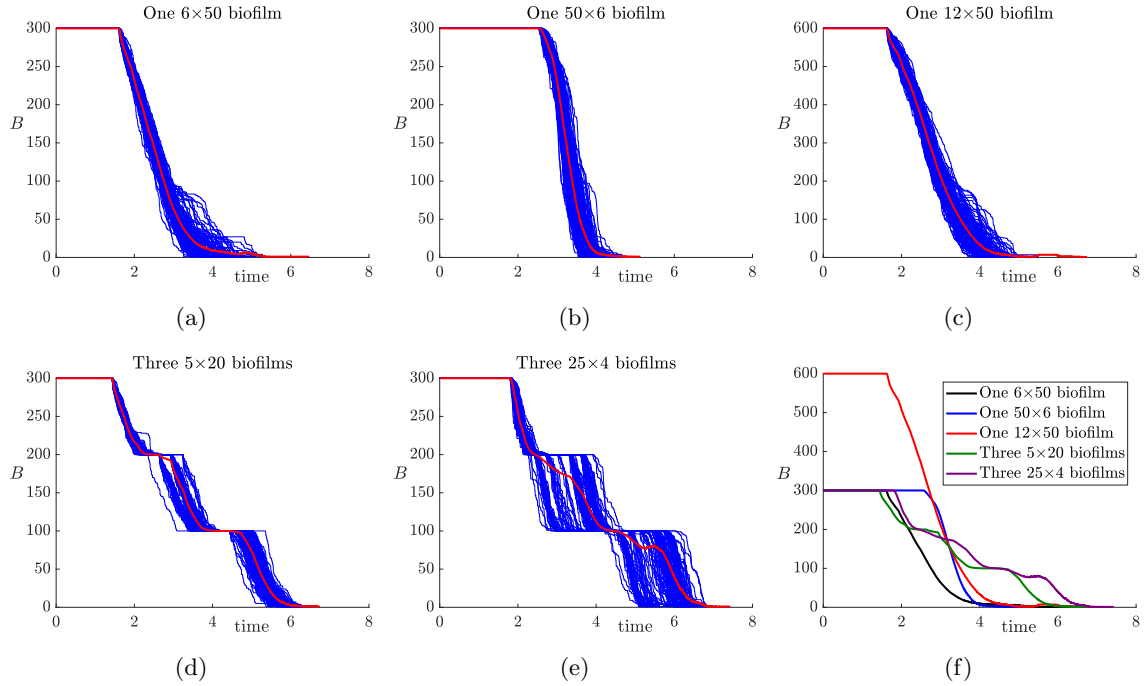


Figure 6.14: (a)-(e) The number of bacteria over time in each simulation (blue) and the mean number of bacteria over all simulations (red) for each artificial biofilm type. The biofilm types are given in the figure titles and are written in the form height $\times$ width. Each biofilm type has 300 bacteria except for the third biofilm type (panel (c)) which has 600 bacteria. (f) The mean curves from (a)-(e) plotted on the same axes. Each case has 150 simulations and all other parameters take their baseline values (see set 4 in Table A.2).

Treatment	Effect(s)	Figure(s)
Inhibit the production of bacterial structural proteins	Reduces the growth rate of bacteria, rendering the infection easier to eradicate by the leukocyte response	6.7, 6.11
Inhibit the production/action of the effector proteins that inhibit pro-inflammatory signalling	Improves the inflammatory response, decreasing the delay in the leukocyte response	6.1–6.7, 6.11
Inhibit the production/action of the effector proteins that inhibit phagocytosis	Improves the efficacy of phagocytosis, rendering the infection easier to eradicate by the leukocyte response	6.1–6.7, 6.11
Increase the recruitment rate/longevity of leukocytes	Improves the efficacy of phagocytosis and the duration of the leukocyte response, rendering the infection easier to eradicate by the leukocyte response	6.7, 6.11, 6.14
Prevent bacteria from clustering close together	Elicits a stronger inflammatory response, reducing the delay in the leukocyte response	6.11–6.14
Increase sensitivity of leukocytes to chemotactic gradients	Allows leukocytes to traverse to bacteria faster, giving the infection less time to grow	6.13, 6.14

Table 6.1: The treatment strategies, highlighted by analysis of the hybrid model (in the absence of antibiotic), that are predicted to be effective, their effects and the Figures that demonstrate them.

6.4 Results in the presence of antibiotic

Exploring the effects of antibiotic treatment on the infection

We now explore the therapeutic effects of aminoglycosides by considering various strategies of antibiotic delivery into the host, seeking those that are most effective at eradicating the infection. The strategies considered are those used by Ternent et al. [85]: (1) one dose, (2) repeated doses and (3) constant delivery. These strategies are compared against the infection with no antibiotic treatment for reference. We are primarily interested in elucidating which strategy is the most effective given a prescribed amount of antibiotic. Unless otherwise stated, all repeated doses are uniform in concentration over time.

One strong antibiotic dose significantly reduces the peak infection size, while several weaker doses eradicate the most infections: Figure 6.15 shows that, in the one dose case, most infections are significantly reduced in size; however, a few infections regrow to larger than the first peak infection size. In the repeated doses case there is a significant spread in infection sizes late in the infection ($t > 4$); however, most infections are eradicated or reduced to small sizes by the cut-off time. There is also a significant spread in infection sizes in the constant delivery case and there are more recurring infections for constant delivery than for either one or repeated doses. Figure 6.16(a) shows that on average, the one dose case makes the initial infection less severe, but results in a poor infection resolution, sometimes leading to a recurrent infection. Regular weaker doses do not reduce the average peak infection size as well as one strong dose, but the average resolution of the infection is the best, with a highest percentage of infections eradicated (88%; see the legend of Figure 6.16(c)). Constant delivery does not reduce the average peak infection size as much as one strong dose does, and has poor resolution; therefore, this strategy is the least effective. Figure 6.16(c) shows that the one dose case has by far the highest concentration of internalized antibiotic for $2.5 < t < 3.5$, leading to the observed smaller and earlier peak infection size. These results demonstrate that, for a given amount of antibiotic, the way in which the antibiotic is administered significantly affects the outcome of the treatment.

The difference between the surviving and eradicated infections is due to the antibiotic aiding the immune response: Figure 6.16(b) shows that of the infections that survive, the infection size is typically the smallest when the repeated dose strategy is applied. The infection size for the one dose and constant delivery strategies is almost as high as that in the absence of antibiotic indicating that these treatment strategies do not resolve the infection in a significant proportion of patients. In the one dose case, the mean infection size is approximately the same for the surviving and eradicated infections until $t \approx 4.5$. This is approximately the time at which the mean total internalized antibiotic concentration decreases below that in the repeated doses and constant delivery cases. However, for repeated doses and constant delivery, the mean infection size for surviving infections diverges from that for eradicated infections at a similar time to that when the antibiotic is absent, implying that the antibiotic does not affect this divergence. This demonstrates that the antibiotic is not solely responsible for the eradication of the infection and works alongside the immune response.

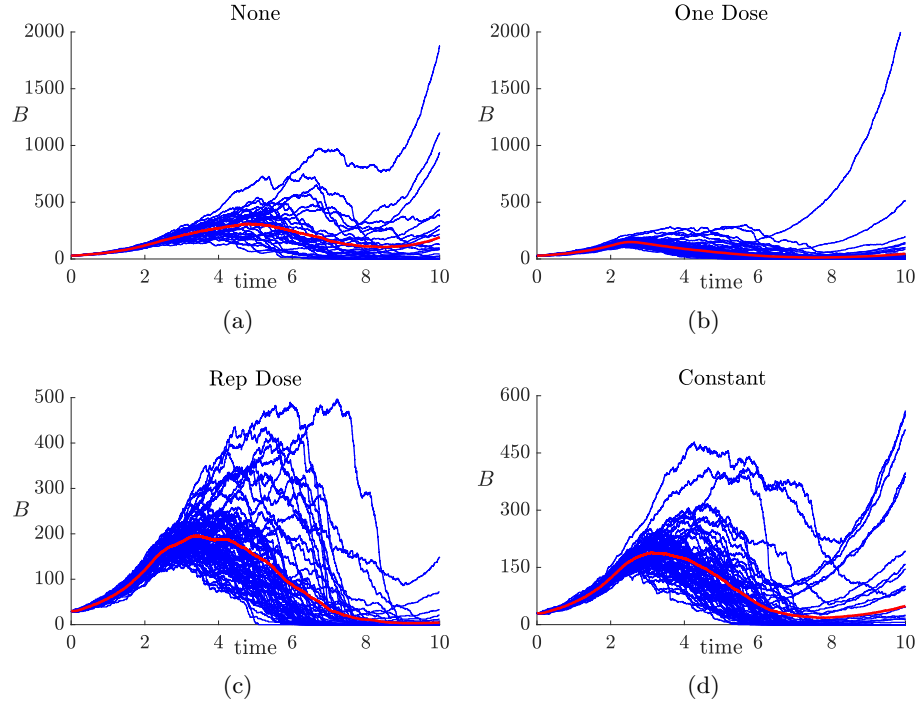


Figure 6.15: The number of bacteria over time in each simulation (blue) and the mean number of bacteria over all simulations (red) for each antibiotic delivery strategy. Each case has 100 simulations and all other parameters take their baseline values (see set 5 in Table A.2).

Exploring the effectiveness of repeated dose strategies

We now explore whether different patterns of repeated doses can reduce the peak infection size and further improve the resolution of the infection. We consider, alongside the uniform dose case (strategy 2a), three non-uniform cases: each dose being half the strength of the previous (strategy 2b), a linear decrease in strength with each dose (strategy 2c) and the same as strategy 2b but with the dose frequency halved (strategy 2d). The total antibiotic concentration administered is kept the same in all strategies and Figure 6.17 gives the times and strengths of the doses for each of the four cases considered.

Regular uniform strength antibiotic doses give a better resolution of the infection than non-uniform dose strategies: Figure 6.18(a) shows that all of the non-uniform dose strategies have a similar mean infection size until $t \approx 8$, demonstrating that distinguishing between them is not important. While the peak infection size is slightly higher in the uniform doses case, the resolution of the average infection is better and Figure 6.18(b) shows that surviving infections are less potent on average. The non-uniform strategies all demonstrate infection recurrence in a small proportion of infections. Comparing Figures 6.18(c) and 6.18(a), we see that having a higher internalized antibiotic concentration early in the infection leads to a lower peak infection size, whereas a higher concentration later leads to better resolution of the infection. Finally, regular uniform doses lead to the most eradicated infections (89%; see the legend of Figure 6.18(c)).

Although any treatment style can eradicate an infection provided enough antibiotic is delivered, the

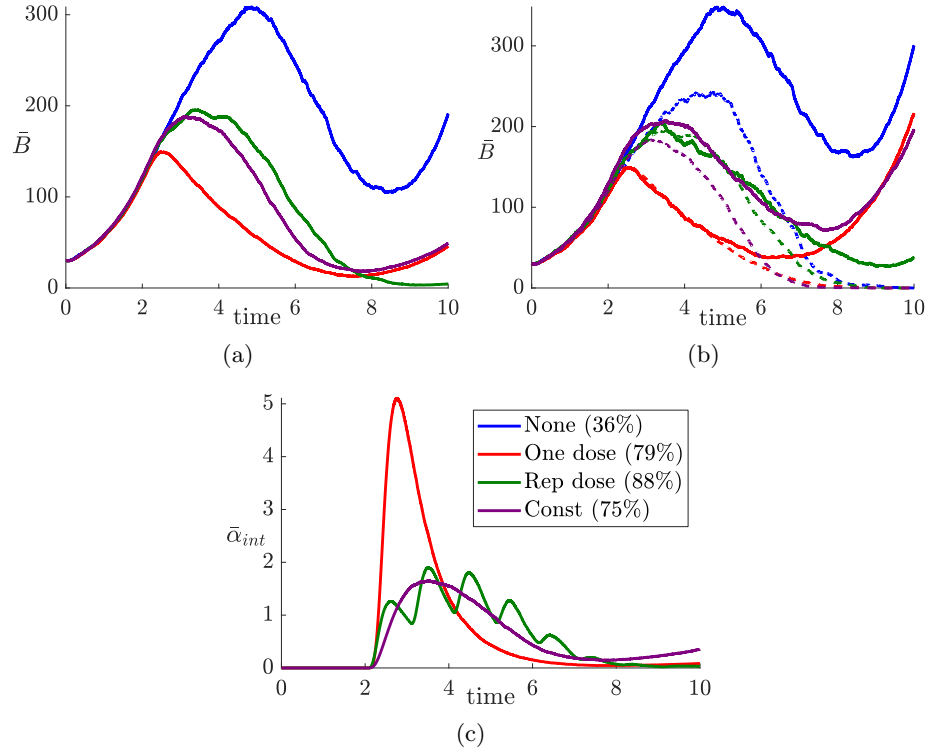


Figure 6.16: The mean number of bacteria over time (a) over all infections, (b) for the surviving (full lines) and eradicated (dashed lines) infections separately. (c) the mean total concentration of internalized antibiotic over all infections for each antibiotic delivery strategy. The bracketed quantities denote the percentage of infections that are eradicated for the respective delivery strategy. Each graph is the mean trajectory of 100 simulations and all other parameters take their baseline values (see set 5 in Table A.2).

clinical safety of very strong doses has not been considered: We now explore how doubling the strength of the antibiotic affects the infection. Figure 6.19 shows that for all antibiotic treatment strategies, the peak infection size is lower, the resolution of the infection better and the percentage of infections eradicated higher. However, we have not considered the clinical safety of very strong doses and we assume that a sufficiently large dose of antibiotic is detrimental to the host (see section 2.5.1).

A shorter delay in the onset of treatment leads to a smaller and earlier peak infection size whereas a longer delay leads to better resolution of the infection: We now explore how the delay in the onset of treatment affects the outcome of the treatment for the non-zero standard delivery strategies (with the original antibiotic dosage). Figure 6.20 shows that the general trend for all three strategies is that a shorter delay leads to a smaller and earlier peak infection size whereas a longer delay leads to better resolution of the infection, particularly in the one dose case. The poorer resolution for shorter delays is due to the antibiotic-mediated killing of pathogens decreasing the intensity of the immune response; a finding that is in keeping with the work of Gjini et al. [86]. A treatment of uniform repeated doses with a short delay gives the best balance between reducing the peak infection size and improving the resolution of the infection, therefore this is the current optimal

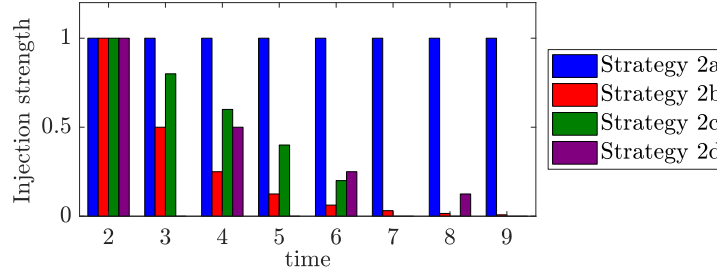


Figure 6.17: The times and strengths of the antibiotic doses for each of the repeated dose strategies.

treatment strategy.

Infrequent stronger doses reduce the peak infection size and frequent weaker doses improve the resolution of the infection: We now explore how the gap in-between uniform repeated doses affects the outcome of the treatment and the results of this can be seen in Figure 6.21. Since the total amount of antibiotic delivered is the same in all cases, more frequent doses are weaker and vice versa. The peak infection size is lower for less regular stronger doses; however, the resolution of the infection is better for more regular weaker doses. In all cases, a larger dose of antibiotic is required to eradicate the infection.

Antibiotics cannot eradicate infections without the immune response: We now consider the direct effects of the antibiotic on the growth of the bacteria. To do this, we instantaneously apply an artificial antibiotic at a constant concentration within all bacteria at a set time point for the remainder of the infection, $t = 3$ chosen to realistically model the delay between clinical diagnosis and treatment commencement. We examine several different artificial antibiotic concentrations and consider them as a percentage of the maximum possible internal concentration ($\max(\alpha_{\text{int}}) = 1$). We run this experiment both in the absence and presence of leukocytes. Figure 6.22 shows that the switch between survival and eradication occurs for an internal antibiotic concentration between 70% and 80% of the maximum possible internal concentration both in the absence and presence of leukocytes; concentrations we assume are too high to sustain clinically. Since the infection size is further reduced in the presence of leukocytes, we infer from these results that an antibiotic treatment cannot eradicate an infection unless it acts in tandem with the immune response.

Antibiotic treatments are more effective if leukocytes can survive for longer, move faster or are more sensitive to chemotactic gradients: We use the same implementation for biofilms as that used in section 6.3 — a region with a non-zero density of bacteria everywhere. At time $t = 2$ (Figure 6.23(a,d,g,j)), there is negligible variation between the delivery strategies, since neither the antibiotic nor the leukocyte response have typically reached the infection by this time (for the parameter values used). At time $t = 4$ (Figure 6.23(b,e,h,k)), the profiles begin to diverge as the differences in antibiotic concentrations become apparent; however, the shapes of all profiles remain similar. The profile is tallest in the absence of antibiotic and shortest in the one dose case, reflecting the higher concentration of antibiotic present early in the infection in the one dose case. In all cases, the middle of the profile is reduced the most initially and the right hand side is reduced more than the

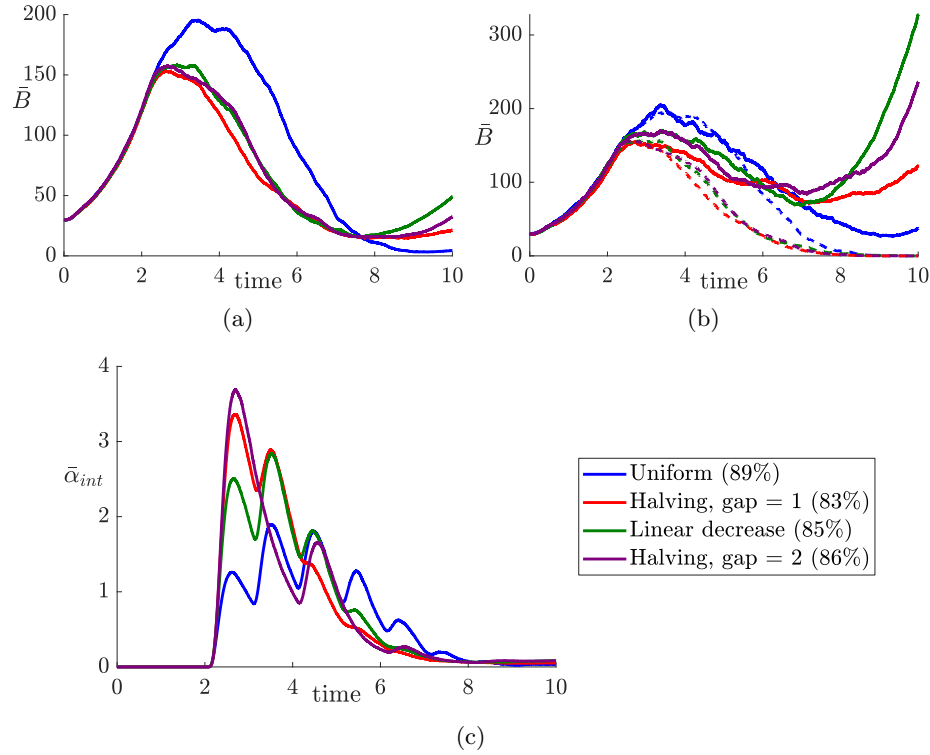


Figure 6.18: The mean number of bacteria over time (a) over all infections, (b) for the surviving infections (full lines) and are eradicated (dashed lines) separately. (c) the mean total concentration of internalized antibiotic over all infections for various repeated dose strategies. The bracketed quantities denote the percentage of infections that are eradicated for the respective delivery strategy. Each graph is the mean trajectory of 100 simulations and all other parameters take their baseline values (see set 5 in Table A.2).

left, due to the advective lumen flow. At time $t = 6$ (Figure 6.23(c,f,i,l)), the profile typically splits into one or two clusters for all treatment strategies, demonstrating that the leukocyte response reduces the infection to smaller clusters. This is significant as Figure 6.14 shows that leukocytes require time to traverse between clusters — time that allows the infection to grow larger and results in sub-maximal effectiveness of the delivered antibiotic. Antibiotic treatments are more effective if leukocytes can survive for longer, move faster or are more sensitive to chemotactic gradients, as this allows them to traverse to and between bacterial clusters more rapidly and remove bacteria for longer.

Results in the presence of antibiotic summary

Analyzing the hybrid model in the presence of the antibiotic has illuminated several additional treatment strategies to those obtained in section 6.3. Antibiotic treatment strategies work by reducing the ability of the infection to grow and produce effector proteins, thereby making it harder for the infection to evade and outlast the leukocyte response. Antibiotic treatments make it more probable that the leukocyte response will eradicate the initial infection, thereby preventing the recurrence of a potentially stronger infection. The hybrid model predicts that:

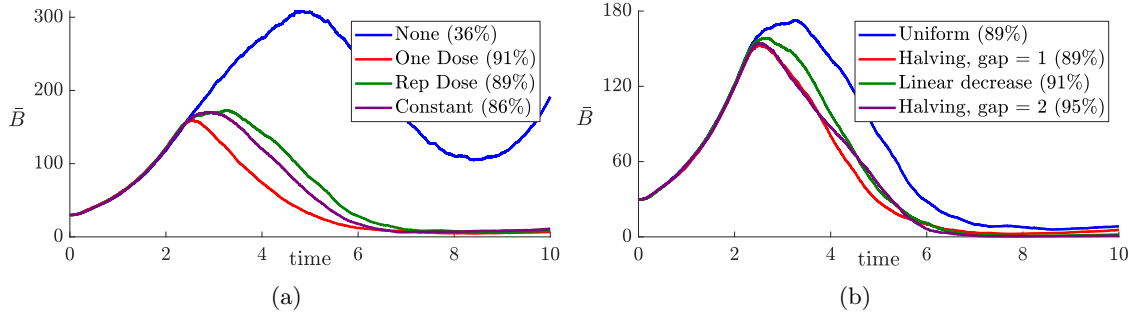


Figure 6.19: The mean number of bacteria over time for (a) the standard antibiotic delivery strategies and (b) the repeated dose strategies, when the total antibiotic delivered is doubled ($\alpha_{\text{tot}} = 2$). The bracketed quantities denote the percentage of infections that are eradicated for the respective delivery strategy. Each graph is the mean trajectory of 100 simulations and all other parameters take their baseline values (see set 6 in Table A.2).

