THE FIGHT AGAINST ANTIMICROBIAL RESISTANCE: OPTIMISING ANTIBIOTIC USAGE TO TREAT BACTERIAL INFECTIONS

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July 2019

DECLARATION

I hereby declare that this dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text and bibliography.

I also declare that this dissertation (or any significant part of my dissertation) is not substantially the same as any that I have submitted, or that is being concurrently submitted, for a degree or diploma or other qualification at the University of Stirling or similar institution.

This dissertation is a record of the work carried out at the University of Stirling between 2013 and 2019, under the supervision of Dr Andrew Hoyle, Professor Gabriela Ochoa, Dr Craig Baker-Austin and Dr Nicholas G.H. Taylor.

Stirling, July 2019

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ABSTRACT

Antibiotic resistance is one of the major health concerns of the 21st century. Antibiotics are essential for the health and well-being of both humans and animals. However, the increase in antibiotic resistant bacteria poses a threat to the continued use of antibiotics to successfully treat bacterial infections. Current research within hospital settings has focused on the use of multi-antibiotic approaches in a variety of treatment patterns. Yet there is limited knowledge on the optimal use of single antibiotic treatments. With the spread of resistance linked to the overuse and misuse of antibiotics, optimal treatment regimens aim to maximise the success of eradicating an infection while minimising the amount of antibiotic required. This thesis therefore aimed to combine mathematical modelling with a genetic algorithm approach to identify optimal dosage regimens for the use of a single antibiotic.

A mathematical model was developed to predict the dynamics of bacterial populations within an infection. A susceptible only infection was initially considered before being extended to include a resistant population. These models were incorporated into a genetic algorithm and used to search for dosage regimens which maximise bacterial eradication and minimise antibiotic use. Taking a theoretical approach, it was found that administering an antibiotic with a high initial dose followed by lowering doses is the optimal treatment regimen. A case study of a *Vibrio anguillarum* infection within *Galleria mellonella* larvae was used to parameterise the one strain bacterial model to a biologically realistic system. The results are consistent with those from the theoretical parameter sets. A tapered treatment regimen maximises the success

of eradicating the bacterial infection while minimising the amount of antibiotic required. Laboratory experiments were performed which provided credibility to the results found.

Finally, the assumption of fixed time intervals between doses was relaxed and the genetic algorithm used to identify both the dose and time intervals of optimal treatment regimens. Varying either the doses or the time intervals separately produced no significant difference in the success of eradicating an infection. When combined, the results showed that significantly better regimens could be identified. These regimens further increased bacterial eradication while using less antibiotic to do so. More work is required to identify a general treatment pattern when both variables are optimised due to the high variability in solutions. However, a shift away from conventional constant dose treatment regimens is required to prolong the future effectiveness of antibiotics.

This research was supported by the University of Stirling via a PhD Impact Collaborative Studentship (Agreement Number DP227AA), and the Centre for Environment, Fisheries and Aquaculture Science (CEFAS).

ACKNOWLEDGMENTS

I want to start by thanking everyone who has supported me in any way throughout this journey. Thank you!

Special thanks have to go to my primary supervisor, Andy, for his mathematical expertise and relentless optimism. I am forever grateful that I was given the opportunity to undertake this PhD. I'd also like to thank my second supervisor, Gabriela, whose knowledge in computing science proved invaluable for this thesis. Thanks must also go to my supervisors from CEFAS, Nick and Craig, who provided the biological expertise and always made me feel so welcome when I visited the lab.

Doing a PhD can be a lonely journey, so I'd like to thank all my fellow PhD students who have provided much needed laughter, food and friendship over the years. A special shout-out to Paul and Adrian who have been here since day one of our Undergraduate degree. To the staff in the Computing Science and Mathematics Department at Stirling, I have thoroughly enjoyed my time at Stirling and you have helped make my time here so enjoyable.

Finally I'd like to thank all my friends and family for their support. To Brian and Craig, I will always be grateful for your friendship, the hikes up Dumyat, office Countdown and the endless laughter. To Jen, who has provided much cake, hot chocolate, Suila dates and Watson duties over the past few months. A huge thank you to my parents who have continued to encourage me to keep pursuing my dreams and my brother for being a continual source of reasoning

when things have seemed impossible. Lastly, I'd like to thank Lisa, Daimen and baby Elliott whose continual support has been unwavering. Sherlock x

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INTRODUCTION

Since their formal discovery in 1928 antibiotics have been at the forefront in the fight against bacterial diseases. However, the increased availability of antibiotics has led to the overuse and often misuse of these substances. This has resulted in a number of diseases such as gonorrhea and tuberculosis becoming increasingly hard to treat due to the emergence of multi-drug resistant bacteria [1, 2, 3, 4, 5]. Resistant bacteria pose significant health and economic burdens and as such have necessitated the research into preventing their spread and prolonging future antibiotic effectiveness. Unfortunately research indicates that the fight against antibiotic resistance will not be won by simply reducing the use of antibiotics [6, 7, 8]. It is estimated that in as little as 20 years we could be returning to a pre-antibiotic era, with antibiotic resistance accounting for approximately 10 million deaths per year globally by 2050 [9].