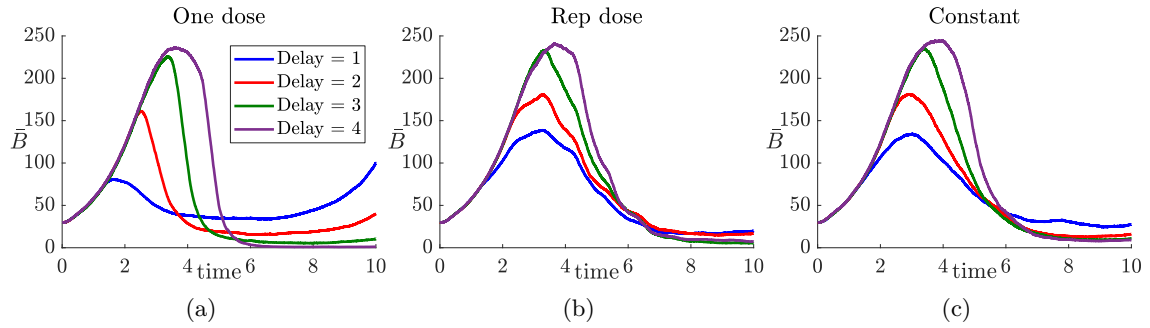


Figure 6.20: The mean number of bacteria over time for $t_1 = 1, 2, 3, 4$, for each antibiotic delivery strategy. Each graph is the mean trajectory of 100 simulations and all other parameters take their baseline values (see set 7 in Table A.2).

- One strong antibiotic dose significantly reduces the peak infection size, but does not resolve the infection well. Regular weaker doses provide better resolution, at the cost of a smaller reduction in the peak infection size.
- Constant delivery is not the most effective antibiotic delivery strategy.
- The antibiotic helps maintain an efficacious immune response.
- Given a prescribed amount of antibiotic, regular weaker doses result in the most infections being eradicated on average.
- Regular uniform doses give a better resolution of the infection than other non-uniform dose strategies.
- Provided enough antibiotic is delivered, any treatment style can eradicate an infection.
- A shorter delay in the onset of treatment leads to a smaller and earlier peak infection size. A longer delay leads to better resolution of the infection. A balance must therefore be struck between reducing the severity of the infection and minimizing the risk of eradication failure.
- Infrequent stronger doses reduce the peak infection size, while frequent weaker doses improve resolution.
- Antibiotic treatments cannot eradicate infection alone; they work in tandem with the immune response.

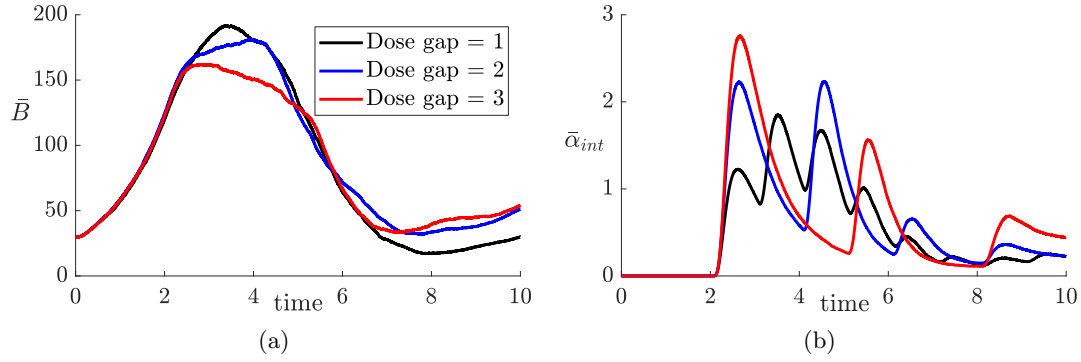


Figure 6.21: (a) The mean number of bacteria and (b) the total concentration of internalized antibiotic over time for uniform repeated doses with $t_{\text{gap}} = 1, 2, 3$. Each graph is the mean trajectory of 100 simulations and all other parameters take their baseline values (see set 5 in Table A.2).

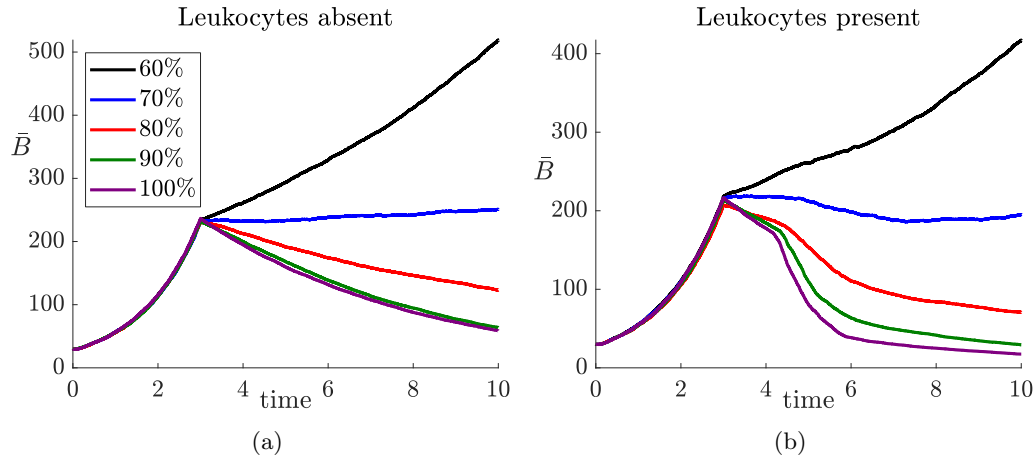


Figure 6.22: The mean number of bacteria over time when the amount of antibiotic internalized by all bacteria is instantaneously set to a constant percentage of the maximum possible at $t = 3$ ($\max\{\alpha_{\text{int}}\} = 1$). This is done in the (a) absence and (b) presence of leukocytes. Each graph is the mean trajectory of 50 simulations and all other parameters take their baseline values (see set 8 in Table A.2).

- Antibiotic treatments are more effective if leukocytes can survive for longer, move faster or are more sensitive to chemotactic gradients.
- For all delivery strategies, the infection is typically split into one or two separated clusters by the leukocyte response.

Here, we discuss caveats of the findings given above. We assume that high doses of antibiotics can lead to infection complications, such as secondary infection due to an imbalance in the gut flora (see section 2.5.1). Not being able to arbitrarily increase the total dose of antibiotic also means that it is not feasible to sustain a high antibiotic concentration within bacteria, meaning that the immune response is necessary to eradicate the infection. It may not always be clinically possible to provide a very short delay in treatment commencement, as the symptoms of infection may not have presented themselves to the host this early. While delaying a treatment leads to better resolution of the infection, the patient will need to endure a more painful and stressful infection. Therefore,

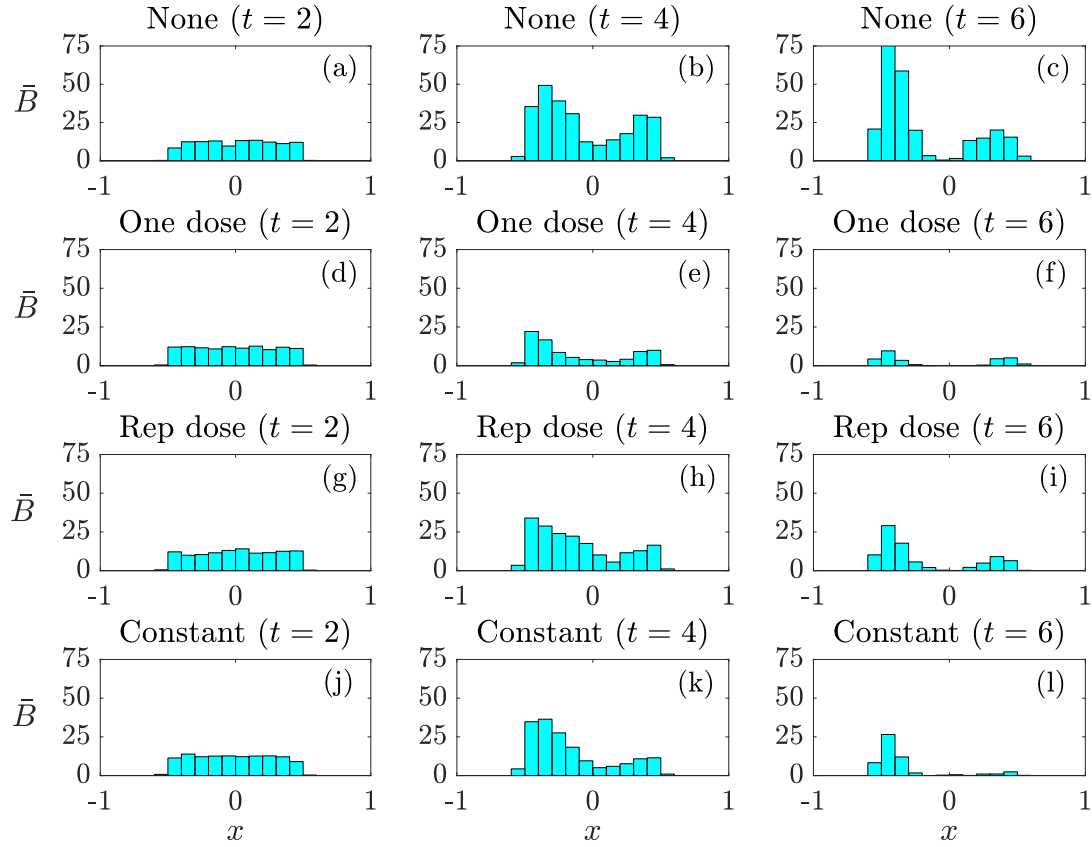


Figure 6.23: Histograms of the average horizontal spatial profile of the bacteria at time points $t = 2, 4, 6$, for each antibiotic delivery strategy. The time point and delivery strategy considered in each plot are given in the plot's title. Each histogram is the mean profile of 100 simulations and all other parameters take their baseline values (see set 5 in Table A.2).

an effective treatment strategy may be to deliver one strong antibiotic dose to prevent the infection from becoming too large, followed by regular weaker doses to eradicate, and prevent the recurrence of, the infection.

6.5 Discussion

In this chapter, we have explored the dynamics of the hybrid model both in the absence and in the presence of antibiotic. The results have highlighted several effective treatment therapies for eradicating EPEC infections.

The four most effective treatment strategies in the absence of antibiotic are; (1) inhibiting the production of bacterial structural proteins, (2) increasing the longevity or the recruitment rate of leukocytes, (3) inhibiting the production or action of the effector proteins that inhibit pro-inflammatory signalling and (4) inhibiting the production or action of the effector proteins that inhibit phagocytosis. Inhibiting the production of structural proteins reduces the growth rate of bacteria, leading to reduced infection survivability. Increasing the longevity or the recruitment rate of leukocytes allows the leukocyte response to remove bacteria for longer. There is a clear monotonically increasing relationship between the production rate of the effector proteins that inhibit pro-inflammatory signalling and both the peak infection size and time. These proteins allow the infection to evade the leukocyte response for longer allowing more time for growth, demonstrating the significant contribution of these effector proteins to the survival of the infection. The model predicts that there is an optimal production rate of the effector proteins that inhibit phagocytosis. This result was surprising, as we expected a monotonically increasing relationship between production and the infection size. Reducing the production rate is predicted by the model to decrease the average number of infections that survive. Together, these results imply that targeting the effector proteins that inhibit phagocytosis will be effective, but less effective than targeting the other three identified targets.

We find that applying any of the above treatment strategies sufficiently strongly is very likely to eradicate the infection; however, the biological feasibility of these treatments has not been considered. If feasible, a healthy resolution is possible in many infections by combining these treatments at non-extreme strengths, therefore minimizing damage done to the host. Exploring whether there is an optimum production rate of the effector proteins that inhibit phagocytosis will yield useful information regarding the effectiveness of treatments targeting these proteins. Care must be taken when boosting the leukocyte response, as raising the leukocyte count too high can result in autoimmune disorders such as leukaemia and inflammatory diseases. A temporary and moderate boost in the longevity or the recruitment rate of leukocytes will safely and effectively contribute to the successful eradication of the infection.

The results obtained by the hybrid model were compared to those obtained by the ODE model. Both models are in agreement that:

- (1) reducing the production rate of bacterial structural proteins, (2) boosting the leukocyte response and (3,4) inhibiting the effector proteins that inhibit pro-inflammatory signalling (E_1) and phagocytosis (E_3) are effective treatments,
- the effector proteins that inhibit host cell apoptosis (E_2) make negligible contribution to the infection,

While the models agree qualitatively on some treatments, they do not always agree quantitatively;

the ODE model predicts that targeting E_3 is more worthwhile than targeting E_1 , whereas the hybrid model predicts the opposite.

The models do not always agree; the replication rate of bacteria increases linearly with the bacterial density in the hybrid model, but plateaus in the ODE model as we have considered the carrying capacity of the infection site. Furthermore, unlike in the hybrid model, the production rates of effector proteins are bacterial density- and inflammation-dependent in the ODE model, these rates based on discussions with experimental collaborators. These remarks demonstrate a limitation of our hybrid model formulation, and future work includes exploring the hybrid model when the propensities for bacterial protein synthesis are density-dependent. There is a discrepancy in the way effector protein degradation is represented by the hybrid model; degradation of E_1 and E_2 is considered for each IEC individually, whereas degradation of E_3 is considered globally for simplicity. The rates are similar when the bacterial density is sufficiently low, but when the density is sufficiently high, degradation of E_3 becomes significantly more likely. This demonstrates another limitation of our hybrid model formulation, and suitably altering the representation of E_3 degradation will make the model more biologically realistic.

We explored how the spatial profile of the infection affects the success of the infection by considering the clustering of bacteria along the IEC layer. We find that the width of clusters has a positive relationship with the strength of the inflammatory response. Clusters that are significantly reduced in width often recur, as the resulting allaying of the inflammatory response gives the infection time to regrow before the leukocyte response returns. Clusters containing more bacteria take longer to be eradicated since there are more bacteria to remove and more bacteria that replicate. Infections that are split into several clusters by the leukocyte response take longer to be eradicated since leukocytes require time to travel to and between clusters.

We showed that aminoglycosides reduce the effector protein-induced delay in leukocyte recruitment activation and attraction. The model predicts that the aminoglycoside delivery strategy that most effectively reduces the peak infection size and increases the potential for eradication is one initial strong dose followed by several regular weaker doses. Without the strong initial dose, the peak infection size is higher and without the subsequent regular weak doses, the infection is often not eradicated, resulting in a recurring infection. This result is also found by Paterson et al. [153] in their study of various antibiotic treatment protocols while considering antibiotic resistance, and by Imran and Smith [154] in their study of how various periodic doses of antibiotic affect the elimination of a microbial population from biofilm. In both of these papers, the subsequent weaker injections taper in strength; however, the essences of the treatments are the same as ours. The regular weaker doses are predicted to be most effective when they are evenly spaced and uniform in strength. Constant delivery, such as via continuous intravenous infusion, is predicted to be less effective than any dose-based strategy.

Given a prescribed amount of antibiotic, shorter delays in treatment onset lead to a smaller and earlier peak infection size, whereas longer delays leads to better infection resolution. This implies that if there is a cap on how much antibiotic a patient can safely have, a delayed treatment is more

likely to clear the infection. Furthermore, short delays may be impractical, as the symptoms of infection may not have presented themselves to the host this early. The mathematical literature both agrees and disagrees with this finding. Gjini and Brito [86] find that the antibiotic-mediated killing of pathogens decreases the intensity of the immune response, leading to sub-optimal treatment conditions; a delay in antibiotic treatment that is sufficiently short allows the immune response and antibiotic to act synergistically, resulting in greater clearance of both antibiotic-sensitive and resistant bacteria. Conversely, D'Agata et al. [84] find that a delay in antibiotic treatment decreases the effectiveness of the treatment, as it can allow the bacteria population size to increase sufficiently far beyond the minimum infective dose before the treatment commences that the antibiotic is unable to reduce the population below the minimum infective dose. However, unlike in our models, it is assumed in this model that the number of phagocytes remains constant (this model-simplifying assumption is made as it allows the threshold of invasion and the threshold of bacterial eradication to become the same). Imran and Smith [154] also find that a delay is not beneficial, and should a delay occur, appropriate adjustments should be made to the dosing strategy, such as increasing the dosing strength or frequency. In clinical practice, deliberate treatment delay is generally not employed. Experimental studies into treatment delay have been carried out, but with the aim of reducing the unnecessary use of antibiotics (e.g. for treatment of viral infections). It was found that delayed treatment is either as or less effective as immediate treatment in acute respiratory and ear infections [155, 156]. While our model predicts that delaying treatment improves infection resolution, this is a risky venture for potentially fatal infections. Clinical exploration of treatment delay on treatment success for EPEC infections identified to be non-fatal may reveal a strategy for more effective aminoglycoside application.

We discussed in the literature review (see section 2.5.1) that, while general antibiotics are delivered in single doses, regular doses and via continuous intravenous infusion, aminoglycosides are generally only delivered in repeated doses. Typical methods of delivery include dose and brief intravenous infusion. The absence of single dose and continuous intravenous infusion treatments in clinical practice suggests that the one dose and constant delivery strategies considered in the model are not effective. Indeed, a detailed analysis of these treatment strategies in the model predicts that repeated doses is the strategy most likely to eradicate the infection.

While we show that an infection is very likely to be eradicated provided enough antibiotic is delivered, we assume that high doses of antibiotics can lead to infection complications, such as secondary infection due to an imbalance in the gut flora (see section 2.5.1). Therefore, the delivery strategy of the antibiotic and the efficacy of the immune response are crucial to the successful eradication of the infection.

Chapter 7

A PDE model for chemotactic leukocyte recruitment

7.1 Overview

In this chapter, we present the formulation and analysis of a one-dimensional continuous analogue of the hybrid model for the chemotactic recruitment of leukocytes, to consider the response of the immune response to an infection in detail. In the hybrid model (Chapter 5), the production of pro-inflammatory mediators by leukocytes (term f_2 in (5.1)) and the chemotactic attraction of leukocytes up gradients in pro-inflammatory mediator concentration (equation (5.41)) are incompatible; the produced pro-inflammatory mediators dilute the chemotactic gradient until the leukocytes become trapped in self-made maxima. To circumvent this, we switched off the production of pro-inflammatory mediators by leukocytes, resulting in leukocytes being chemotactically attracted only by the pro-inflammatory mediators produced by IECs. Here, we develop a PDE model with the aim of finding a condition for leukocyte entrapment. This model accounts for the spatial aspects considered in the hybrid model, but in a more analytically and computationally tractable framework.

We first formulate the model and then analyse it via numerical simulations and stability analysis. We find for certain parameter regimes that the concentrations of pro-inflammatory mediators and leukocytes diverge to infinity, suggesting model instability; however due to time constraints, we were unable to complete a comprehensive stability analysis. Future work includes addressing this instability, and an analytical procedure for this is given in Appendix A.10.

7.2 Model formulation

We consider a one-dimensional continuous analogue of the hybrid model for the chemotactic recruitment of leukocytes and consider the concentrations of pro-inflammatory mediators, $P(x, t)$, and leukocytes, $L(x, t)$, as quantities that vary continuously in time and space. The mechanisms for the production, diffusion and degradation of pro-inflammatory mediators, as well as the recruit-

ment, diffusion, chemotaxis and death of leukocytes, are accounted for. The epithelium and the endothelium are defined to be at $x = 0$ and $x = h$ respectively and the boundaries of the system are defined to be these layers.

As in the hybrid model, P are produced constantly by IECs (due to the presence of bound bacteria), diffuse freely through the domain and are transported out of the system through the endothelium at a rate proportional to their local concentration. L are recruited into the system through the endothelium at a rate proportional to the local concentration of P , diffuse freely through the domain and are chemotactically attracted up gradients in P . We assume that since the IECs are the sole producers of the chemoattractant, L do not travel past the epithelium and this is reflected by imposing a no flux condition at $x = 0$. We assume for simplicity that all diffusion and chemotactic parameters are constants and that L produce P at their current location at a rate proportional to the local magnitude of the chemotactic gradient, $\left| \frac{\partial P}{\partial x} \right|$. Finally, P and L degrade and die at linear rates respectively. The two variable system of PDEs governing these dynamics is:

$$\frac{\partial P}{\partial t} = d_P \frac{\partial^2 P}{\partial x^2} + rL \left| \frac{\partial P}{\partial x} \right| - k_P P, \quad P(0, t) = P^*, \quad \frac{\partial P}{\partial x}(h, t) = -\alpha P(h, t), \quad (7.1a)$$

$$\frac{\partial L}{\partial t} = d_L \frac{\partial^2 L}{\partial x^2} - c \frac{\partial}{\partial x} \left(L \frac{\partial P}{\partial x} \right) - k_L L, \quad \frac{\partial L}{\partial x}(0, t) = 0, \quad \frac{\partial L}{\partial x}(h, t) = \beta P(h, t), \quad (7.1b)$$

where the definition of each parameter is given in Table 7.1.

Parameter	Description
d_P	The diffusion coefficient of pro-inflammatory mediators
r	The production rate of pro-inflammatory mediators by leukocytes
k_P	The natural degradation rate of pro-inflammatory mediators
d_L	The diffusion coefficient of leukocytes
c	The chemotactic parameter
k_L	The natural death rate of leukocytes
α	The flux rate of pro-inflammatory mediators out through the endothelium
β	The flux rate of leukocytes in through the endothelium
P^*	The strength of the production of pro-inflammatory mediators by IECs

Table 7.1: Descriptions of the parameters in (7.1).

Non-dimensionalising via the scalings $P = P^*p$, $L = \beta P^*hl$, $x = hz$ and $t = (\tau/k_P)$, we obtain the following non-dimensional system of PDEs:

$$\frac{\partial p}{\partial \tau} = d_p \frac{\partial^2 p}{\partial z^2} + \lambda l \left| \frac{\partial p}{\partial z} \right| - p, \quad p(0, \tau) = 1, \quad \frac{\partial p}{\partial z}(1, \tau) = -wp(1, \tau), \quad (7.2a)$$

$$\frac{\partial l}{\partial \tau} = d_l \frac{\partial^2 l}{\partial z^2} - \phi \frac{\partial}{\partial z} \left(l \frac{\partial p}{\partial z} \right) - k_l l, \quad \frac{\partial l}{\partial z}(0, \tau) = 0, \quad \frac{\partial l}{\partial z}(1, \tau) = p(1, \tau), \quad (7.2b)$$

where the dimensionless parameters in (7.2) are given by:

$$d_p = \frac{d_P}{k_P h^2}, \quad \lambda = \frac{r \beta P^*}{k_P}, \quad d_l = \frac{d_L}{k_P h^2}, \quad \phi = \frac{c P^*}{k_P h^2}, \quad k_l = \frac{k_L}{k_P}, \quad w = \alpha h.$$

Given the absence of relevant experimental data to inform these values, we choose values (through trial and error) that yield qualitatively sensible solutions (where they exist). The parameter values and initial conditions used, unless otherwise stated, are given by:

$$\lambda = 1, \quad \phi = 1, \quad d_p = 0.1, \quad d_l = 0.1, \quad k_l = 0.1, \quad w = 0.2, \quad (7.3a)$$

$$p(z, 0) = \begin{cases} 1, & z = 0, \\ 0, & 0 < z \leq 1, \end{cases} \quad l(z, 0) = 0. \quad (7.3b)$$

In the following sections, we explore (7.2) through numerical simulations and stability analysis. The results will then be used to seek a condition for leukocyte entrapment.

7.3 Model analysis

7.3.1 Numerical simulations

Since we are interested in resolving the incompatibility of production of p by l and chemotactic recruitment of l , we explore solutions of (7.2) as the parameters controlling the production, λ , and chemotactic pull, ϕ , are varied. We denote the steady state profiles of $p(z, \tau)$ and $l(z, \tau)$ as $\bar{p}(z)$ and $\bar{l}(z)$ respectively, and we numerically integrate (7.2) from $t = 0$ to $t = 10^{40}$. If $|p(z, t)|, |l(z, t)| > 500$ for any $z \in [0, 1]$, then we infer that the solution is diverging and, therefore, that a steady solution has not been found. We therefore abort simulations if $|p(z, t)|, |l(z, t)| > 500$ is satisfied for any $z \in [0, 1]$ and report the value of t at which this occurs. To check the existence of $\bar{p}(z)$ and $\bar{l}(z)$, we consider:

$$\max \left(\left| \frac{p(z, t_n) - p(z, t_{n-1})}{t_n - t_{n-1}} \right| \right) < 10^{-20}, \quad (7.4a)$$

$$\max \left(\left| \frac{l(z, t_n) - l(z, t_{n-1})}{t_n - t_{n-1}} \right| \right) < 10^{-20}, \quad (7.4b)$$

where t_n and t_{n-1} are the ultimate and penultimate time points used in the numerical solver. If (7.4) are both satisfied for all $z \in [0, 1]$, then we infer that a stable steady state has been found (and vice versa).

Figure 7.1 shows numerical solutions of (7.2) for selected values of λ and ϕ . When $\lambda = 0, \phi = 0$

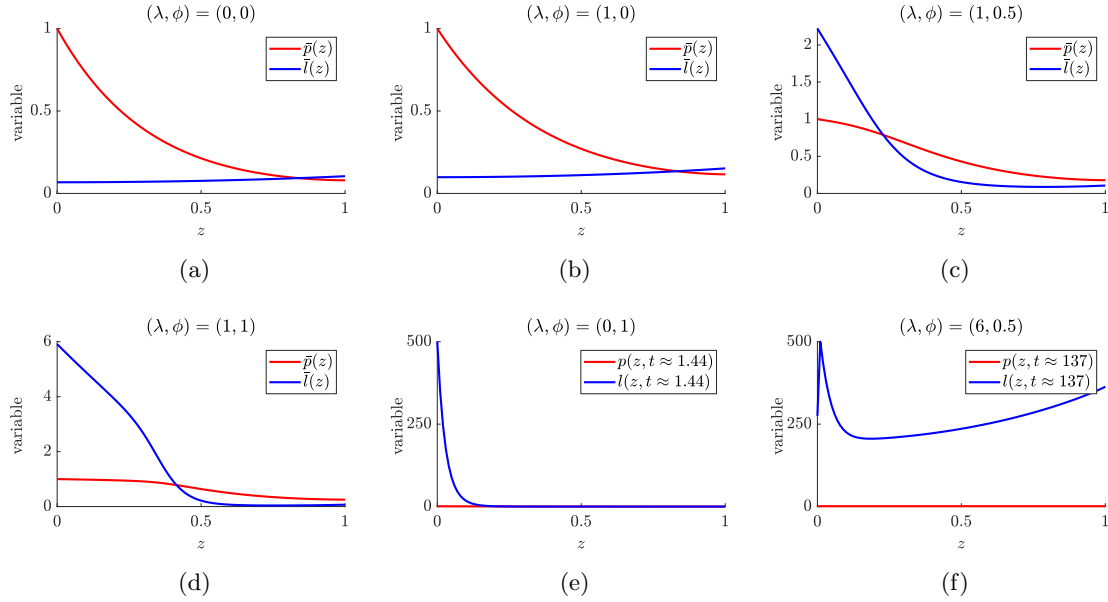


Figure 7.1: Numerical solutions to (7.2) for various values of λ and ϕ , the values used given in the respective plot title. Initial conditions and other parameter values are given by (7.3).

(Figure 7.1(a)), $\bar{l}(z = 0)$ is positive due to diffusion; however, the value is low due to the absence of chemotaxis. When $\lambda = 1, \phi = 0$ (Figure 7.1(b)), the steady state profiles are qualitatively similar to those in Figure 7.1(a), but are generally slightly higher in value everywhere; the increased production of p results in more effective recruitment of l . However, $\bar{l}(z = 0)$ is still low due to the absence of chemotaxis. When $\lambda = 1, \phi = 0.5$ (Figure 7.1(c)), $\bar{l}(z = 0)$ is higher, demonstrating effective chemotaxis. When $\lambda = 1, \phi = 1$ (Figure 7.1(d)), $\bar{l}(z = 0)$ is even higher, demonstrating more effective chemotaxis. Interestingly, $\bar{l}(z = 1)$ is lower for the larger value of ϕ (compare Figures 7.1(c) and (d)), demonstrating the strength of the chemotactic attraction of l from a large distance for large ϕ . When $\lambda = 0, \phi = 1$ (Figure 7.1(e)), $l = 500$ for some $z \in [0, 1]$ at $t \approx 1.44$. We observe similar behaviour when $\lambda = 6, \phi = 0.5$ (Figure 7.1(f)) — $l = 500$ for some $z \in [0, 1]$ at $t \approx 137$. In these cases, a steady solution has not been found, implying that the steady state of (7.2) is either unstable or non-existent. Since a stable steady state cannot be obtained for all parameter values, we infer that (7.2) is unstable in certain regions of parameter space.

7.3.2 Stability analysis

The existence of stable steady states through numerical integration

Figure 7.2 shows a plot of where a stable steady state of (7.2) was found numerically for a range of λ and ϕ value combinations. There are distinct regions of (λ, ϕ) space where a stable solution is and is not found respectively, with some manifold(s) separating the two regions. We infer that (7.2) is unstable for some open connected region in (λ, ϕ) space.

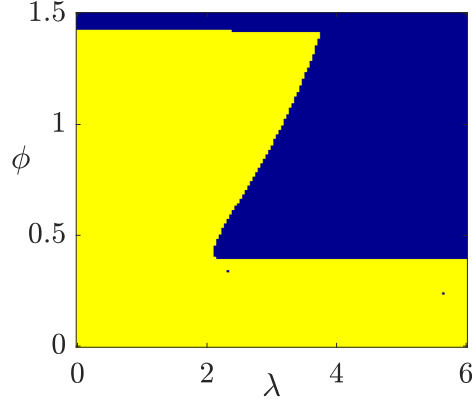


Figure 7.2: Plot of where a stable steady state of (7.2) was found numerically for a range of λ and ϕ value combinations. Yellow and blue regions indicate parameter space where a stable solution was and was not found respectively. Initial conditions and other parameter values are given by (7.3).

Solving (7.2) as a BVP

We now consider solving (7.2) as a BVP to elucidate whether an unstable steady state exists in the regions of parameter space that do not yield a stable steady state. The steady state(s) of (7.2) are given by:

$$0 = d_p \frac{d^2 \bar{p}}{dz^2} + \lambda \bar{l} \left| \frac{d\bar{p}}{dz} \right| - \bar{p}, \quad (7.5a)$$

$$0 = d_l \frac{d^2 \bar{l}}{dz^2} - \phi \frac{d}{dz} \left(\bar{l} \frac{d\bar{p}}{dz} \right) - k_l \bar{l}, \quad (7.5b)$$

$$\bar{p}(0) = 1, \quad \frac{d\bar{p}}{dz}(1) = -w\bar{p}(1), \quad \frac{d\bar{l}}{dz}(0) = 0, \quad \frac{d\bar{l}}{dz}(1) = \bar{p}(1). \quad (7.5c)$$

Substituting $\bar{u} = (d\bar{p}/dz)$ and $\bar{v} = (d\bar{l}/dz)$ into (7.5), we obtain:

$$\frac{d\bar{p}}{dz} = \bar{u}, \quad \frac{d\bar{u}}{dz} = \frac{1}{d_p} \left(\bar{p} - \lambda \bar{l} |\bar{u}| \right), \quad (7.6a)$$

$$\frac{d\bar{l}}{dz} = \bar{v}, \quad \frac{d\bar{v}}{dz} = \frac{1}{d_l} \left[\phi \left(\bar{u} \bar{v} + \frac{\bar{l}}{d_p} \left(\bar{p} - \lambda \bar{l} |\bar{u}| \right) \right) + k_l \bar{l} \right], \quad (7.6b)$$

$$\bar{p}(0) = 1, \quad \bar{u}(1) = -w\bar{p}(1), \quad \bar{v}(0) = 0, \quad \bar{v}(1) = \bar{p}(1). \quad (7.6c)$$

Equations (7.6) are a BVP from which $\bar{p}$ and $\bar{l}$ can be computed numerically, regardless of stability. Unfortunately, a solution could not be found for all (λ, ϕ) space (results not shown). This is due to either the sensitivity of the BVP solver (BVP4c in MATLAB) to initial guesses for the solution, or the non-existence of steady states for certain regions of (λ, ϕ) space.

Stability analysis via perturbation theory

We analyze the stability of the steady state of (7.2), given by (7.5), by formulating the associated eigenvalue problem under the assumption that perturbations from the steady state can be written as Fourier series. We linearise about $\bar{p}$ and $\bar{l}$ via:

$$p(z, \tau) = \bar{p}(z) + \epsilon \tilde{p}(z, \tau),$$

$$l(z, \tau) = \bar{l}(z) + \epsilon \tilde{l}(z, \tau),$$

where $0 < \epsilon \ll 1$ and:

$$\tilde{p} = e^{\sigma\tau} \sum_{j=1}^{\infty} a_j \cos(j\pi z), \quad (7.7a)$$

$$\tilde{l} = e^{\sigma\tau} \sum_{j=1}^{\infty} b_j \cos(j\pi z). \quad (7.7b)$$

In (A.21), σ is the perturbation growth rate, $j \in \mathbb{N}$ are the wave numbers of the perturbations and a_j, b_j are the respective eigenmodes for the p and l perturbations respectively. As $j \rightarrow \infty$, the eigenvalue problem is infinite-dimensional. We do not include the derivation of the eigenvalue problem here for brevity and refer the reader to Appendix A.10 for the derivation.

Davidson and Savory [157] apply the same perturbation expansion in their model for chemotaxis. They make the assumption, based on an orders of magnitude argument, that the most-positive eigenvalues come from the top-left corner of the Jacobian matrix, allowing the infinite-dimensional Jacobian to be approximated by a finite-dimensional matrix, permitting further stability analysis. However, when we make this assumption, the approximation to the Jacobian matrix does not converge as the number of dimensions increases, rendering the assumption invalid. An explanation as to why the assumption does not work is also given in Appendix A.10.

Despite numerous efforts, we have been unsuccessful at undertaking a comprehensive stability analysis due to time constraints. We have therefore been unable to investigate the chemotactic recruitment of leukocytes or find a condition for leukocyte entrapment. To address this issue, further work includes investigating the stability of (7.2) more thoroughly, for example by considering alternative methods by which the infinite-dimensional Jacobian matrix can be simplified or approximated.

7.4 Discussion

In the hybrid model (Chapter 5), we found that pro-inflammatory mediator production by leukocytes and the chemotaxis of leukocytes lead to leukocyte entrapment, disabling them from eradicating the infection. To circumvent this, we disabled the production of pro-inflammatory mediators by leukocytes. To explore this antagonism in detail, we developed the one-dimensional PDE model presented in this chapter (equations (7.2)), considering the non-dimensionalised concentrations of

pro-inflammatory mediators and leukocytes as varying continuously in time and space.

Numerical simulations of (7.2) demonstrated a stable steady state, as well as sensible chemotaxis dynamics, for particular combinations of the production rate of pro-inflammatory mediators by leukocytes (λ) and the chemotactic parameter (ϕ); however, concentrations diverge to infinity for other combinations. This implies that for certain parameter regimes, the steady state of (7.2) is either unstable or non-existent; therefore, the model is globally unstable. To investigate whether an unstable steady state exists in the regions of parameter space that do not yield a stable steady state, we solved (7.2) as a BVP; however, a solution could not be found for all (λ, ϕ) space (results not shown). We infer from these findings that either the BVP solver is very sensitive to initial guesses for the solution, or there are no steady states of (7.2) in certain parameter regimes.

We further analyzed the stability of the steady state of (7.2) by formulating the associated eigenvalue problem, under the assumption that perturbations from the steady state can be written as Fourier series (see Appendix A.10 for the derivation of the problem). We followed the method used by Davidson and Savory [157] in the stability analysis of their model for chemotaxis, in which an orders of magnitude argument led to the assumption that the most-positive eigenvalues come from the top-left corner of the Jacobian matrix. This allowed the infinite-dimensional Jacobian to be approximated by a finite-dimensional matrix, permitting further stability analysis. However, when we make this assumption, the approximation to the Jacobian matrix does not converge as the number of dimensions increases, thus rendering the assumption invalid (see Appendix A.10). Future work includes addressing this stability analysis in further detail.

Chapter 8

The formation of bacterial biofilms and their effects on the infection

8.1 Overview

Once bacteria have attached to a surface, they secrete extracellular polymeric substances that embed the infection within a slimy extracellular matrix (ECM). This ECM makes the bacteria more resistant to anti-microbial strategies, such as leukocytes and antibiotics.

In this Chapter, we adapt the hybrid model developed in Chapter 5 to include a simple representation of the dynamics of bacterial biofilms (see section 2.6). We implicitly represent the ECM by considering the proximity of bacteria to other bacteria, and model the effects of biofilms by assuming that the following processes are impeded within the biofilm: (1) the transport of inflammatory mediators and antibiotic via diffusion and advection, (2) the movement of leukocytes and (3) phagocytosis. We present the formulation of the biofilm model, followed by simulations to demonstrate the effects of biofilms on the virulence of the infection and on the effectiveness of the immune response and antibiotic treatment. We then compare the results with those obtained through analysis of the original hybrid model, under the same parameter set. Finally, we discuss possible treatment strategies for eradicating EPEC biofilms based on our results.

8.2 Model development

8.2.1 Biofilms and their effects

The biofilm model builds on the original hybrid model. We first prescribe a set of rules under which bacteria are considered to be sufficiently close to each other for a biofilm to be formed between them.

For each bacterium, we find the bacteria within a distance of one additional cell diameter, i.e. 3rad_B , where rad_B denotes the radius of the bacteria (Figure 8.1). The bacteria within this critical distance are assumed to be sufficiently close for a biofilm to be formed between them. We apply

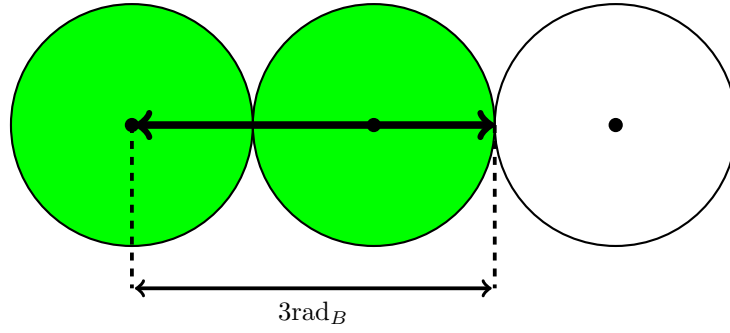


Figure 8.1: A caricature showing that 3rad_B is the maximum distance that includes nearest neighbours but excludes next-nearest neighbours; the middle bacterium is included in the biofilm of the left bacterium, but the right bacterium is not.

volume exclusion so that bacteria within 2rad_B of each other repel. If bacteria are between 2rad_B and 3rad_B of each other, the viscoelasticity of the ECM between them results in attraction, and we assume the attraction is strongest for some intermediate distance. If the bacteria are more than 3rad_B apart, then we assume there is negligible influence between the bacteria.

To model the effects of biofilms on transport, leukocyte movement and phagocytosis, we introduce a biofilm density, denoted $\hat{\phi}(x, y, t)$. This density is given by:

$$\hat{\phi}(x, y, t) = \iint_{\Omega} \mathbf{x}_B \, d\Omega, \quad (8.1)$$

where $\mathbf{x}_B$ represents the positions of the bacteria and Ω denotes the space spanned by the region of interest.

We now consider the inhibition of transport (diffusion and advection). Biofilm density-dependent diffusion parameters for inflammatory mediators and antibiotic, denoted $\mathcal{D}_I$ and $\mathcal{D}_\alpha$ respectively, are introduced and take the form:

$$\mathcal{D}_I = D_I \left(\frac{q_B^{n_B}}{q_B^{n_B} + \hat{\phi}^{n_B}} \right), \quad (8.2)$$

$$\mathcal{D}_\alpha = D_\alpha \left(\frac{q_B^{n_B}}{q_B^{n_B} + \hat{\phi}^{n_B}} \right), \quad (8.3)$$

where D_I and D_α denote the free-flow diffusion coefficients given in the original hybrid model (see section 5.2.2), q_B denotes the threshold biofilm density at which diffusion is reduced and n_B denotes the Hill parameter for diffusion inhibition in a biofilm.

Similarly, the advective flow in the lumen (due to the digestive system), given by (5.9), is replaced with the biofilm density-dependent flow given by:

$$\mathbf{u}(x, y, t) = \theta(y - y^*) \left(\frac{(y - y^*)}{\phi_u + (y - y^*)} \right) \left(\frac{q_B^{n_B}}{q_B^{n_B} + \hat{\phi}^{n_B}} \right) \hat{\mathbf{x}}. \quad (8.4)$$

$\mathbf{u}$ now depends on x and t due to the dependence on $\hat{\phi}(x, y, t)$, representing impedance of flow by biofilms.

We model the inhibition of leukocyte movement in a similar way — let $\mathcal{L}_{\text{mot}}$ denote the biofilm density-dependent motility rate of leukocytes and be given by:

$$\mathcal{L}_{\text{mot}} = L_{\text{mot}} \left(\frac{q_B^{n_B}}{q_B^{n_B} + \hat{\phi}^{n_B}} \right), \quad (8.5)$$

where L_{mot} denotes the free-flow motility rate of leukocytes given in section 5.4.3. We assume that since bacteria bind to the epithelium and therefore already have restricted movement before biofilms can form, the effects of biofilms on bacterial movement are negligible. We highlight that the effects of biofilms on the internalization of pro-inflammatory mediators and antibiotic by bacteria are implicitly accounted for by the reduced capacity of diffusion within biofilms.

We also model the inhibition of phagocytosis in a similar way. The probability that phagocytosis occurs in time interval $(t, t + \Delta t]$, denoted $\mathcal{P}$ and given by equation (5.44) (see section 5.4.3), is replaced with:

$$\mathcal{P}(\text{phag in } t \leq \Delta t) = \left(1 - (1 - Q)^k \right) \left(\frac{q_E^{n_E}}{q_E^{n_E} + \hat{\phi}^{n_E}} \right), \quad (8.6)$$

where q_E denotes the threshold biofilm density at which phagocytosis efficacy is reduced and n_E denotes the Hill parameter for phagocytosis inhibition in a biofilm.