Antibiotic resistance is not only of great concern within the human population but also has a significant impact within agriculture and aquaculture. With the growth of the population and the increased demand for meat, the use of antibiotics in food animals continues to increase [10]. Antibiotics are used extensively in these industries to treat infections, prevent diseases and promote the growth of livestock. With the overuse of antibiotics linked to increases in resistant bacteria their use in healthy animals to promote growth is controversial [11]. Due to the importance of antibiotics for human health some countries have tight legislation surrounding the use of antibiotics within animal husbandry [12]. However, this is not widespread with antibiotic use in

some countries completely unregulated.

The 'prudent' use of antibiotics has long been recommended [13] as a way in which to slow the spread of antibiotic resistance. However, for the 'prudent' use of antibiotics to be effective in the fight against resistance the treatment regimens under which they are administered must be optimal. Optimal antibiotic treatment strategies consist primarily of two variables: the dose and the duration of treatment. For most antibiotics the drug developer identifies a conventional treatment regimen which is implemented by doctors and veterinary surgeons when prescribing these antibiotics [14]. Conventional treatment regimens usually consist of a fixed dose administered for a specified duration. Drug efficiency studies are used to determine the dose and duration for these treatment regimens. However, one limitation of this approach is that it only provides information for the regimen being analysed and offers no indication for other potential regimens [15]. While conventional treatment regimens may be effective they may not be the optimal duration or dose at which to administer antibiotics to prevent the spread of resistance.

Mathematical modelling uses mathematical terms to represent the behaviour of a real world system. It can be used to develop scientific understanding, predict the effect of change within a system and even aid in decision making. Mathematical models are used extensively in engineering, economics and natural science. Real world systems are very complex and as such a large element of compromise is required when creating mathematical models. By only including the pivotal concepts of a system and excluding the rest, mathematical models are able to simplify these complex systems. In 2001 three mathematical models were used to predict the disease dynamics and inform control measures during the foot-and-mouth outbreak in the UK [16]. As long

as the assumptions and limitations of the model are understood before interpreting the results, mathematical models can be a great asset in comparing and identifying optimal treatment strategies.

Broadly, this thesis therefore aims to combine mathematical modelling with a computational optimisation technique to identify optimal antibiotic dosage regimens which maximise antibiotic treatment success and minimise antibiotic use. This will primarily be done theoretically, but a biological study was carried out to show that the results are credible.

1.1 BIOLOGICAL BACKGROUND

1.1.1 *Bacteria*

First discovered by Anton van Leeuwenhoek in the 1670's bacteria have been found to live virtually everywhere [17, 18, 19]. Bacteria are referred to as the simplest form of life as they are prokaryotic, single celled, organisms. This means that they contain no nucleus or membrane bound organelles and instead their genetic material is contained in a single loop of DNA. (Figure 1.1)

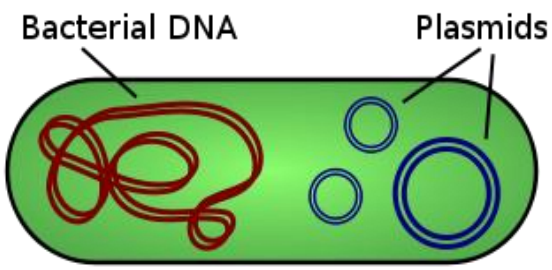

Figure 1.1: A bacterial cell showing the bacterial DNA and the independent plasmids.

Fewer than 100 species of bacteria are estimated to cause infectious diseases in humans [20]. With several thousand species of bacteria existing within the human digestive system alone, the majority of bacteria are harmless. In fact, bacteria make up a large part of the human microbiome. These bacteria are beneficial colonisers and are essential for human development, immunity and nutrition [21, 22, 23]. Many bacteria have been found to not only be harmless but to actually be beneficial to the environment in which they live [24, 25, 26]. Processes such as nutrient cycling, food production and digestion would not be possible without bacteria. Even just the presence of non-pathogenic bacteria can help prevent diseases by occupying places that pathogenic bacteria want to invade [27]. We would not exist without bacteria.