Since the influence of the biofilm is likely to be strong only for a sufficiently high cell density, we set $n_B, n_E = 3$ so that the Hill functions in (8.2)–(8.6) exhibit switch-like behaviour. Finally, as we showed in Chapter 6 that the optimum antibiotic treatment strategy is repeated uniform strength doses, we employ this strategy to investigate the effects of biofilms on antibiotic efficacy using this model.

8.2.2 Pro-inflammatory mediator production

We remind the reader that the region of interest is a two dimensional transverse section of the intestines, extending from the lumen to the underlying blood supply, and indefinitely into the far field in the x and positive y directions (see section 5.2.1). We redefine $P(x, y, t)$ and $A(x, y, t)$ as the *concentrations* of pro- and anti-inflammatory mediators respectively, and therefore, $P, A \in [0, \infty)$. The background and stimulated production of pro-inflammatory mediators, f_1 , and the inhibition of production by anti-inflammatory mediators, f_3 , given in equation (5.1), are respectively replaced with:

$$f_1 = \left(\Phi \begin{cases} 1, & \text{for } y = y^*, \\ 0, & \text{otherwise,} \end{cases} + C_b \begin{cases} 1, & \text{for } \mathbf{x} = \mathbf{x}_B, \\ 0, & \text{otherwise,} \end{cases} \right), \quad (8.7)$$

$$f_3 = \frac{\psi_A^2}{\psi_A^2 + A}, \quad (8.8)$$

where $\mathbf{x} = (x, y, t)$, y^* denotes the y -coordinate of the epithelium, $\mathbf{x}_B$ gives the positions of the bound bacteria, Φ and C_b denote the background and stimulated production rates of pro-inflammatory mediators respectively and ψ_A denotes the anti-inflammatory mediator concentration at which inhibition is half-maximal.

8.3 Model implementation

To implement the forming of biofilms, we replace the bacterial volume exclusion algorithm from the original hybrid model (Algorithm 4) with the biofilm forming algorithm described above (section 8.2), where the speed and direction of movement of a bacterium are dependent on the distance between it and other bacteria. We denote the position of bacterium i at the k th time point as (x_{Bi}^k, y_{Bi}^k) . We first ensure that no bacteria have identical positions by executing equations (5.45) (see section 5.4.5) for all pairs of bacteria with identical positions. The unit vector connecting the centres of bacteria i and j , in the direction of j , is given by:

$$\hat{\mathbf{u}} = \frac{(x_{Bj}^k - x_{Bi}^k, y_{Bj}^k - y_{Bi}^k)}{d},$$

where d is the distance between the centres of the bacteria. The speed of outward movement of a pair of interacting bacteria, $v(x)$, is governed by:

$$v(x) = \begin{cases} v_{\max} \left(1 - \frac{x^2}{\beta_1^2} \right), & 0 \leq x < 2\text{rad}_B, \\ v_{\min} \left(\frac{x - \beta_1}{\beta_2 - \beta_1} \right), & 2\text{rad}_B \leq x < 2.5\text{rad}_B, \\ v_{\min} \left(\frac{x - \beta_3}{\beta_2 - \beta_3} \right), & 2.5\text{rad}_B \leq x < 3\text{rad}_B, \\ 0, & x \geq 3\text{rad}_B, \end{cases} \quad (8.9)$$

where x is the distance between the centres of the bacteria, $v_{\max} > 0$ and $v_{\min} < 0$. Figure 8.2 shows the shape of $v(x)$. For simplicity, the speed of movement decreases quadratically with distance when cells are overlapping to represent stronger repulsion. For further simplicity, the speed of movement is linear with distance when cells are not overlapping, the distance giving strongest attraction set at $x = 2.5\text{rad}_B$. The distance of bacterial movement is therefore $\Delta t v(x)$, and the bacteria move half this distance in opposing directions along $\hat{\mathbf{u}}$. The general biofilm forming algorithm (for n interacting bacteria, where $n \in \mathbb{Z} \geq 2$) is given by Algorithm 6. For a group of bacteria in sufficiently close proximity, Algorithm 6 acts over time to pull and push the bacteria until there

are no gaps or overlaps between the bacteria (see Figure 8.3).

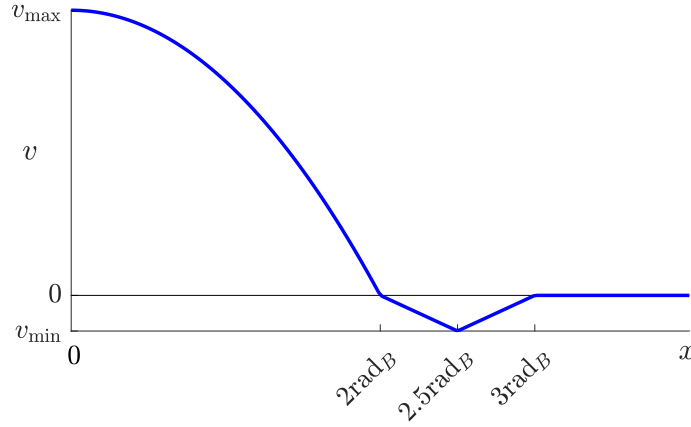


Figure 8.2: A graph showing how the movement speed of an interacting pair of bacteria, $v(x)$, varies with the distance between the cells under the biofilm forming algorithm.

Algorithm 6 Algorithm for bacterial movement in a biofilm

1. Compute the matrix containing the distances between the centres of all pairs of bacteria, denoted $\mathbf{B}$, and only consider the strictly lower triangular part, denoted $\mathbf{B}_L$.
2. **while** $\min(\mathbf{B}_L) < 3\text{rad}_B$, **then**:
 - (a) Find the indices i, j such that $(B_L)_{ij} = 0$. Put these pairs of indices into two lists, denoted $\mathbf{p}$ and $\mathbf{q}$, the elements of which are i and j respectively. Apply equations (5.46).
 - (b) Find the indices i, j such that $(B_L)_{ij} < 3\text{rad}_B$. Put these pairs of indices into two lists, denoted $\mathbf{m}$ and $\mathbf{n}$, the elements of which are i and j respectively.
 - (c) Calculate the speed of movement for all pairs of bacteria $\mathbf{v}_{mn} = v(x)(\mathbf{B}_L)_{mn}$, where $v(x)$ is given by (8.9).
 - (d) Calculate the unit vectors connecting the centres of bacteria $\mathbf{m}$ and $\mathbf{n}$, pointing towards bacteria $\mathbf{m}$:

$$\hat{\mathbf{u}}_{mn} = \frac{(\mathbf{x}_{B\mathbf{m}}^k - \mathbf{x}_{B\mathbf{n}}^k, \mathbf{y}_{B\mathbf{m}}^k - \mathbf{y}_{B\mathbf{n}}^k)}{(\mathbf{B}_L)_{mn}}. \quad (8.10)$$

- (e) Perform

$$(\mathbf{x}_{B\mathbf{m}}^{k+1}, \mathbf{y}_{B\mathbf{m}}^{k+1}) = (\mathbf{x}_{B\mathbf{m}}^k, \mathbf{y}_{B\mathbf{m}}^k) + 0.5\Delta t \mathbf{v}_{mn} \hat{\mathbf{u}}_{mn}, \quad (8.11a)$$

$$(\mathbf{x}_{B\mathbf{n}}^{k+1}, \mathbf{y}_{B\mathbf{n}}^{k+1}) = (\mathbf{x}_{B\mathbf{n}}^k, \mathbf{y}_{B\mathbf{n}}^k) - 0.5\Delta t \mathbf{v}_{mn} \hat{\mathbf{u}}_{mn}. \quad (8.11b)$$

- (f) Recalculate $\mathbf{B}$.
-

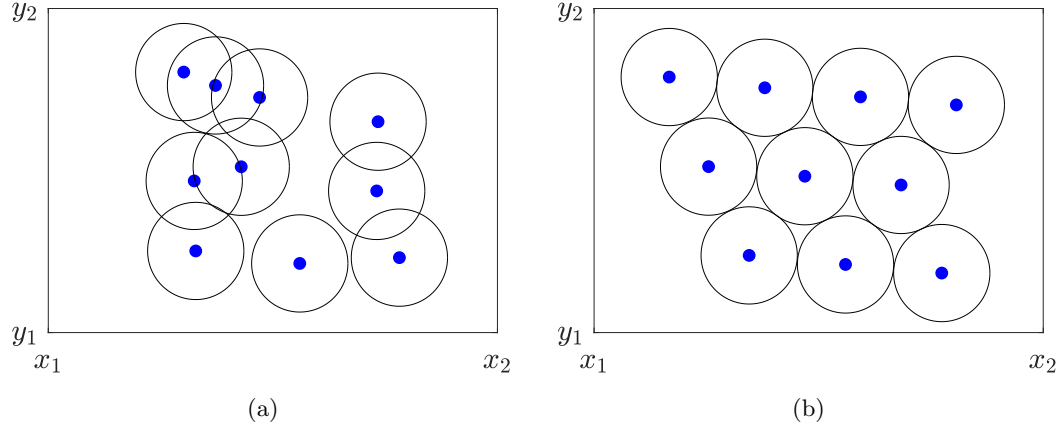


Figure 8.3: A simulation showing a group of bacteria (a) before and (b) after the biofilm forming algorithm has been repeatedly applied until equilibrium is reached. Blue filled circles represent the bacteria's centres and black circles represent the surfaces of the bacteria.

To implement (8.1), we discretise $\hat{\phi}(x, y, t)$ the same way inflammatory mediator presence and antibiotic concentration are discretised in the hybrid model (see section 5.3.1), the approximation to $\hat{\phi}(x_i, y_j, t_k)$ denoted $\hat{\phi}_{i,j}^k$. For bacterium m , the four enveloping mesh points are calculated at the current time point and are denoted (x_m^1, y_m^1) , (x_m^2, y_m^2) , (x_m^3, y_m^3) and (x_m^4, y_m^4) respectively. $\hat{\phi}_{i,j}^k$ is then given by:

$$\hat{\phi}_{i,j}^k = \sum_{m=1}^N \sum_{l=1}^4 \begin{cases} 1, & \text{for } (x_i - x_m^l)^2 + (y_j - y_m^l)^2 = 0, \\ 0, & \text{otherwise,} \end{cases} \quad (8.12)$$

where N is the number of bacteria. $\hat{\phi}_{i,j}^k$ counts how many bacteria have (x_i, y_j) as an enveloping mesh point at $t = t_k$. The corresponding discretisation of $\mathcal{D}_I$ and $\mathcal{D}_\alpha$ (see 8.2) is then given by:

$$\mathcal{D}_{I,i,j}^k = D_I \left(\frac{q_B^{n_B}}{q_B^{n_B} + (\hat{\phi}_{i,j}^k)^{n_B}} \right), \quad (8.13)$$

$$\mathcal{D}_{\alpha,i,j}^k = D_\alpha \left(\frac{q_B^{n_B}}{q_B^{n_B} + (\hat{\phi}_{i,j}^k)^{n_B}} \right). \quad (8.14)$$

The discretisation of $\mathbf{u}$ (see 8.4) is given by:

$$\mathbf{u}_{i,j}^k = \theta(y_j - y^*) \left(\frac{(y_j - y^*)}{\phi_u + (y_j - y^*)} \right) \left(\frac{q_B^{n_B}}{q_B^{n_B} + (\hat{\phi}_{i,j}^k)^{n_B}} \right) \hat{\mathbf{x}}, \quad (8.15)$$

that of $\mathcal{L}_{\text{mot}}$ (see 8.5) given by:

$$\mathcal{L}_{\text{mot},i,j}^k = L_{\text{mot}} \left(\frac{q_B^{n_B}}{q_B^{n_B} + \hat{\phi}_{i,j}^k} \right), \quad (8.16)$$

and that of (8.6) given by:

$$\mathcal{P}(\text{phag in } t \leq \Delta t) = \left(1 - (1 - Q)^k\right) \left(\frac{q_E^{n_E}}{q_E^{n_E} + \hat{\phi}_{i,j}^{n_E}}\right). \quad (8.17)$$

$P(x, y, t)$ and $A(x, y, t)$ are discretised as they are in the hybrid model (see section 5.3.1).

8.4 Results

We simulate the biofilm model and consider, over time, the infection size, the pro-inflammatory mediator concentration (specifically at $(|x| \leq 1, y)$ and $(|x| \leq 1, y = 0)$) and the internalized concentration of antibiotic. We run these simulations to find a relationship between the biofilm density and the success of the infection. In each experiment, we run the simulation 80 times and calculate mean solutions, giving us more accurate results. As discussed in section 8.2, the optimum antibiotic treatment strategy is repeated uniform strength doses; therefore, we employ this in the simulations that follow. We simulate the model in the absence and presence of antibiotic and/or biofilm¹. We assume that the virulence of the infection is positively correlated with the size of the infection, allowing us to infer predictions about the virulence based on changes in infection size. Due to the absence of relevant experimental data, the parameters that are present in the original hybrid model keep their original values, while new parameters are given values (through trial and error) that yield qualitatively sensible results.

The biofilm may not provide significant protection against the antibiotic: Figure 8.4(a) shows that the mean infection size when the antibiotic and biofilm are both absent (black line) is significantly higher than when the antibiotic and biofilm are both present (green line). Figure 8.4(d) shows that when the biofilm is present, the average internalized concentration of antibiotic is only slightly lower (from administration until $t \lesssim 8$), implying that either (1) the biofilm does not effectively prevent the transport of antibiotic into the biofilm or (2) the antibiotic can effectively attack the edges of the biofilm and work through to the centre. In either case, comparing the difference in infection size (with the biofilm present) between when the antibiotic is absent and present (Figure 8.4(a), blue and green lines respectively), we see that the biofilm does not provide significant protection against the antibiotic (at least in this parameter regime).

The biofilm increases the virulence of the infection, and is therefore an effective treatment target: In Figure 8.4(a), we see that for $t \gtrsim 5$, the mean infection size is higher when the biofilm is present (compare black curve with blue, and green curve with red), demonstrating that the biofilm likely contributes to the virulence of the infection. Since the biofilm does not provide significant protection against the antibiotic (in this parameter regime), it may provide significant protection against the immune response. We predicted in section 6.4 that the antibiotic needs to work in tandem with the immune response in order to be effective; therefore by providing protection against the immune response, the biofilm will provide protection against the antibiotic indirectly. Since the infection size is lowest when the antibiotic is present and the biofilm is absent (Figure 8.4(a), red

¹We remark that in the absence of biofilm, the model reduces to the hybrid model.

line), inhibiting the ability of EPEC to form a biofilm and applying aminoglycosides is an effective combination treatment strategy.

The biofilm unintentionally promotes the leukocyte response: Figure 8.4(b) shows that the mean pro-inflammatory mediator concentration close to the infection is higher when the biofilm is present (blue and green lines), due to the reduced diffusive flux in and out of the biofilm. Inflammation is lower when the antibiotic is present (black and red lines) as a consequence of the reduced infection size. The increased concentration of pro-inflammatory mediators strengthens the chemotactic attraction of leukocytes towards the bacteria. Figure 8.4(c) shows that the average pro-inflammatory mediator concentration across the endothelium close to the infection is also higher when the biofilm is present (blue and green lines). This is due to the reduced transport of the pro-inflammatory mediators produced at the epithelium into the biofilm. This leads to increased transport towards the endothelium, which then leads to faster leukocyte recruitment activation. These results suggest that the presence of a biofilm promotes the leukocyte response; however, the decrease in susceptibility to phagocytosis and antibiotics offered by the biofilm vastly outweighs the unintentional by-product of a stronger leukocyte response.

A biofilm affords comprehensive protection provided it can inhibit phagocytosis sufficiently well: The results in Figure 8.4 correspond to $q_E = 2$. We now set $q_E = 1$, making the biofilm more resistant to phagocytosis. Figure 8.5 shows that in this parameter regime, the infection size increases continually in both the absence and presence of antibiotic when the biofilm is present (blue and green lines respectively). This indicates that if a biofilm can inhibit phagocytosis sufficiently well, it can also withstand the adverse effects of the antibiotic and therefore survive. This result emphasises the importance of disabling EPEC's ability to form a biofilm.

8.5 Discussion

In this Chapter, we have adapted the hybrid model (presented in Chapter 5) to accommodate the formation of bacterial biofilms in EPEC infections, to explore the effects of biofilms on infection virulence and on the effectiveness of the immune response and antibacterial treatment. We model biofilms by assuming that bacteria in sufficiently close proximity aggregate such that there are no gaps or overlaps between neighbouring bacteria. To include the effects of biofilms, we introduced a biofilm density based on the proximity of bacteria to other bacteria. We assume the transport rates of inflammatory mediators and antibiotic, the motility rate of leukocytes and the probability of successful phagocytosis are biofilm density-dependent, resulting in impeded transport, movement and phagocytosis within biofilms. The inhibitions are implemented using Hill functions that exhibit switch-like behaviour, allowing each of the above mechanisms to be significantly inhibited when a critical biofilm density is attained. The developed biofilm model was then simulated to seek a relationship between biofilm density and infection success.

We showed that the infection is more virulent when it can form a biofilm and that the biofilm-afforded resistance to aminoglycosides is dependent on the ability of the biofilm to inhibit phagocytosis. Particularly strong biofilms can withstand both aminoglycoside treatment and the leukocyte

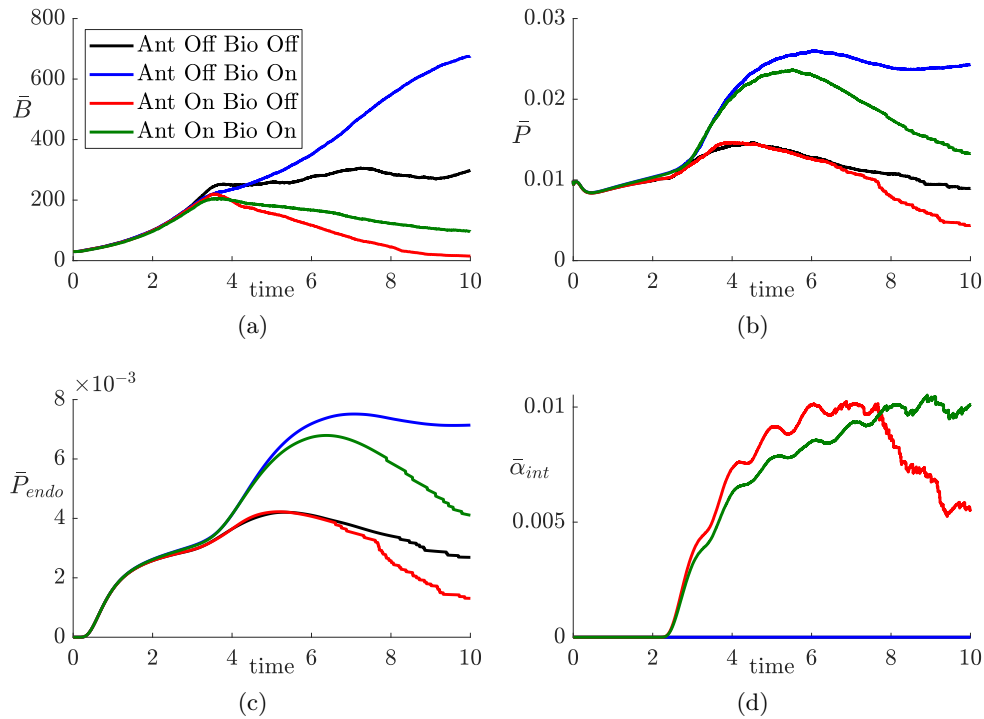


Figure 8.4: The mean (a) population of bacteria over time, (b) concentration of pro-inflammatory mediators over ($|x| \leq 1, y$), (c) concentration of pro-inflammatory mediators over the endothelium, ($|x| \leq 1, y = 0$), and (d) concentration of internalized antibiotic, for each combination of antibiotic (Ant) and biofilm (Bio) presence and absence. Each graph is the mean of 80 simulations and all other parameters take their baseline values (given in Appendix A.9).

response, making eradication via aminoglycoside therapy alone ineffective. The amount of aminoglycoside internalized by bacteria is only slightly reduced when in a biofilm, demonstrating that the biofilm-afforded inhibition of leukocyte movement and phagocytosis is of importance to the infection. We also showed that due to the reduced transport of pro-inflammatory mediators through the biofilm, there is increased transport towards the endothelium, leading to faster leukocyte recruitment activation. However, the decrease in susceptibility to phagocytosis and aminoglycosides offered by the biofilm vastly outweighs the unintentional by-product of a stronger leukocyte response. Our results emphasize the importance of disabling the ability of EPEC to form biofilms and predict that combining this with an aminoglycoside treatment effectively eradicates the infection.