However a small number of bacteria are pathogens and it is these bacteria which can cause disease. Pathogenic bacteria cause disease by either directly destroying tissue cells, becoming so numerous that the host system cannot function or by producing toxins which kill other cells [28]. As bacterial cells contain all the genetic material necessary to reproduce they are able to undertake a simple form of asexual reproduction known as binary fission. During binary fission a cell replicates its DNA and then elongates and splits itself in two, ensuring each daughter cell has a copy of the DNA. This process highlights the ease at which bacterial infections can take hold. With an optimal generation time of 20 minutes it would take 1 bacterium less than 7 hours to replicate to over 1 million cells.

Bacterial cells also contain separate, circular pieces of DNA called plasmids (Figure 1.1). Plasmids are extra-chromosomal DNA elements which exist and replicate independently of the host bacterial genome. They consist of a phosphate backbone typically composed of essential genes which control core

plasmid functions such as plasmid replication, stability and transfer. They also contain genes which are non-essential to the plasmid but may encode selectively advantageous traits to the host cell in certain environments e.g virulence factors, antibiotic resistance and the ability to degrade environmental pollutants [29, 30, 31].

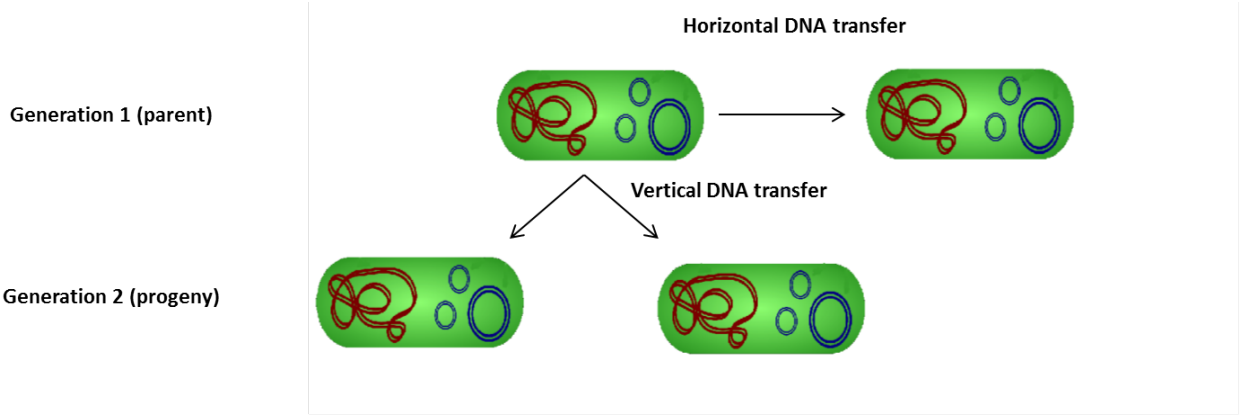

Figure 1.2: Diagram showing the horizontal transmission of plasmids to an unrelated bacterial cell and the vertical transfer of plasmids to both daughter cells during cell division.

Plasmids are able to pass vertically to each new daughter cell along with the DNA during bacterial fission. This means that once a bacterial cell possesses a plasmid its offspring will also possess a copy of that plasmid. An additional feature of plasmids is their ability to not only transfer vertically but also horizontally between bacterial cells (Figure 1.2). Horizontal gene transfer (HGT) is independent of reproduction and means that cells can obtain plasmids at any point in their life cycle. There are 3 main mechanisms: transformation, transduction and conjugation. Transformation is where competent bacteria uptake free DNA from the environment. While in the environment this free DNA is subject to DNase which can break the DNA down. Transduction is the transfer of DNA via a virus. Transduction does not require cell-to-cell contact and, because of the bacteriophage, is protected from DNase. Conjugation is the

transfer of a copy of a plasmid from one cell to another via direct cell-to-cell contact [32].

1.1.2 *Antibiotics*

Antimicrobials are substances that kill or inhibit the growth of micro-organisms, such as bacteria, fungi and viruses. Metals, such as silver and copper, have long been used in medicine and agriculture for their antimicrobial effects [33]. Antibiotics are a sub-set of antimicrobials. They are chemical substances which are used to treat bacterial infections and diseases. Antibiotics can be natural, semi-synthetic or synthetic in origin. The first antibiotic, Penicillin, was discovered in 1928 and paved the way in revolutionising the way bacterial infections were treated. Since then humans have found and synthesised a number of additional antibiotic compounds. The increase in availability of antibiotics has contributed to increased survival rates in areas where bacterial infections are likely complications, such as surgery and cancer chemotherapy [34].