The model we have developed is simple and therefore has limitations. The ECM is modelled as a homogeneous substance for simplicity, therefore the non-uniform density of biofilms, due to the varying densities of the constituents of the ECM, is not accounted for. In quorum sensing, bacteria use secreted signalling molecules to alter gene expression in a density-dependent manner. Biologically, biofilm formation and growth are stimulated and controlled by quorum sensing, whereas in our model, formation and growth are simply dependent on the density of bacteria. Detailed modelling of quorum sensing (e.g. in [116, 38]) will make our biofilm model more biologically realistic and will potentially highlight quorum-sensing related treatment strategies. Physiologically, the

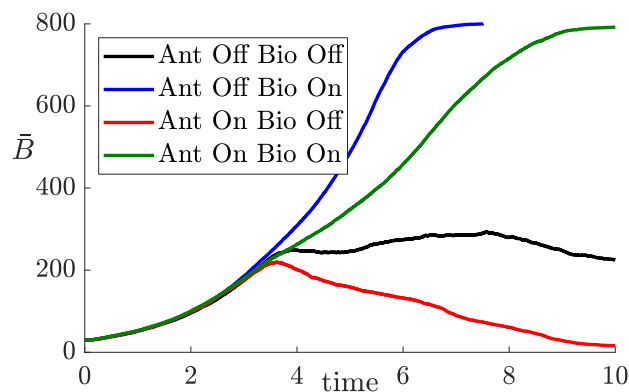


Figure 8.5: The mean population of bacteria over time for each combination of antibiotic (Ant) and biofilm (Bio) presence and absence. Each graph is the mean of 80 simulations and all other parameters are identical to those in Figure 8.4, except that $q_E = 1$ instead of 2.

bacteria towards the centres of biofilms often display minimized metabolic activity, giving rise to antibiotic ineffectiveness as well as reduced replication and phagocytosis. Detailed modelling of the non-uniform metabolic activity of biofilm-bound bacteria may shed more light on how to effectively treat biofilm-related diseases.

Chapter 9

Conclusions and further work

9.1 Preamble

In this thesis, we have considered various mathematical models for pathogen-host dynamics, with a focus on EPEC infections. The motivation for this work was the high number of global EPEC-related deaths (approximately 800,000 annually) combined with the lack of a comprehensive understanding of the infection strategy. Furthermore, there are, to our current knowledge, no existing mathematical models specific to the infection strategy of EPEC, allowing us to undertake novel mathematical research on the topic. The goal of this thesis was to develop and analyse models to predict clinically feasible treatment strategies that will eradicate EPEC infections. Due to the unavailability of experimental data, we are currently unable to perform a comparison between our results and those from the literature, making this thesis a theoretical study.

In the main body of this chapter, we briefly recap the models we have developed and the results they yielded. We then discuss the implications of our results by highlighting possible clinical treatments, while considering their advantages and drawbacks. To conclude this chapter, we consider possible extensions of the work presented here, both experimentally and mathematically.

9.2 Models and results

In Chapter 2, we presented a literature review of the known mechanisms involved in EPEC infections, in particular the inhibitory action of secreted effector proteins. We also reviewed the innate immune response, bacteria protein synthesis, the actions of various classes of antibiotic and the formation and consequential effects of bacterial biofilms. We discussed the recent experimental findings by Easton et al. at the Centre for Biomolecular Sciences (CBS), University of Nottingham; that EPEC internalize host pro-inflammatory cytokines, reducing their sensitivity to aminoglycosides (unpublished). Together with experimental collaborators, we hypothesized that the internalization of host cytokines alters the secretion rates of effector proteins. We reviewed several mathematical models from the literature that describe the above mechanisms and we used them, together with the biological literature and the above experimental findings, to inform the formulation and architecture of our models.

Throughout this thesis, we make the biologically realistic assumption that the background production of pro-inflammatory mediators is much smaller in magnitude than the stimulated production in an otherwise healthy person. In Chapter 3, we develop a simple ODE population-level model for a general bacterial infection and its interaction with the evoked immune response. We explored this model analytically, by considering the steady states and their stability; and numerically, by considering parameter sensitivity analysis, bifurcation analysis and simulation. We found that there are two clinically feasible steady states — the disease-free state and the diseased state. We also found that only one of these states is stable, the disease-free state being stable when the bacteria cannot grow sufficiently fast to overcome the immune response. The two steady states exchange stability at a transcritical bifurcation when any of: (1) the bacterial growth rate; (2) the degradation rate of pro-inflammatory mediators; (3) the recruitment rate of leukocytes or (4) the death rate of leukocytes pass a critical value. The absence of bi-stability suggests that for the eradication of an otherwise potent infection to occur, the above parameter values must be altered. We make the assumption that the infection is eradicated if it reaches a sufficiently low density, due to the continuous ODE representation of the infection breaking down. Under this assumption, sufficiently increasing the stimulated production rate of pro-inflammatory mediators eradicates the infection. A two-parameter sensitivity analysis showed that: (1) simultaneously decreasing the growth rate of bacteria and the degradation rate of either pro-inflammatory mediators or leukocytes or (2) simultaneously increasing the attraction rate of leukocytes and the stimulated production rate of pro-inflammatory mediators leads to a significant reduction in the pathogen size, the latter highlighting the synergy between the inflammatory response and the leukocyte response.

We then developed in Chapter 4 an ODE model describing an EPEC-specific infection, based on the literature and the recent experimental findings discussed earlier. This ODE model uses the model developed in Chapter 3 as a foundation and couples the cell-scale dynamics between EPEC, the immune response and aminoglycoside therapy with sub-cellular bacterial protein synthesis. This model also makes a distinction between inflammation-stimulated and necrosis-driven production of pro-inflammatory mediators. We analyse this model in a similar fashion to the general infection model, under the assumption that the infection is eradicated if it reaches a sufficiently low density. We showed that there are at least two clinically feasible steady states — the disease-free state and diseased state(s). We did not observe the existence of multiple diseased states for physiologically realistic parameter values; however, we were unable to prove this analytically. We were able to show analytically that the net strength of the inflammation-stimulated immune response must out-compete the net growth of the bacteria for the infection to be eradicated. Interestingly, this condition is equivalent to the analytic condition derived for the general infection model, demonstrating that targeting the EPEC-specific mechanisms does not influence the eradication of the infection. The disease-free state exchanges stability with a diseased state at a transcritical bifurcation when any of the bacterial growth or death rates or the leukocyte recruitment or death rates pass a critical value. We showed via bifurcation analysis and simulation that inhibiting the effector proteins that inhibit phagocytosis (E_3) decreases the infection size significantly. Inhibiting the effector proteins that inhibit pro-inflammatory signalling (E_1) noticeably reduces the infection size, while inhibiting those that inhibit apoptosis (E_2) makes negligible difference to the size of infection. Sufficiently

reducing the rate of internalization of pro-inflammatory mediators by bacteria also reduces the infection size significantly. We were able to show via numerical simulation that decreasing the synthesis rate of bacterial mRNA is as effective at reducing the infection size as decreasing the growth rate of bacteria. We also predict that: (1) increasing the sensitivity of bacteria to aminoglycosides; (2) increasing the maximum attainable permeability of bacterial membranes (through the insertion of incorrect structural proteins) and (3) increasing the longevity of aminoglycoside molecules reduce the infection size significantly. We showed that administering aminoglycosides earlier leads to a reduction in the infection size and that it is more effective to strongly target two aspects of the infection than to weakly target three.

To model the spatial and individual cell dynamics of an EPEC infection more realistically, in Chapter 5 we coupled a volume-excluding agent-based representation of bacterium, leukocyte and effector protein behaviour, with a continuum description of the inflammatory and antibiotic responses. In particular, this hybrid model includes: (1) a stochastic sub-cellular gene regulatory network for bacterial protein synthesis and replication; (2) reaction-diffusion PDEs for the production, degradation and transport of inflammatory mediators and antibiotic and (3) leukocyte recruitment to the infection site in response to inflammatory status via a chemotactically-biased random walk. We simulated the model and recorded the relevant variables over time. We averaged the data from many simulations to obtain more accurate results, the results presented in Chapter 6. We found that, in the absence of antibiotic: (1) inhibiting the production of bacterial structural proteins; (2) increasing the longevity or the attraction rate of leukocytes and (3) inhibiting the production or action of E_1 all reduce the infection size significantly. An interesting finding is the difference in effectiveness of inhibiting the production or action of E_3 between the ODE model and the hybrid model. While the ODE model predicts a monotonically increasing relationship between the production rate of E_3 and the size of the infection, the hybrid model predicts that an intermediate production rate gives the optimum infection size. Despite this, the percentage of infections that are eradicated increases as the production rate of E_3 decreases. This discrepancy between the two models suggests that the spatial dependence of many mechanisms and cell-cell interactions is significant regarding the effectiveness of E_3 . The ODE and hybrid models are in agreement that reducing the growth rate of bacteria, increasing the longevity of leukocytes and inhibiting E_1 reduce the infection size significantly and that inhibiting E_2 has negligible effect. Another interesting finding is that the trend in the percentage of infections that are eradicated as the production rates of structural proteins and E_1 are varied is switch-like, resembling a transcritical bifurcation. This implies that if the natural value for either parameter is close to the switch value, then many infections that would survive without intervention can be eradicated with a small reduction in that parameter. We demonstrated that the spatial profile of the infection affects its survivability, with infections that are arranged into several populous clusters that are narrow at the interface of the epithelium being the most likely to survive. We inferred from our results that, given a prescribed amount of aminoglycoside, the administration strategy that gives the highest chance of infection eradication, as well as the lowest peak infection size, is one strong initial dose followed by several weaker, uniform strength, evenly spaced doses. The hybrid model also predicts that continuous infusion is less effective than any dose-based strategy and that, despite resulting in a higher peak infection size, a delayed treatment is more likely to clear the infection. This last finding contrasts

that of the ODE model, which predicts that the effectiveness of the treatment decreases as the delay increases. This result discrepancy is due to the spatial and individual cell dependence of the hybrid model, features that are absent from the ODE model.

In Chapter 7, we developed a PDE model to explore in detail the dynamics between pro-inflammatory mediator production by leukocytes and the chemotactic attraction of leukocytes. We analysed this model numerically via simulations, bifurcation analysis and solving the boundary value problem (BVP) for the steady state profiles. We find for certain parameter regimes that the concentrations of pro-inflammatory mediators and leukocytes diverge to infinity, suggesting model instability. We explored the stability of the model analytically by assuming that the perturbations can be written as Fourier series but; due to time constraints, we were unable to find an explanation for the observed instability.

In Chapter 8, we adapted the hybrid model to simply model EPEC biofilms. We formulated a biofilm-forming algorithm and considered the effects of biofilms on infection virulence and on the effectiveness of the immune response and antibiotic treatment. We assume that the virulence of the infection is positively correlated with the size of the infection, allowing us to infer predictions about the virulence based on changes in infection size. Via numerical simulation, we showed that the infection is more virulent when it is able to form a biofilm and that particularly strong biofilms are able to simultaneously withstand the immune response and a course of aminoglycosides. Finally, we demonstrated that the biofilm-afforded reduction in leukocyte mobility and protection against phagocytosis are particularly important for infection survivability.

9.3 Treatment strategies

Most effective treatments: Together, our results predict that the two most effective treatment strategies are reducing the net growth rate of bacteria and reducing the bacterial mRNA synthesis rate. Bacteriostatic and bactericidal antibiotics reduce the growth rate and increase the death rate of bacteria respectively and inhibiting the production of bacterial structural proteins is an effective bacteriostatic strategy. A treatment that can sequester locally free nutrients, particularly sugars, phosphates and nitrogen, will effectively shut down mRNA synthesis. The effects of this treatment on the host have not been considered, but if it is clinically shown that there are no serious detrimental effects, then we predict the treatment to be very effective.

Immune-boosting treatments: Treatments that can sufficiently increase the lifespan of leukocytes or pro-inflammatory mediators, or increase the sensitivity of leukocyte attraction or recruitment activation to the presence of pro-inflammatory mediators are predicted to be effective. We have not considered the clinical feasibility of these immune-boosting methods, therefore exploring methods for their implementation experimentally, considering effectiveness and ease/expense of application, will be beneficial. Treatments that boost the immune response must be carefully planned and monitored to ensure that the immune response does not enter a state of overdrive, as this could lead to aseptic health problems. As examples, a hyperactive inflammatory response can lead to arthritis and hepatitis, while a hyperactive leukocyte response can lead to leukaemia and leukosta-

sis (a condition that can lead to respiratory failure). Ensuring that the effects of immune-boosting treatments are temporary will reduce the risk of complications such as these. We therefore predict that combining a mild immune-boosting treatment with an aggressive bacteriostatic or bactericidal antibiotic therapy, or an mRNA synthesis inhibition therapy, will not only be more effective than any individual treatment, but will be safer than an aggressive immune-boosting treatment.

Effector protein inhibition: Since inhibiting the production or action of E_1 or E_3 has been shown to reduce the infection size significantly, we suggest that clinicians combine these treatments with a more direct treatment, such as an antibiotic or mRNA synthesis inhibition. Furthermore, since some infections that would survive without intervention can be eradicated with a mild treatment of a drug that inhibits the synthesis or action of E_1 or the synthesis of structural proteins, these treatments will give, in these cases, a very high benefit to cost/risk ratio; making it an appealing choice for clinical use. As with immune-boosting strategies, the clinical feasibility of effector protein-inhibiting treatments is not known. An interesting experimental study would be comparing the effectiveness and ease/expense of application of immune-boosting and effector protein-inhibiting therapies to see which are the most beneficial accessory treatments regarding safety and cost-effectiveness. We predict that effector protein-inhibiting therapies will be safer, since immune-boosting therapies can lead to auto-immune diseases.

Antibiotic treatments: We predict that antibiotic treatments are most effective when administered regularly, with an initial strong dose, followed by weaker doses. This result is in keeping with other mathematical studies [153, 154]. Importantly, our prediction is also in agreement with clinical practice; a significant proportion of antibiotics are indeed delivered clinically in a course of doses. Possible alternatives to inhibiting protein synthesis are to render secreted proteins ineffective or break them down. If feasible, these strategies are appealing as the bacteria still produce the proteins, draining their nutrient/energy supplies, thus making the infection less virulent. Experimental exploration into the feasibility of these strategies will therefore potentially lead to novel antibacterial strategies. We showed that diagnosing the infection sooner leads to a more significant reduction in the infection size; however, this strategy will be infeasible if the physical symptoms that lead to correct diagnosis are not yet present. Once an EPEC infection is suspected, correct diagnosis and treatment should be undertaken as soon as possible. Our research predicts that the deliberate delaying of an antibiotic treatment improves the effectiveness of the treatment at eradicating an infection, as it allows the antibiotic and the immune response to act at their peak strengths in synergy. This finding is in both agreement [86] and disagreement [84, 154] with the mathematical literature. A longer delay results in the peak infection size being higher, potentially fatally high in many cases. Furthermore, there is no clinical evidence that delaying an antibiotic treatment improves the effectiveness of the treatment; therefore, we do not put confidence in this prediction. Finally, clinical care needs to be taken with high doses of antibiotics to reduce the risk of infection complications, such as secondary infection due to an imbalance in the gut flora.

Biofilm inhibition: Disabling the ability of EPEC to form biofilms was shown to be an effective strategy and can be achieved by inhibiting molecules that are pivotal in bacterium-bacterium communication, such as quorum-sensing autoinducers. Recent experimental research has shown

promising findings in the development of autoinducer inhibitors [158], demonstrating the clinical feasibility of this strategy.

Other treatments: An increase in the sensitivity of bacteria to aminoglycosides and in the maximum attainable permeability of bacterial membranes can be achieved if it is possible to create a higher affinity for the binding of aminoglycoside molecules to bacterial ribosomes and force incorrect bacterial structural proteins to fold into shapes that span a greater volume respectively; however, we have not considered the clinical feasibility of these treatment strategies. Another strategy that may be clinically feasible is the development of a drug that prevents the internalization of pro-inflammatory mediators by EPEC. We predict that simply sequestering pro-inflammatory mediators will not be sufficient, however, as this will make leukocyte recruitment and attraction less effective.

Treatment combinations: In addition to being more likely to eradicate infections and safer, we predict that treatment combinations will be more effective long-term since they are less likely to promote the spread of resistance genes, due to the reduced emphasis on directly attacking the bacteria. Being effective long-term is an invaluable quality given the reducing effectiveness of current antibiotics and the low number of novel antibiotic types being produced. The most effective treatment combination for a patient may depend on individual host factors such as the patient's immune response strength and the virulence of their particular strain, therefore an informed decision may need to be taken in each case in clinical practice. Strongly targeting more than two aspects of the infection will typically be unnecessary and expensive, but may be necessary for patients who have a particularly virulent strain of EPEC, a weak or compromised immune response or other medical complications.

9.4 Further work

The research presented in this thesis has opened many avenues of further research, both experimental and theoretical.

Revisions of current model implementations: The most effective revision for our current models is to fit them to the relevant experimental data, when it becomes available. This will allow comparisons between our models to be more reliable and predictions made by our models to be more valid. Should data become available on the relationships between effector protein production and infection size and inflammation, then the production rates of effector proteins can be modelled more realistically. Accounting for limiting factors, for example by including a carrying capacity, will allow the replication of bacteria in the hybrid and biofilm models to be more physiologically realistic. In the hybrid model, degradation of E_1 and E_2 is considered for each IEC individually, whereas degradation of E_3 is considered globally for simplicity. Revising this implementation so that the degradation rates of all effector protein groups are comparable for all protein densities will improve the validity of the model, especially at large infection sizes. In its current state, the hybrid model is computationally expensive for large numbers of bacteria, due to a Gillespie algorithm being executed for each individual bacterium. Improving the efficiency of the implemented Gillespie algorithm, such as by implementing a variable time step (tau-leaping) [122], will reduce

the computational expense of the model, allowing detailed analyses to be undertaken faster.

More detailed modelling: In the analysis of the biofilm model, it was shown that, in a cluster of bacteria, the inner bacteria are safer from phagocytosis due to the vastly restricted mobility of leukocytes. This effect can be encapsulated in the ODE models by making the phagocytosis term a non-linear function of the infection size (under the assumption that the bacteria always form a biofilm). An alternative method is to split the bacteria population into two phenotypes — ordinary bacteria and persister cells, where the rate of phagocytosis is significantly smaller for persister cells. Biologically, biofilm formation and growth are stimulated and controlled by quorum sensing molecules (autoinducers), whereas in our model, formation and growth are simply dependent on the density of bacteria. The formation of biofilms can therefore be modelled in more detail by including autoinducer density and making the distinction between ordinary and biofilm-bound bacteria.

Identifying the impact of pro-inflammatory mediator production by leukocytes on leukocyte chemotaxis will allow the implementation of both mechanisms in the hybrid and biofilm models. This will make the models more biologically realistic and may highlight novel treatments regarding leukocyte behaviour or inform changes to the already elucidated strategies. This can be achieved by thoroughly investigating the stability of the PDE model presented in Chapter 7, in particular, the apparent lack of global stability. We seek parameter regimes where the model is stable analytically, and to understand why the model becomes unstable. It would then be of theoretical and biological interest to relate predictions made by this model to those made by the hybrid model (Chapter 6).

Experimental studies: Many experimental studies that will be informative in the elucidation of novel treatment strategies have been highlighted in the previous section. In particular, an interesting experimental study would be comparing the effectiveness and ease/expense of application of immune-boosting and effector protein-inhibiting therapies to see which are the most beneficial accessory treatment strategies. We also expect that comparing the effects of drugs that inhibit production against those that inhibit action will be informative. The discovery of a drug that can sequester locally free nutrients without harming the host should be undertaken, as this is predicted by our research to be one of the most effective strategies. We suggest that the finding of an optimal production rate of E_3 be explored in experimental detail as, if true, it will have important consequences for treatments that inhibit the production of these proteins. Continued active research into the inhibition of quorum sensing molecules will potentially unlock new effective therapies; therapies that are thought to lead to drug resistance at a much slower rate than typical antibiotics due to their indirect approach to inhibition.

Other treatment strategies: In this thesis, we have considered various treatment strategies, including antibiotic, immune-boosting and virulence-inhibiting strategies; however, there are numerous other external treatment strategies that can be considered. Hussey et al. [159] show that *Streptococcus pneumoniae* and *Staphylococcus aureus* can have their biofilm structures altered and thus their susceptibility to antibiotics altered, both positively and negatively, in the presence of the air pollutant black carbon. This has numerous consequences, particularly on the treatment of existing infections and the spreading of infections to other parts of the body (notably the lungs). To our current

knowledge, this has yet to be modelled mathematically with specificity to bacterial infections.

An alternative, albeit quite controversial, treatment method is phage therapy; a popular therapy in Georgia and Russia [160]. It involves administering bacteriophages (phages) — a type of virus that only infects particular species of bacteria. Once a phage has attached to and injected its genetic material into a bacterium, the phage reproduces via two different cycles — the lytic cycle and the lysogenic cycle. In the lytic cycle, the phage uses the bacterium's metabolic machinery to create a new generation of phages. The phages then burst out of the bacterium via lysis and then repeat the cycle in other bacteria. In the lysogenic cycle, the phage's DNA integrates itself into the bacterium's DNA, causing the bacterium to inadvertently replicate the phage's DNA over many generations. Certain stress conditions then lead to the phage undergoing the lytic cycle [161, 162, 160].

While phage therapy is reputed in the countries where it is used, it is not well used in the Western world. This is due to antibiotics having a much broader spectrum and also possibly due simply to the negative public perception of viruses [163]. Also, due to phages being bacterium-specific, it becomes economically necessary to use phage combinations (cocktails). This has several consequences, notably the costs of creating, maintaining and storing these cocktails; however, in the absence of novel antibiotics, phage therapy is a possible solution. It is already well studied mathematically [164, 165], therefore the available models can be adapted and applied to EPEC-specific infections to study whether phage therapy would be a viable strategy.

To conclude, we believe that the work presented in this thesis gives a thorough insight into the mathematical modelling of EPEC-specific infections, as well as a plethora of possible effective treatment strategies for combating these infections. We are hopeful that our predictions will correctly inform future experimental work in the search for clinically effective treatments for EPEC infections.

Appendix A

Appendix

A.1 Showing that equation (3.10) only has one root with $p > 0$:

We first rewrite equation (3.10) in the following form:

$$p \left(1 + \left(\frac{r_a}{d_a} \right)^2 \frac{p^4}{(1 + p^2)^2} \right) = \frac{\phi}{d_p} + \frac{c_b}{d_p} \left(1 + \frac{vr_l}{d_l} p \right) \left(\frac{(r_b - 1) - \frac{r_l}{d_l} p}{\frac{r_l}{d_l} p + 1} \right). \quad (\text{A.1})$$

The left hand side (LHS) of (A.1) increases monotonically from 0 with p (varying linearly for $p \ll 1$ and $p \gg 1$). Conversely, the right hand side (RHS) is positive at $p = 0$ and decays linearly with p for large p . The above arguments hold since all parameters are positive and $r_b > 1$ in the diseased state. To make the equation easier to deal with, the following groupings are made:

$$A = \frac{r_l}{d_l}, \quad C = (r_b - 1), \quad D = \frac{\phi}{d_p}, \quad E = \frac{c_b}{d_p}.$$

We now consider the gradient of the RHS:

$$\begin{aligned} \frac{d\text{RHS}}{dp} &= \frac{d}{dp} \left[D + E (1 + Avp) \left(\frac{C - Ap}{Ap + 1} \right) \right], \\ &= \frac{d}{dp} E \left(\frac{C + A(Cv - 1)p - A^2vp^2}{Ap + 1} \right), \\ &= E \frac{(Ap + 1)(A(Cv - 1) - 2A^2vp) - A(C + A(Cv - 1)p - A^2vp^2)}{(Ap + 1)^2}. \end{aligned}$$

The denominator is always positive. Define $f(p)$ as the numerator after taking out a factor of AE :

$$f(p) = (Ap + 1)(Cv - 1 - 2Avp) - (C + A(Cv - 1)p - A^2vp^2)$$

The $A(Cv - 1)p$ terms cancel, leaving:

$$\begin{aligned} f(p) &= (Cv - 1) - 2A^2vp^2 - 2Avp - C + A^2vp^2, \\ &= (Cv - 1) - C - 2Avp - A^2vp^2, \\ &= G - 2Avp - A^2vp^2. \end{aligned} \tag{A.2}$$

Where $G = (Cv - 1) - C = (r_b - 1)v - r_b$. Equation (A.2) is an 'n' shaped parabola. If $G \leq 0$, then for $p > 0$, $f(p)$ is always negative, meaning that the gradient of the RHS is always negative and therefore the RHS of equation (A.1) is monotonically decreasing. This therefore means that there can only be one intersection between the LHS (which is monotonically increasing and has a monotonically increasing gradient) and the RHS. Figure A.1 (a) shows an example of this.

For $G > 0$, then there will be two stationary points defined by $A^2vp^2 + 2Avp - G = 0$. Using the quadratic formula:

$$\begin{aligned} p &= \frac{-2Av \pm \sqrt{4A^2v^2 + 4A^2vG}}{2A^2v}, \\ &= \frac{-1 \pm \sqrt{1 + Gv^{-1}}}{A}. \end{aligned}$$

Since $A, G, v > 0$, it can be seen that there is one positive root and one negative root. Therefore the RHS of equation (A.1) will have exactly one turning point with positive p , along with a positive value for $p = 0$. Since the function becomes negative and linear in p as $p \rightarrow \infty$ (see main body of text), the gradient must remain negative after this turning point¹. This, together with the properties of the LHS discussed earlier, implies that there is exactly one intersection between the LHS and the RHS. Figure A.1 (b) shows an example of this.

¹This implies that the turning point must be either a local maximum or a point of inflexion

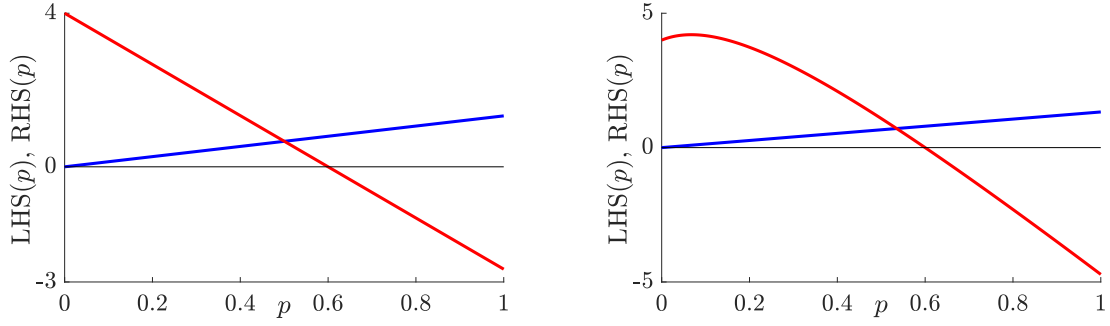


Figure A.1: Example graphs for the LHS (blue) and the RHS (red) of equation (3.10) for **(a)** $G = (r_b - 1)v - r_b \leq 0$ and **(b)** $G > 0$.

A.2 EPEC ODE model — Jacobian elements

$$J_{11} = \frac{r_b(1 + (1 - \lambda)b_2)}{(1 + B)^2} - qp - l(1 + e_3)^{-1} - d_b, \quad J_{12} = \frac{r_b(\lambda(1 + b_1) - b_1)}{(1 + B)^2}, \quad J_{13} = -qb_1,$$

$$J_{14} = 0, \quad J_{15} = -b_1(1 + e_3)^{-1}, \quad J_{16} = 0, \quad J_{17} = 0, \quad J_{18} = lb_1(1 + e_3)^{-2}, \quad J_{19} = 0,$$

$$J_{21} = -\frac{(1 - \lambda)r_b b_2}{(1 + B)^2} + qp, \quad J_{22} = \frac{(1 - \lambda)r_b(1 + b_1)}{(1 + B)^2} - l(1 + e_3)^{-1} - d_b, \quad J_{23} = qb_1,$$

$$J_{24} = 0, \quad J_{25} = -b_2(1 + e_3)^{-1}, \quad J_{26} = 0, \quad J_{27} = 0, \quad J_{28} = lb_2(1 + e_3)^{-2}, \quad J_{29} = 0,$$

$$J_{31} = c_b(1 + vl)(1 + a^2)^{-1}(1 + e_1)^{-1} - qu_{bp}p, \quad J_{32} = c_b(1 + vl)(1 + a^2)^{-1}(1 + e_1)^{-1},$$

$$J_{33} = -(qu_{bp}b_1 + 1), \quad J_{34} = -\left[\phi + c_b(1 + vl)B\right] \frac{2a}{(1 + a^2)^2}(1 + e_1)^{-1},$$

$$J_{35} = c_b v B(1 + a^2)^{-1}(1 + e_1)^{-1}, \quad J_{36} = -\left[\phi + c_b(1 + vl)B\right] (1 + a^2)^{-1}(1 + e_1)^{-2},$$

$$J_{37} = 0, \quad J_{38} = 0, \quad J_{39} = s_n,$$

$$J_{41} = 0, \quad J_{42} = 0, \quad J_{43} = \frac{2r_a p}{(1 + p^2)^2}, \quad J_{44} = -d_a,$$

$$J_{45} = 0, \quad J_{46} = 0, \quad J_{47} = 0, \quad J_{48} = 0, \quad J_{49} = 0,$$

$$J_{51} = s_\alpha(1 + e_2)^{-1}, \quad J_{52} = J_{51}, \quad J_{53} = r_l, \quad J_{54} = 0, \quad J_{55} = -d_l,$$

$$J_{56} = 0, \quad J_{57} = -s_\alpha B(1 + e_2)^{-2}, \quad J_{58} = 0, \quad J_{59} = 0,$$

$$\begin{aligned}
J_{61} &= c_{11}, & J_{62} &= c_{12}, & J_{63} &= 0, & J_{64} &= 0, \\
J_{65} &= 0, & J_{66} &= -d_{e1}, & J_{67} &= 0, & J_{68} &= 0, & J_{69} &= 0,
\end{aligned}$$

$$\begin{aligned}
J_{71} &= c_{21}, & J_{72} &= c_{22} \frac{\psi_{y1}^2}{\psi_{y1}^2 + p^2} \frac{\psi_{y2}^2 - b_2^2}{(\psi_{y2}^2 + b_2^2)^2}, & J_{73} &= c_{22} \frac{-2\psi_{y1}^2 p}{(\psi_{y1}^2 + p^2)^2} \frac{b_2}{\psi_{y2}^2 + b_2^2}, & J_{74} &= 0, \\
J_{75} &= 0, & J_{76} &= 0, & J_{77} &= -d_{e2}, & J_{78} &= 0, & J_{79} &= 0,
\end{aligned}$$

$$\begin{aligned}
J_{81} &= c_{31}, & J_{82} &= \frac{c_{32}p}{\psi_z + p}, & J_{83} &= \frac{c_{32}\psi_z}{(\psi_z + p)^2} b_2, & J_{84} &= 0, \\
J_{85} &= 0, & J_{86} &= 0, & J_{87} &= 0, & J_{88} &= -d_{e3}, & J_{89} &= 0,
\end{aligned}$$

$$\begin{aligned}
J_{91} &= e_2^2(\psi_n^2 + e_2^2)^{-1}, & J_{92} &= J_{91}, & J_{93} &= 0, & J_{94} &= 0, \\
J_{95} &= 0, & J_{96} &= 0, & J_{97} &= 0, & J_{98} &= 0, & J_{99} &= -d_n.
\end{aligned}$$

A.3 EPEC ODE model — reducing the Jacobian matrix at the disease free state

The Jacobian of system (4.10) is given by:

$$\mathbf{J} = \begin{bmatrix} J_{11} & J_{12} & J_{13} & 0 & J_{15} & 0 & 0 & J_{18} & 0 \\ J_{21} & J_{22} & J_{23} & 0 & J_{25} & 0 & 0 & J_{28} & 0 \\ J_{31} & J_{32} & J_{33} & J_{34} & J_{35} & J_{36} & 0 & 0 & J_{39} \\ 0 & 0 & J_{43} & J_{44} & 0 & 0 & 0 & 0 & 0 \\ J_{51} & J_{52} & J_{53} & 0 & J_{55} & 0 & J_{57} & 0 & 0 \\ J_{61} & J_{62} & 0 & 0 & 0 & J_{66} & 0 & 0 & 0 \\ J_{71} & J_{72} & J_{73} & 0 & 0 & 0 & J_{77} & 0 & 0 \\ 0 & J_{82} & J_{83} & 0 & 0 & 0 & 0 & J_{88} & 0 \\ J_{91} & J_{92} & 0 & 0 & 0 & 0 & 0 & 0 & J_{99} \end{bmatrix}. \quad (\text{A.3})$$

Evaluating $\det[\mathbf{J} - s\mathbf{I}]$ at the disease free state gives:

$$\begin{vmatrix} J_{11} - s & J_{12} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ J_{21} & J_{22} - s & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ J_{31} & J_{32} & J_{33} - s & J_{34} & 0 & J_{36} & 0 & 0 & J_{39} \\ 0 & 0 & J_{43} & J_{44} - s & 0 & 0 & 0 & 0 & 0 \\ J_{51} & J_{52} & J_{53} & 0 & J_{55} - s & 0 & 0 & 0 & 0 \\ J_{61} & J_{62} & 0 & 0 & 0 & J_{66} - s & 0 & 0 & 0 \\ J_{71} & J_{72} & 0 & 0 & 0 & 0 & J_{77} - s & 0 & 0 \\ 0 & J_{82} & 0 & 0 & 0 & 0 & 0 & J_{88} - s & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & J_{99} - s \end{vmatrix}. \quad (\text{A.4})$$

Since column 5 has only one non-zero entry in it, (A.4) can be written as $(J_{55} - s)$ multiplied by the minor determinant obtained when the 5th row and 5th column are removed. The same operation can be applied to columns 7 and 8 sequentially, giving:

$$(J_{55} - s)(J_{77} - s)(J_{88} - s) \begin{vmatrix} J_{11} - s & J_{12} & 0 & 0 & 0 & 0 \\ J_{21} & J_{22} - s & 0 & 0 & 0 & 0 \\ J_{31} & J_{32} & J_{33} - s & J_{34} & J_{36} & J_{39} \\ 0 & 0 & J_{43} & J_{44} - s & 0 & 0 \\ J_{61} & J_{62} & 0 & 0 & J_{66} - s & 0 \\ 0 & 0 & 0 & 0 & 0 & J_{99} - s \end{vmatrix}$$

Similarly, the 6th row of this reduced matrix has only one entry and so can be further reduced to:

$$(J_{55} - s)(J_{77} - s)(J_{88} - s)(J_{99} - s) \begin{vmatrix} J_{11} - s & J_{12} & 0 & 0 & 0 \\ J_{21} & J_{22} - s & 0 & 0 & 0 \\ J_{31} & J_{32} & J_{33} - s & J_{34} & J_{36} \\ 0 & 0 & J_{43} & J_{44} - s & 0 \\ J_{61} & J_{62} & 0 & 0 & J_{66} - s \end{vmatrix}.$$

Setting this equal to zero immediately gives:

$$s_5 = J_{55} = -d_l, \quad s_7 = J_{77} = -d_{e2}, \quad s_8 = J_{88} = -d_{e3}, \quad s_9 = J_{99} = -d_n.$$

To calculate the remaining eigenvalues, the remaining determinant is expanded as follows:

$$\begin{vmatrix} J_{11} - s & J_{12} & 0 & 0 & 0 \\ J_{21} & J_{22} - s & 0 & 0 & 0 \\ J_{31} & J_{32} & J_{33} - s & J_{34} & J_{36} \\ 0 & 0 & J_{43} & J_{44} - s & 0 \\ J_{61} & J_{62} & 0 & 0 & J_{66} - s \end{vmatrix} = 0,$$

$$(J_{11} - s) \begin{vmatrix} J_{22} - s & 0 & 0 & 0 \\ J_{32} & J_{33} - s & J_{34} & J_{36} \\ 0 & J_{43} & J_{44} - s & 0 \\ J_{62} & 0 & 0 & J_{66} - s \end{vmatrix} - J_{12} \begin{vmatrix} J_{21} & 0 & 0 & 0 \\ J_{31} & J_{33} - s & J_{34} & J_{36} \\ 0 & J_{43} & J_{44} - s & 0 \\ J_{61} & 0 & 0 & J_{66} - s \end{vmatrix} = 0,$$

$$\left((J_{11} - s)(J_{22} - s) - J_{12}J_{21} \right) \begin{vmatrix} J_{33} - s & J_{34} & J_{36} \\ J_{43} & J_{44} - s & 0 \\ 0 & 0 & J_{66} - s \end{vmatrix} = 0,$$

$$(J_{66} - s) \left((J_{11} - s)(J_{22} - s) - J_{12}J_{21} \right) \begin{vmatrix} J_{33} - s & J_{34} \\ J_{43} & J_{44} - s \end{vmatrix} = 0,$$

$$(J_{66} - s) \left((J_{11}J_{22} - J_{12}J_{21}) - (J_{11} + J_{22})s + s^2 \right) \left((J_{33}J_{44} - J_{34}J_{43}) - (J_{33} + J_{44})s + s^2 \right) = 0. \quad (\text{A.5})$$

From (A.5), we obtain:

$$\begin{aligned} s_6 &= J_{66} = -d_{e1}, \\ 2s_{1,2} &= (J_{11} + J_{22}) \pm \sqrt{(J_{11} + J_{22})^2 - 4(J_{11}J_{22} - J_{12}J_{21})}, \\ 2s_{3,4} &= (J_{33} + J_{44}) \pm \sqrt{(J_{33} + J_{44})^2 - 4(J_{33}J_{44} - J_{34}J_{43})}. \end{aligned}$$

The analysis from here continues in the main text (see section 4.3.2).

A.4 A toy model of pro-inflammatory mediator production by leukocytes

A.4.1 Introduction

A simple toy model was developed in one spatial dimension and in time to explore the clashing dynamics between pro-inflammatory mediator production by leukocytes and the movement of leukocytes up chemotactic gradients. It is found that for parameter regimes that allow a noticeable increase in the concentration of pro-inflammatory mediators due to production by leukocytes, the leukocytes either get trapped in self-made maxima of pro-inflammatory mediators or move in a jerky

unrealistic fashion, implying that the production of pro-inflammatory mediators by leukocytes and the chemotactic attraction of leukocytes cannot be reconciled in the toy model and therefore in the hybrid model. This then implies that more in-depth modelling and biological knowledge are required to reconcile the conflicting mechanisms, however, this would over-complicate the model. We therefore omit the mechanism of pro-inflammatory mediator production by leukocytes from the hybrid mode and as a result of this, all pro-inflammatory mediator production in the hybrid model is done by IECs. In this case, leukocytes are chemotactically attracted to the bacteria since they are in general attached to the IECs, giving realistic results. We now present the formulation of the toy model and the analysis undertaken on it to reach the results discussed above.

A.4.2 Model formulation

Let $P = P(x, t)$ denote the non-dimensional concentration of pro-inflammatory mediators over one spatial dimension and time, where $x, P \in [0, 1]$. Let the initial profile of pro-inflammatory mediators be given by $P(x, 0) = P_0(x)$, where $P_0(x)$ is the steady state solution of the diffusion equation with constant rate of diffusion and a linear degradation term. The profile given by this initial condition is a simple one dimensional portrayal of the pro-inflammatory mediator profile in the hybrid model before pro-inflammatory mediator production by leukocytes occurs (neglecting more complicated dynamics such as lumen flow, internalization and inhibition by effector proteins). This is to account for the delay between pro-inflammatory mediator production and their diffusion to the endothelium, as occurs in the hybrid model. We set the IEC layer to be at $x = 1$ and the endothelium to be at $x = 0$. We assume that the IEC layer acts as a constant source of pro-inflammatory mediators therefore we set $P(1, t) = 1$. For the endothelium, we use the Robin boundary condition that is used in the hybrid model, given by the third equation of (5.3), replacing y with x . This lets the flux of pro-inflammatory mediators out into the blood supply be proportional to the concentration at the endothelium. $P_0(x)$ is therefore given by:

$$0 = D_P \frac{d^2 P_0}{dx^2}(x) - k_P P_0(x), \quad P_0(1) = 1, \quad \frac{\partial P_0}{\partial x}(0) = \alpha P_0(0), \quad (\text{A.6})$$

where D_P and k_P are the diffusion coefficient and degradation rate of pro-inflammatory mediators respectively and $\alpha = R_P/D_P$ where R_P is the pro-inflammatory mediator endothelial flux coefficient. Other mechanisms such as lumen flow, internalization and inhibition have been omitted as they would cause ambiguity in the findings. The general solution of the first equation in (A.6) is given by:

$$P_0 = Ae^{Kx} + Be^{-Kx}, \quad (\text{A.7})$$

where $K = \sqrt{k_P/D_P}$ and A and B are arbitrary constants. Applying the boundary conditions of (A.6) to (A.7) gives:

$$1 = Ae^K + Be^{-K},$$

$$K(A - B) = \alpha(A + B).$$

Solving these equations for A and B gives:

$$A = \frac{e^{-K}}{1 + K^*e^{-2K}}, \quad B = \frac{K^*e^{-K}}{1 + K^*e^{-2K}}, \quad (\text{A.8})$$

where $K^* = (K - \alpha)/(K + \alpha)$. Substituting (A.8) into (A.7) gives the profile for $P_0(x)$:

$$P_0(x) = \frac{e^{-K(1-x)} + K^*e^{-K(1+x)}}{1 + K^*e^{-2K}}. \quad (\text{A.9})$$

We now introduce an individual leukocyte into the model. We define the centre of the leukocyte to initially be at $x_C \in (0, 1)$ and the surface of the leukocyte to be at $(x_L, x_R) = (x_C - \text{rad}_L, x_C + \text{rad}_L)$ where rad_L is the radius of the leukocyte. The gradient of P across the leukocyte is therefore given by:

$$G(x_C, t) = \frac{P(x_R, t) - P(x_L, t)}{2\text{rad}_L}. \quad (\text{A.10})$$

This is also the chemotactic gradient across the leukocyte. It is assumed that leukocytes only produce pro-inflammatory mediators when they are being chemotactically attracted. To model this, the rate of production of pro-inflammatory mediators by the leukocyte is proportional to the chemotactic gradient G and to implement this, the pro-inflammatory mediator concentration across the leukocyte is increased by an amount proportional to this gradient. The production of pro-inflammatory mediators by the leukocyte is therefore modelled by:

$$P(x, t + \Delta t) = P(x, t) + \begin{cases} \Delta t r_P G(x_C, t) & x \in [x_L, x_R], \\ 0 & \text{otherwise,} \end{cases} \quad (\text{A.11})$$

where Δt is the time step and r_P is the production rate of pro-inflammatory mediators by the leukocyte. (A.11) increases the pro-inflammatory mediator concentration local to the leukocyte while preserving the gradient across the leukocyte (see Figure A.2).

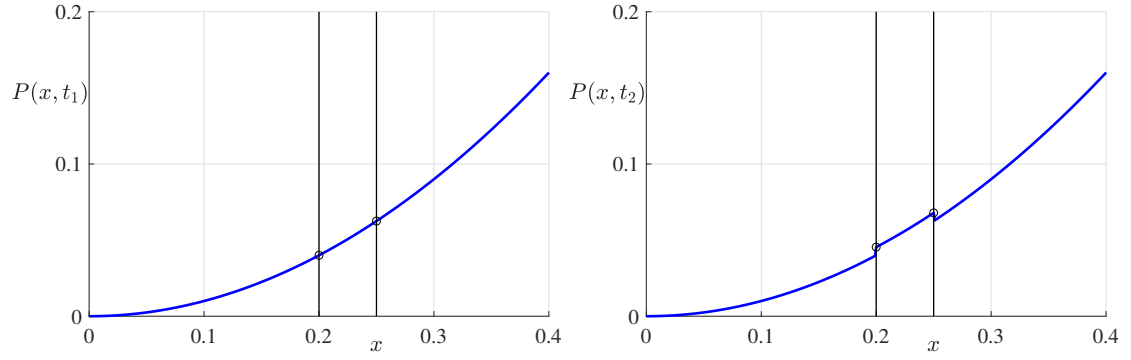


Figure A.2: Graphs showing the effect of (A.11). $(\Delta t, x_C, \text{rad}_L, r_P, L_{\text{mot}}) = (0.01, 0.225, 0.025, 0.012, 0.01)$. The black circles and lines represent the positions of the left and right boundaries of the leukocyte.

The leukocyte then moves up the chemotactic gradient by an amount proportional to the gradient:

$$x_C \rightarrow x_C + \Delta t L_{\text{mot}} G(x_C, t), \quad (\text{A.12})$$

where L_{mot} is the motility rate of the leukocyte. This therefore also shifts the position of x_L and x_R (see Figure A.3). The algorithm given by equations (A.10) and (A.11) is repeated until $x_R \geq 1$, signifying successful chemotaxis. Numerically, this algorithm is implemented by discretizing x onto a uniform mesh from 0 to 1 with mesh gap widths Δx and discretizing time with time step Δt . If the gradient of pro-inflammatory mediators across the leukocyte becomes non-positive, then it is reasoned that chemotaxis has failed and the algorithm is aborted. A pseudo-code for this algorithm is:

- Define parameter values and leukocyte centre x_C .
- Define $P(x, 0) = P_0(x)$ and $(x_L, x_R) = (x_C - \text{rad}_L, x_C + \text{rad}_L)$.
- Calculate using the closest mesh points:

$$G(x_C, t) = \frac{P(x_R, t) - P(x_L, t)}{2\text{rad}_L}.$$

- **while** $x_R < 1$ and $G(x_C, t) > 0$,
 apply (A.11) and (A.12),
 move x_C to the nearest mesh point and update (x_L, x_R) and $G(x_C, t)$.

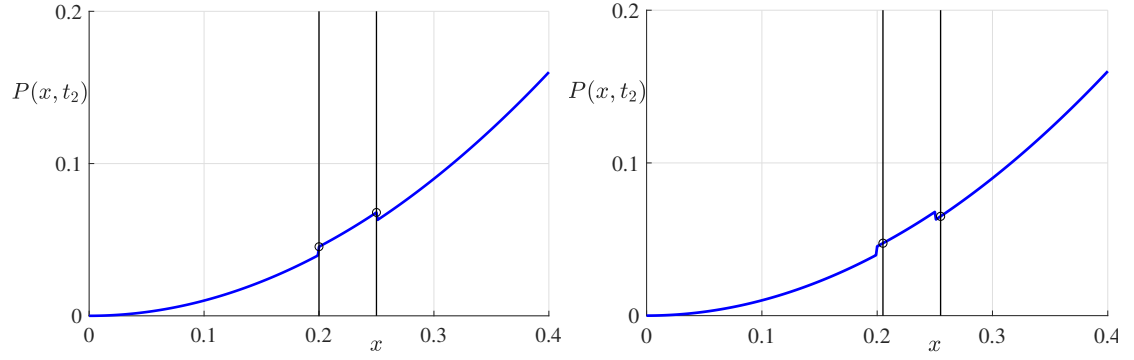


Figure A.3: Graphs showing the effect of transformation (A.12). $(\Delta t, x_C, \text{rad}_L, r_P, L_{\text{mot}}) = (0.01, 0.225, 0.025, 0.012, 0.01)$. The black circles and lines represent the positions of the left and right boundaries of the leukocyte.

Now that we have formulated the model, we now move to analyze it to gain a better understanding of the antagonism between pro-inflammatory mediator production by leukocytes and the chemotactic movement of leukocytes.

Analysis

A description of each of the toy model's parameters and their default values are given in Table A.1. After a single iteration of the algorithm, the gradient of pro-inflammatory mediators across the leukocyte's surface $G(x_C, t)$ will typically become shallower due to x_R moving outside of the region of x values that were given an elevated pro-inflammatory mediator concentration by (A.11). As the algorithm is repeated, $G(x_C, t)$ becomes shallower and often becomes non-positive after sufficiently many iterations (and the pro-inflammatory mediator concentration in-between x_L and x_R is not necessarily monotonic). This implies that the leukocyte has become stuck in a self-made maxima of pro-inflammatory mediators and that chemotaxis has failed. An example of this is seen in Figure A.4. This suggests, at least for these parameter values, that the production of pro-inflammatory mediators by leukocytes and the movement of leukocytes up chemotactic gradients are unintentionally antagonistic to each other.

Δt	0.01	Time step.
x_C	0.225	Position of the leukocyte's centre.
rad_L	0.025	Radius of the leukocyte
D_P	0.033	Diffusion rate of pro-inflammatory mediators.
k_P	0.1	Natural degradation rate of pro-inflammatory mediators.
α	30.3	Rate of flux of pro-inflammatory mediators through the left boundary.
r_P	0.1	Production rate of pro-inflammatory mediators by leukocytes.
L_{mot}	0.3	Motility rate of leukocytes.

Table A.1: Descriptions of what each parameter in the chemotaxis toy model functions as, along with its default value.

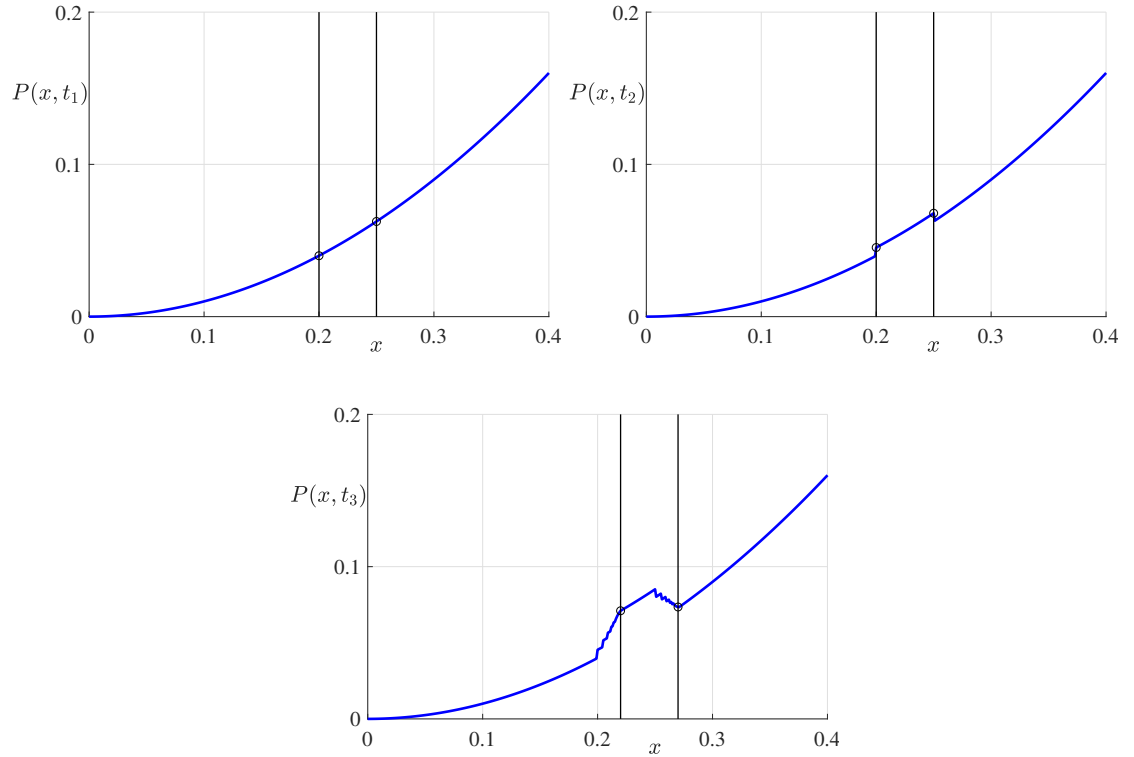


Figure A.4: Graphs showing how typically after sufficiently many iterations of the toy model algorithm, the gradient of pro-inflammatory mediators across the surface of the leukocyte becomes non-positive, disabling chemotaxis. The black circles and lines represent the positions of the left and right boundaries of the leukocyte respectively. Parameter values are given in Table A.1.

The parameters r_P and L_{mot} are critical to pro-inflammatory mediator production by leukocytes and chemotactic attraction of leukocytes respectively, therefore they will be considered in detail.

Figure A.5 shows the pro-inflammatory mediator profile before and after the toy model algorithm is applied for various r_P and L_{mot} values. When r_P and L_{mot} are both sufficiently low (top left panel), the leukocyte successfully travels to the IEC layer ($x = 1$), albeit slowly and with little effect on the pro-inflammatory mediator profile. When L_{mot} is high (top right panel), the leukocyte successfully travels to the IEC layer much faster than in the first case (0.27 units of time compared with 5.01), but with even less effect on the pro-inflammatory mediator profile. For these two cases, the model does not represent the production of pro-inflammatory mediators by leukocytes well, as evidenced by the negligible change in the pro-inflammatory mediator profile. When L_{mot} is low but r_P is high (bottom left panel), the leukocyte produces pro-inflammatory mediators effectively, but gets stuck in a self made maximum of pro-inflammatory mediators, leading to chemotaxis failing. When r_P and L_{mot} are both high (bottom right panel), the leukocyte successfully travels to the IEC layer very quickly and with a noticeable effect on the pro-inflammatory mediator profile, however, the movement is discontinuous and jerky and is deemed to be unrealistic. The latter two cases suggest that in the parameter regimes that give a noticeable change in the pro-inflammatory mediator profile due to production by a leukocyte, the leukocyte either gets trapped in a self-made maximum of pro-inflammatory mediators or moves in a jerky unrealistic fashion. Together these results imply that the production of pro-inflammatory mediators by leukocytes and the movement of leukocytes up chemotactic gradients cannot be reconciled by the current modelling framework and that more detailed modelling of the two mechanisms is required in order for them to be reconciled. These implications are also assumed to be applicable to the hybrid model. We therefore omit the mechanism of pro-inflammatory mediator production by leukocytes from the hybrid model and as a result of this, all pro-inflammatory mediator production in the hybrid model is done by IECs. In this case, leukocytes are chemotactically attracted to the bacteria since they are in general attached to the IECs, which is a realistic result. It is assumed that modelling the conflicting mechanisms in more biological detail will complicate the model and since the model is currently giving realistic results, no further mechanisms or details will be added to the model.

Finally, Figure A.6 shows snapshots over time of the region of interest during a hybrid model simulation, demonstrating leukocyte entrapment in two spatial dimensions.

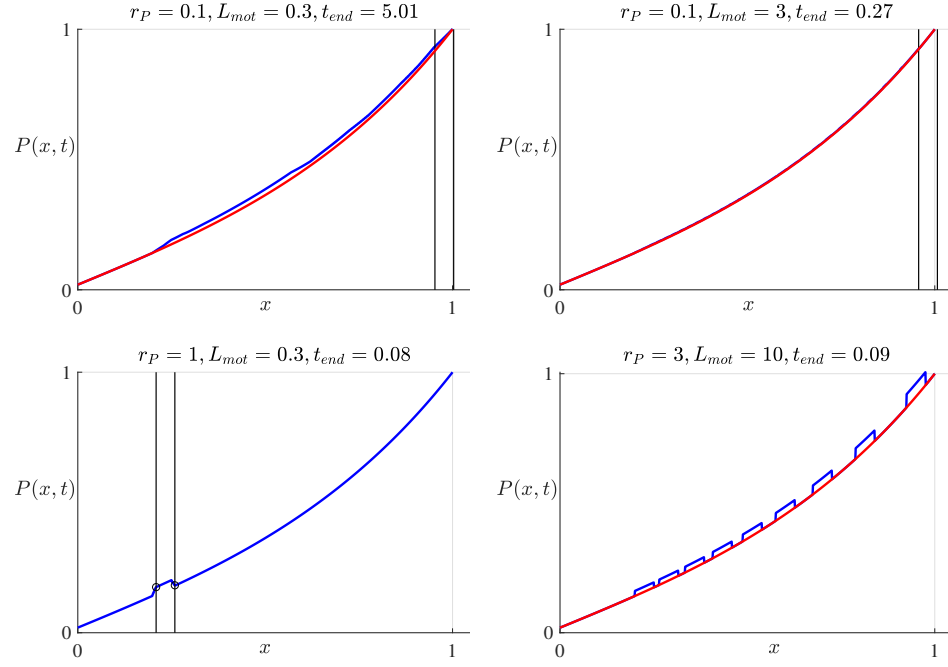


Figure A.5: Graphs of the toy model showing the P profile and the position of the leukocyte in space and time for various r_P and L_{mot} values. The red and blue lines represent the P profile before and after the toy model algorithm is applied respectively and the black circles and lines represent the positions of the left and right boundaries of the leukocyte. The r_P and L_{mot} values used are given in the graph titles and all other parameter values are given in Table A.1.

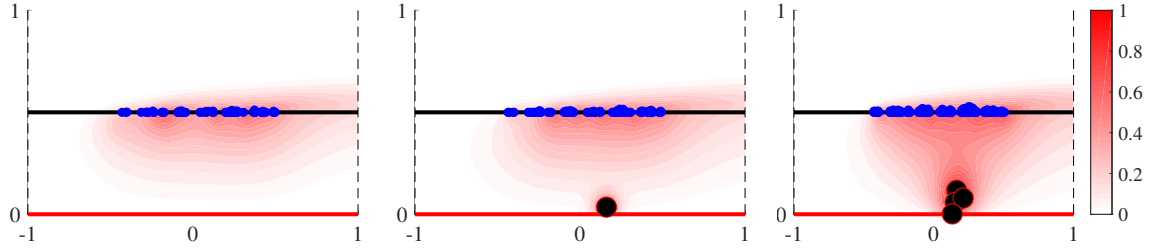


Figure A.6: Snapshots of a hybrid model simulation showing examples of leukocytes getting stuck in self-made maxima of pro-inflammatory mediators due to their own production of pro-inflammatory mediators. Blue and black circles represent bacteria and leukocytes respectively, the full black and red lines represent the epithelium and the endothelium respectively and the red cloud represents pro-inflammatory mediator presence.

A.4.3 Summary

In this section, we formulated and analyzed a toy model that explores the dynamics of pro-inflammatory mediator production by leukocytes and chemotactic movement of leukocytes in more detail than the hybrid model. The model only considers one leukocyte and it is found that either (1) the leukocyte successfully travels to the IEC layer but with negligible effects on the pro-inflammatory mediator profile, (2) the leukocyte produces pro-inflammatory mediators sufficiently well that the chemotactic gradient across the leukocyte becomes non-positive and the leukocyte therefore becomes stuck in a self-made local maximum of pro-inflammatory mediators, or (3) successfully reaches the

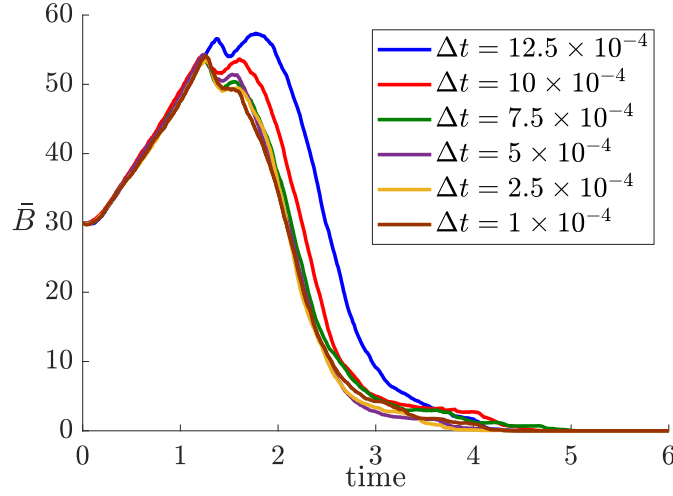


Figure A.7: The mean number of bacteria over time for varying time step sizes. Each graph is the mean of 100 simulations and all other parameters take their baseline values (see set 2 in Table A.2).

IEC layer whilst producing a noticeable change in the pro-inflammatory mediator profile, but moves in a discontinuous, jerky and unrealistic fashion. Since none of these results are realistic, it is assumed that the production of pro-inflammatory mediators by leukocytes and the movement of leukocytes up chemotactic gradients cannot be reconciled by the current modelling framework and that more detailed modelling of the two mechanisms is required in order for them to be reconciled. These implications are also assumed to be applicable to the hybrid model. We therefore omit the mechanism of pro-inflammatory mediator production by leukocytes from the hybrid model and allow all pro-inflammatory mediator production to be done by IECs. Since the model gives realistic results in the absence of this mechanism, more detailed modelling and additional mechanisms are not needed in the hybrid model.

A.5 Hybrid model - showing numerical convergence for a decreasing time-step

Figure A.7 shows the mean number of bacteria over time for varying time step sizes. The model converges sufficiently well as the time step $\Delta t \rightarrow 0$; however, there are slight discrepancies in the mean number of bacteria over time, particularly for $t > 3$, due to the stochastic implementation of some of the model's mechanisms. As a balance between accuracy and computation cost, a value of $\Delta t = 5 \times 10^{-4}$ is taken in all baseline parameter sets.

A.6 Hybrid model - deriving expression for an approximation to the second derivative for a non-uniform mesh

Here, we only consider the x direction for simplicity. Let the approximation for the second derivative of P at point x_i , denoted Q_i , be the weighted sum of the values of P at x_i and its neighbours:

$$Q_i = aP_{i-1} + bP_i + cP_{i+1}. \quad (\text{A.13})$$

P_{i-1} and P_{i+1} can be approximated by the following Taylor expansions:

$$P_{i-1} \approx P_i - \Delta x_{i-1}P'_i + \frac{(\Delta x_{i-1})^2}{2}P''_i, \quad (\text{A.14a})$$

$$P_{i+1} \approx P_i + \Delta x_iP'_i + \frac{(\Delta x_i)^2}{2}P''_i. \quad (\text{A.14b})$$

By substituting (A.14) into (A.13), it can be shown that:

$$Q_i = \sigma_0P_i + \sigma_1P'_i + \sigma_2P''_i + O\left((\Delta x_{i-1})^3, (\Delta x_i)^3\right),$$

where

$$\sigma_0 = a + b + c, \quad \sigma_1 = -a\Delta x_{i-1} + c\Delta x_i, \quad \sigma_2 = \frac{1}{2}\left((\Delta x_{i-1})^2a + (\Delta x_i)^2c\right).$$

For Q_i to approximate the second derivative of P_i , we require $\sigma_0, \sigma_1 = 0$ and $\sigma_2 = 1$. This gives:

$$a + b + c = 0, \quad (\text{A.15a})$$

$$-a\Delta x_{i-1} + c\Delta x_i = 0, \quad (\text{A.15b})$$

$$\frac{1}{2}\left((\Delta x_{i-1})^2a + (\Delta x_i)^2c\right) = 1. \quad (\text{A.15c})$$

Solving (A.15) gives:

$$a = \frac{2}{\Delta x_{i-1}(\Delta x_{i-1} + \Delta x_i)}, \quad b = -\frac{2}{\Delta x_{i-1}\Delta x_i}, \quad c = \frac{2}{\Delta x_i(\Delta x_{i-1} + \Delta x_i)}.$$

Substituting (A.16) into (A.13) gives:

$$\frac{\partial^2 P_i}{\partial x^2} \approx \frac{2}{\Delta x_{i-1}(\Delta x_{i-1} + \Delta x_i)}P_{i-1} - \frac{2}{\Delta x_{i-1}\Delta x_i}P_i + \frac{2}{\Delta x_i(\Delta x_{i-1} + \Delta x_i)}P_{i+1}. \quad (\text{A.17})$$

(A.17) allows for a horizontally non-uniform mesh in our two dimensional diffusion finite difference scheme.

A.7 Hybrid model - deriving the probability of phagocytosis

We seek a probability $\mathcal{P}$, dependent on the number of E_3 and the time step Δt , that governs whether or not phagocytosis is successful in the time window $(t^*, t^* + \Delta t]$, where t^* is the current time. Suppose that bacterium i , positioned at (x_i, y_i) , is the closest bacterium to the leukocyte and that $|(L_x, L_y) - (x_i, y_i)| < \text{rad}_L$. We define the probability that bacterium i is phagocytosed in an infinitesimally small time step t_ϵ to be Q , where:

$$Q(\bar{E}_{3,i}) = \frac{\phi_3^{-1}}{\phi_3^{-1} + \bar{E}_{3,i}},$$

$\bar{E}_{3,i}$ is the number of E_3 contained within the i th bacterium and ϕ_3 , analogous to ϕ_1 and ϕ_2 , is a parameter measuring the efficacy of E_3 at preventing phagocytosis. It is worth noting that $Q(i_3)$ is a decreasing Hill function of $\bar{E}_{3,i}$ and that only integer values of $\bar{E}_{3,i}$ are allowed. In the absence of E_3 , the probability of phagocytosis is 1. By making ϕ_3 sufficiently large, the probability is drastically reduced even in the presence of just one E_3 . It is desired that the probability of phagocytosis in the presence of $\bar{E}_{3,i}$ in a small time window is small. We now need to find the probability of phagocytosis in time window Δt in terms of Q . For this to work, Δt must be an integer multiple of t_ϵ , say k . It is assumed for simplicity that the probability of being phagocytosed is uniform across the whole time step (see Figure A.8).

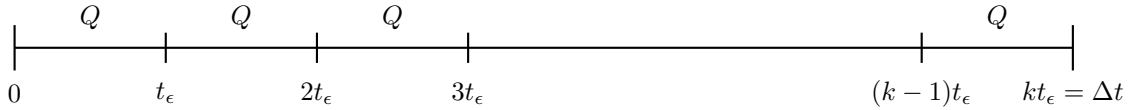


Figure A.8: Splitting up the time step Δt into k uniform regions, each with probability of successful phagocytosis equal to Q for simplicity.

Here, we use the symbol $\mathcal{P}$ to denote probability. Given that $\mathcal{P}(\text{phag in } t \leq t_\epsilon) = Q$, Q is small and $t > 0$,

$$\begin{aligned} \mathcal{P}(\text{phag in } t \leq \Delta t) &= \mathcal{P}(\text{phag in } t \leq t_\epsilon) \\ &\quad + \mathcal{P}(\text{phag in } t_\epsilon < t \leq 2t_\epsilon \text{ and not phag in } t \leq t_\epsilon) \\ &\quad + \mathcal{P}(\text{phag in } 2t_\epsilon < t \leq 3t_\epsilon \text{ and not phag in } t \leq 2t_\epsilon) \\ &\quad + \dots \\ &\quad + \mathcal{P}(\text{phag in } (k-1)t_\epsilon < t \leq \Delta t \text{ and not phag in } t \leq (k-1)t_\epsilon), \\ &= Q + Q(1-Q) + Q(1-Q)^2 + \dots + Q(1-Q)^{k-1}, \\ &= Q \sum_{n=0}^{k-1} (1-Q)^n. \end{aligned}$$

It can be seen that the probability of phagocytosis follows a geometric distribution and can be written as:

$$\mathcal{P}(\text{phag in } t \leq \Delta t) = Q \frac{1 - (1 - Q)^k}{1 - (1 - Q)} = 1 - (1 - Q)^k. \quad (\text{A.18})$$

A.8 Hybrid model default parameter sets

Parameter	Definition	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8
Space and time									
$\Delta x_{\min}$	Minimum horizontal mesh spacing	0.02							
$\Delta x_{\max}$	Maximum horizontal mesh spacing	0.5							
Δy	Vertical mesh spacing	0.01							
Δt	Time step	$5e^{-4}$							
y^*	y -coordinate of IEC layer	0.5							
x_l	left boundary of central region	-1							
x_r	left boundary of central region	1							
$x_{\max}$	Largest value of x	≈ 5.03	≈ 4.24						
$y_{\max}$	Largest value of y	1							
$t_{\max}$	Maximum time the simulation runs for	8	10						
Bacteria									
N_B^0	Number of bacteria at $t = 0$	30							
(x_B, y_B)	Initial position of each bacterium	$(\mathcal{U}(-0.5, 0.5), y^* + 3\text{rad}_B \mathcal{U}(0, 1))$							
rad_B	Radius of bacteria	0.003							
B_{mot}	Directed motility rate of bacteria	20							
r_B^*	Scale factor of how strong directed motion is compared to Brownian motion	20							
ϕ_B	The distance above the IEC layer at which bacterial Brownian motion is half-maximal	0.01							
Effector proteins									
N_{eff}	Number of all EP groups at $t = 0$	0							
ϕ_1	Efficacy of E_1 at inhibiting pro-inflammatory signalling	100	100	12		15			7
ϕ_2	Efficacy of E_2 at inhibiting host cell apoptosis	100	100	5000				$1e4$	
ϕ_3	Efficacy of E_3 at inhibiting phagocytosis	100	100	3000		5000		$2.5e4$	$5e4$

Parameter	Definition	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8
Leukocytes									
N_L^0	Number of leukocytes at $t = 0$	0							
rad_L	Radius of leukocytes	0.06		0.03					0.08
L_{mot}	Motility rate of leukocytes	1							
$\sigma_{\text{mot},L}$	Standard deviation of the angles of movement of leukocytes due to chemotaxis	$\sqrt{0.6}$							
$\mu_{\text{deg},L}$	Mean lifespan of a leukocyte	4		5				4	
$\sigma_{\text{deg},L}$	Standard deviation of leukocyte lifespans	0.5							
$t_{L,\text{rep}}$	Leukocyte attraction time (deterministic)	0.3							
Inflammatory mediators and apoptosis markers									
$P(x, y, 0)$	Concentration of PIMs at $t = 0$	0							
$A(x, y, 0)$	Concentration of AIMs at $t = 0$	0							
Φ	Background production rate of PIMs	0		1		0			
D_I	Diffusion coefficient of IMs	0.033							
d_I	Natural degradation rate of IMs	0.1							
R_I	IM endothelial flux coefficient	0.5		1					
$r_{\text{int},P}$	Internalization rate of PIMs by bacteria	$1e^{-4}$		20					0.01
P_{crit}	Concentration of internalized PIMs required for a bacterium to be classed as a B_2	0.1							
P_{endo}^*	Concentration of PIMs at the endothelium required for leukocyte attraction to be activated	0.035							
r_A	Production rate of AIMs	0.8							
ϕ_A	Concentration of PIMs in an enveloping 3×3 grid at which AIM production is half-maximal	6							
$m(x, y, 0)$	Concentration of AMs at $t = 0$	0							
ϕ_u	Distance above the IEC layer at which the flow in the lumen is half-maximal	0.3							

Parameter	Definition	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8
Antibiotic									
$\alpha(x, y, 0)$	Concentration of antibiotic at $t = 0$	–				0			–
D_α	Diffusion coefficient of antibiotic	–				0.1			–
d_α	Natural degradation rate of antibiotic	–				0.2			–
R_α	Antibiotic endothelial flux coefficient	–				10			–
$r_{\text{int},\alpha}$	Internalization rate of antibiotic by bacteria	–				80			–
w_1	Number of incorrect structural proteins at which the permeability of a bacterial membrane is half-maximal	–				3			–
z_0	A measure of how well antibiotic is internalized by a bacterium with a fully intact membrane	–				0.3			–
ϕ_Λ	A measure of how well antibiotic flows through a permeable bacterial membrane (a smaller value $\implies$ better flow)	–				0.1			–
α_{tot}	Total amount of antibiotic delivered into the host	–				1	2		–
z_i	Peak antibiotic concentration of the i 'th dose	–				1			–
z_C	Antibiotic concentration of a constant delivery	–				0.125	0.25		–
t_1	Time of first antibiotic delivery	–				2			–
t_{gap}	Time gap between repeated doses	–				1			–
α_0	Proportion of internalized antibiotic that is unaffected by the inhibitory action of internalized PIMs	–				0.1			–
q_α	Concentration of internalized antibiotic at which protein synthesis inhibition is half-maximal	–				0.03			–
q_P	Concentration of internalized PIMs at which antibiotic inhibition is half-maximal	–				1.5		1	–

Parameter	Definition	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8
Intestinal epithelial cells									
H^0	Health value of IECs at $t = 0$	1							
H_{apop}	Health value at which AM presentation is activated	0.25							
r_{dam}	The rate of damage to an IEC due to one bacterium	0.05				0.2			
t_a	Time for an apoptosed IEC to repair	2							
t_n	Time for a necrosed IEC to repair	3							
t_f	Time for a fully functioning IEC to repair (if it was infinitesimally close to undergoing necrosis)	5							

Parameter	Definition	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8
Sub-cellular gene network									
$\theta_{\max}$	Number of polymerases found in a bacterium	80							
$R_{\max}$	Number of ribosomes found in a bacterium	45							
Γ^*	Number of structural proteins required to form a new bacterium	10							
b_E	Rate at which polymerases bind to DNA strands	250							
r_0	Transcription rate of mRNA	2.5							
b_1	Rate at which free ribosomes bind to mRNA strands	250							
$r_{1,\text{base}}$	Translation rate of E_1 by B_1	0.375		0.6	0	0.6			
$r_{2,\text{base}}$	Translation rate of E_2 by B_1	0.375		0.6		0	0.6		
$r_{3,\text{base}}$	Translation rate of E_3 by B_1	0.375		0.6					
r^*	Ratio of the production rate of EPs by B_2 to that by B_1	1.5							
r_Γ	Translation rate of (all) structural proteins	0.225		0.33					
r_B	Rate at which structural proteins fuse to become new bacteria	125		220	0	220			
d_0	Natural degradation rate of (unbound) mRNA strands	25							
d_1	Natural degradation rate of E_1 (global)	8							
d_2	Natural degradation rate of E_2 (global)	8							
d_3	Natural degradation rate of E_3 (per bacterium)	0.2							
d_Γ	Natural degradation rate of correct structural proteins	0.15		0.3					
d_B	Natural degradation rate of bacteria	0.2	0.15	0.2	0	0.2			
d_X	Natural degradation rate of incorrect structural proteins	0.5	0.3	0.5					

Table A.2: The default parameter regimes used in the analysis of the hybrid model. A blank parameter value entry implies that the parameter value used in the relevant set is the same as the value used in the previous set. A dash implies that the parameter is not used in the respective parameter set. ye^x is shorthand for $y \times 10^x$. Note that the value for $\Delta x_{\min}$ given is valid for $x \in [-1, 1]$ and this region includes most of the infection dynamics. EP, PIM, AIM, AM, IEC are acronyms for effector protein, pro-inflammatory mediator, anti-inflammatory mediator, apoptosis marker and intestinal epithelial cell respectively.

A.9 Biofilm model baseline parameter values

For the definitions of all hybrid model parameters, see Table A.2.

Original parameters:

$\Delta x_{\min} = 0.02$, $\Delta x_{\max} = 0.5$, $\Delta y = 0.01$, $\Delta t = 5 \times 10^{-4}$, $y^* = 0.5$, $x_{\max} \approx 4.244$, $y_{\max} = 1$, $x_l = -1$, $x_r = 1$, $t_{\max} = 10$, $N_B^0 = 30$, $(x_B, y_B) = (\mathcal{U}(-0.5, 0.5), y^* + 3\text{rad}_B \mathcal{U}(0, 1))$, $\text{rad}_B = 0.003$, $B_{\text{mot}} = 20$, $r_B^* = 20$, $\phi_B = 0.01$, $N_{\text{eff}} = 0$, $\phi_1 = 0.3$, $\phi_2 = 5000$, $\phi_3 = 5000$, $N_L^0 = 0$, $\text{rad}_L = 0.03$, $L_{\text{mot}} = 1$, $\sigma_{\text{mot},L} = \sqrt{0.6}$, $\mu_{\text{deg},L} = 5$, $\sigma_{\text{deg},L} = 0.5$, $t_{L,\text{rep}} = 0.3$, $P(x, y, 0) = 0$, $A(x, y, 0) = 0$, $\Phi = 1$, $D_I = 0.028$, $d_I = 0.1$, $R_I = 1$, $r_{\text{int},P} = 100$, $P_{\text{crit}} = 0.1$, $P_{\text{endo}}^* = 0.0024$, $r_A = 0.8$, $\phi_A = 0.5$, $m(x, y, 0) = 0$, $\phi_u = 0.3$, $\alpha(x, y, 0) = 0$, $D_\alpha = 0.1$, $d_\alpha = 0.2$, $R_\alpha = 10$, $r_{\text{int},\alpha} = 80$, $w_1 = 3$, $z_0 = 0.3$, $\phi_\Lambda = 0.1$, $\alpha_{\text{tot}} = 1$, $z_i = 1$, $t_1 = 2$, $t_{\text{gap}} = 1$, $\alpha_0 = 0.1$, $q_\alpha = 0.03$, $q_P = 1.5$, $H^0 = 1$, $H_{\text{apop}} = 0.25$, $r_{\text{dam}} = 0.2$, $t_a = 2$, $t_n = 3$, $t_f = 5$, $\theta_{\max} = 80$, $R_{\max} = 45$, $\Gamma^* = 10$, $b_E = 250$, $r_0 = 2.5$, $b_1 = 250$, $r_{1,\text{base}} = 0.6$, $r_{2,\text{base}} = 0.6$, $r_{3,\text{base}} = 0.6$, $r^* = 1.5$, $r_\Gamma = 0.3$, $r_B = 220$, $d_0 = 25$, $d_1 = 8$, $d_2 = 8$, $d_3 = 0.2$, $d_\Gamma = 0.3$, $d_B = 0.22$, $d_X = 0.5$.

Biofilm parameters:

$v_{\max} = 4$, $v_{\min} = -0.5$, $\beta_1 = 6 \times 10^3$, $\beta_2 = 7.5 \times 10^3$, $\beta_3 = 9 \times 10^3$, $q_B = 10$, $n_B = 3$, $q_E = 2$, $n_E = 3$, $c_b = 1.2$.

A.10 Stability analysis of (7.2)

The steady state(s) of (7.2) are given by:

$$0 = d_p \frac{d^2 \bar{p}}{dz^2} + \lambda \bar{l} \left| \frac{d\bar{p}}{dz} \right| - \bar{p}, \quad (\text{A.19a})$$

$$0 = d_l \frac{d^2 \bar{l}}{dz^2} - \phi \frac{d}{dz} \left(\bar{l} \frac{d\bar{p}}{dz} \right) - k_l \bar{l}, \quad (\text{A.19b})$$

$$\bar{p}(0) = 1, \quad \frac{d\bar{p}}{dz}(1) = -w\bar{p}(1), \quad \frac{d\bar{l}}{dz}(0) = 0, \quad \frac{d\bar{l}}{dz}(1) = \bar{p}(1), \quad (\text{A.19c})$$

implying that the concentrations of p and l in a non-trivial steady state are non-uniform. In order to investigate the linear stability of non-uniform steady states, we must formulate the associated eigenvalue problem and to do this, we follow the method used in [157]. Linearising about $\bar{p}, \bar{l}$ via:

$$p(z, \tau) = \bar{p}(z) + \epsilon \tilde{p}(z, \tau),$$

$$l(z, \tau) = \bar{l}(z) + \epsilon \tilde{l}(z, \tau),$$

where $0 < \epsilon \ll 1$, we find the perturbations $\tilde{p}, \tilde{l}$ satisfy:

$$\frac{\partial}{\partial t} \begin{bmatrix} \tilde{p} \\ \tilde{l} \end{bmatrix} = \begin{bmatrix} d_p \frac{\partial^2}{\partial z^2} - \lambda \bar{l} \frac{\partial}{\partial z} - 1 & -\lambda \frac{d\bar{p}}{dz} \\ -\phi \left(\frac{d\bar{l}}{dz} \frac{\partial}{\partial z} + \bar{l} \frac{\partial^2}{\partial z^2} \right) & d_l \frac{\partial^2}{\partial z^2} - \phi \left(\frac{d\bar{p}}{dz} \frac{\partial}{\partial z} + \frac{d^2 \bar{p}}{dz^2} \right) - k_l \end{bmatrix} \begin{bmatrix} \tilde{p} \\ \tilde{l} \end{bmatrix}. \quad (\text{A.20})$$

We make the standard assumption that the perturbations can be written as Fourier series:

$$\tilde{p} = e^{\sigma\tau} \sum_{j=1}^{\infty} a_j \cos(j\pi z), \quad (\text{A.21a})$$

$$\tilde{l} = e^{\sigma\tau} \sum_{j=1}^{\infty} b_j \cos(j\pi z), \quad (\text{A.21b})$$

where σ is the perturbation growth rate, $j \in \mathbb{N}$ are the wave numbers of the perturbations and a_j, b_j are the respective eigenmodes for the pro-inflammatory mediator and leukocyte perturbations respectively. Multiplying (A.20) by $\cos(j\pi z)$ and integrating with respect to z between 0 and 1 yields the following relation connecting σ to the perturbation modes:

$$\frac{\sigma}{2} \begin{bmatrix} a_i \\ b_i \end{bmatrix} = \begin{bmatrix} A_{i(1,1)} & A_{i(1,2)} \\ A_{i(2,1)} & A_{i(2,2)} \end{bmatrix} \begin{bmatrix} a_i \\ b_i \end{bmatrix} \quad \text{when } j = i, \quad (\text{A.22a})$$

$$\begin{bmatrix} 0 \\ 0 \end{bmatrix} = \begin{bmatrix} B_{ij(1,1)} & B_{ij(1,2)} \\ B_{ij(2,1)} & B_{ij(2,2)} \end{bmatrix} \begin{bmatrix} a_j \\ b_j \end{bmatrix} \quad \text{when } j \neq i, \quad (\text{A.22b})$$

where:

$$A_{i(1,1)} = \int_0^1 -2 \cos^2(i\pi z) \left(1 + d_p i^2 \pi^2 \right) + \lambda \bar{l} \sin(2i\pi z) i\pi \, dz, \quad (\text{A.23a})$$

$$A_{i(1,2)} = -2\lambda \int_0^1 \frac{d\bar{p}}{dz} \cos^2(i\pi z) \, dz, \quad (\text{A.23b})$$

$$A_{i(2,1)} = \phi \int_0^1 \frac{d\bar{l}}{dz} \sin(2i\pi z) i\pi + 2\bar{l} \cos^2(i\pi z) i^2 \pi^2 \, dz, \quad (\text{A.23c})$$

$$A_{i(2,2)} = \int_0^1 -2 \cos^2(i\pi z) \left(k_l + d_l i^2 \pi^2 \right) + \phi \left(\frac{d\bar{p}}{dz} \sin(2i\pi z) i\pi - 2 \frac{d^2 \bar{p}}{dz^2} \cos^2(i\pi z) \right) \, dz, \quad (\text{A.23d})$$

$$B_{ij(1,1)} = \int_0^1 \left(-\cos(j\pi z) (1 + d_p j^2 \pi^2) + \lambda \bar{l} \sin(j\pi z) j\pi \right) \cos(i\pi z) \, dz, \quad (\text{A.23e})$$

$$B_{ij(1,2)} = -\lambda \int_0^1 \frac{d\bar{p}}{dz} \cos(j\pi z) \cos(i\pi z) \, dz, \quad (\text{A.23f})$$

$$B_{ij(2,1)} = j\pi\phi \int_0^1 \left(\frac{d\bar{l}}{dz} \sin(j\pi z) + \bar{l} \cos(j\pi z) j\pi \right) \cos(i\pi z) dz, \quad (\text{A.23g})$$

$$B_{ij(2,2)} = \int_0^1 \left(-\cos(j\pi z) \left(k_l + d_l j^2 \pi^2 \right) + \phi \left(\frac{d\bar{p}}{dz} \sin(j\pi z) j\pi - \frac{d^2 \bar{p}}{dz^2} \cos(j\pi z) \right) \right) \cos(i\pi z) dz. \quad (\text{A.23h})$$

In obtaining (A.23), we have made use of the following identities:

$$\begin{aligned} \int_0^1 \sin(j\pi z) \sin(i\pi z) &= 0, \\ \int_0^1 \cos(j\pi z) \cos(i\pi z) &= \begin{cases} 0, & j = i, \\ \frac{1}{2}, & j \neq i. \end{cases} \end{aligned}$$

The system (A.22) can be written compactly as an eigenvalue problem:

$$\sigma \mathbf{a} = \mathbf{Q} \mathbf{a}, \quad (\text{A.24})$$

where:

$$\mathbf{a} = [\mathbf{a}_1, \mathbf{a}_2, \mathbf{a}_3 \dots]^T, \quad \mathbf{Q} = \begin{bmatrix} \mathbf{A}_1 & \mathbf{B}_{12} & \mathbf{B}_{13} & \cdots \\ \mathbf{B}_{21} & \mathbf{A}_2 & \mathbf{B}_{23} & \cdots \\ \mathbf{B}_{31} & \mathbf{B}_{32} & \mathbf{A}_3 & \cdots \\ \vdots & \vdots & \vdots & \ddots \end{bmatrix},$$

$$\mathbf{a}_i = [a_i, b_i]^T, \quad \mathbf{A}_i = \begin{bmatrix} A_{i(1,1)} & A_{i(1,2)} \\ A_{i(2,1)} & A_{i(2,2)} \end{bmatrix}, \quad \mathbf{B}_{ij} = \begin{bmatrix} B_{ij(1,1)} & B_{ij(1,2)} \\ B_{ij(2,1)} & B_{ij(2,2)} \end{bmatrix}.$$

The growth rate σ is computed from $\det|\mathbf{Q} - \sigma \mathbf{I}| = 0$ and the steady state given by the numerical solution of (7.2) is stable provided that $\max(\Re(\sigma)) < 0$. However, since j in (A.21) is unbounded, we must truncate $\mathbf{Q}$ in order to determine σ numerically.

In [157], the authors show for the equivalent matrix $\mathbf{Q}$ in their model that by taking $i, j \rightarrow \infty$, the most-positive eigenvalues come from the top-left corner of $\mathbf{Q}$, allowing the approximation of $\mathbf{Q}$ by taking $i, j = 1, 2, \dots, K$, where K is a large positive integer. Asserting $i, j \gg 1$ in (A.23), we obtain:

$$A_{i(1,1)} = \mathcal{O}(i^2), \quad A_{i(1,2)} = \mathcal{O}(1), \quad A_{i(2,1)} = \mathcal{O}(i^2), \quad A_{i(2,2)} = \mathcal{O}(i^2), \quad (\text{A.25a})$$

$$B_{ij(1,1)} = \mathcal{O}(j^2), \quad B_{ij(1,2)} = \mathcal{O}(1), \quad B_{ij(2,1)} = \mathcal{O}(j^2), \quad B_{ij(2,2)} = \mathcal{O}(j^2). \quad (\text{A.25b})$$

From (A.25), we find that the matrix reduction applied in [157] cannot be applied to (A.24) because the terms of the largest magnitude are not restricted to the diagonal of $\mathbf{Q}$. Since we cannot make this matrix reduction, we can only assume (rather than state) that the most-positive eigenvalues are located towards the top-left corner of $\mathbf{Q}$. We take $i, j = 1, 2, \dots, 200$ and consider the steady states $\bar{p}$ and $\bar{l}$ and $\max(\Re(\sigma))$ as λ and ϕ are varied.

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