Antibiotics target bacterial cells in two main ways: they prevent the growth and reproduction of the bacterial cell (bacteriostatic) or they actively kill the bacterial cell (bactericidal). The bacteriostatic or bactericidal nature of antibiotics can differ depending on the infection they are being used to treat. Antibiotics can interfere with the cell wall synthesis, inhibit protein synthesis, interfere with nucleic acid synthesis or inhibit metabolic pathways [35]. Targeting structures present in bacterial cells or bacterium-specific targets within processes common to both bacterial and human cells means that the antibiotic will not harm human cells. Some antibiotics can be used to target specific bacteria. Unfortunately it can be time consuming to correctly identify the

bacteria causing the infection and often treatment must be started before it can be identified. Broad spectrum antibiotics target a wide range of bacterial strains and are ideal for initial antibiotic therapy. They can be effective in treating bacterial infections but will also kill harmless and even beneficial bacteria.

While all antibiotics target bacterial cells, it requires a certain concentration of antibiotic to be present before it will negatively impact the bacteria. The minimum concentration of antibiotic required to inhibit visible growth of the bacteria is known as the minimum inhibitory concentration (MIC) point. If the antibiotic is present in a concentration above the MIC of the bacteria then it will be killed off. However, concentrations below a bacteria's MIC threshold results in bacteria persisting despite the presence of antibiotic. This highlights the importance of getting antibiotic prescriptions correct.

1.1.3 *Resistance*

Antibiotic resistance is defined as the ability for bacteria to survive and reproduce in the presence of a higher concentration of antibiotic. This is indicated by an increase in the MIC of the bacteria. Despite the discovery of many more antibiotics, bacteria have evolved resistance to every antibiotic in clinical use [36]. Figure 1.3 identifies the year in which an antibiotic was introduced and the year in which resistance was first observed. Resistant bacteria may still be controlled by antibiotics but a higher dosage will be required. These higher concentrations of antibiotic may be harmful to, or not well tolerated by, humans rendering the antibiotic useless.

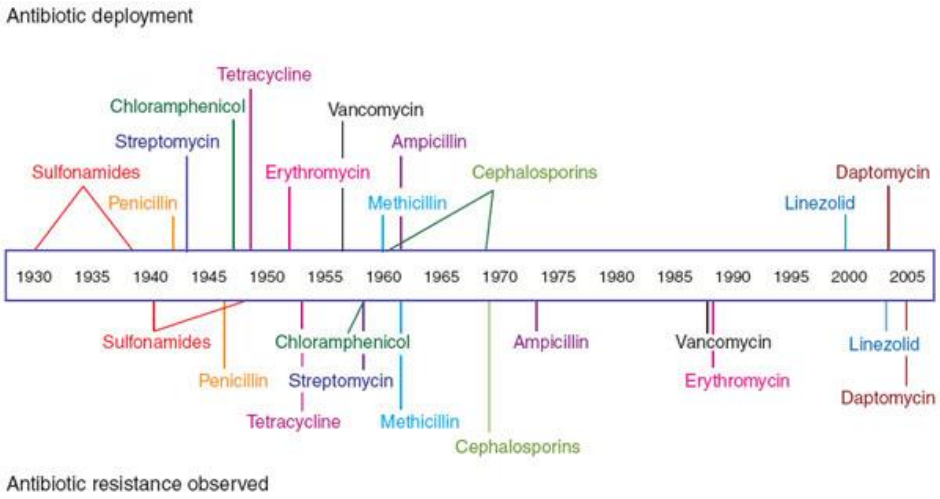

Figure 1.3: Timeline showing the year of introduction of antibiotics on the top and the year resistance was first observed on the bottom. Obtained from Caltworthy et al. (2007).

Resistance can either be a natural (intrinsic) trait of the bacteria or acquired through mutations or gene transfer. Intrinsic resistance, where a cell is naturally resistant, may be due to the bacteria lacking the target site of the antibiotic molecule or possessing an efflux pump which can pump the antibiotic back out of the cell. Baker-Austin et al [37] highlights the possibility that these intrinsic mechanisms may have occurred due to cross-resistance. In the presence of heavy metals, or other toxins, bacteria develop mechanisms to protect themselves but in some cases these mechanisms also work in providing resistance to antibiotics. Acquired resistance requires either mutations in existing genetic material or the acquisition of additional genetic material from another source, such as plasmids. These additional genes can encode for traits that the bacteria did not originally possess without the need to wait for a suitable mutation on the chromosome.

The importance of plasmids and horizontal gene transfer in the development and spread of antibiotic resistance was not initially recognised. It has since become evident that they play a major role [38]. Bacteria which produce antibiotics are generally resistant to the antibiotics they produce. This indicates that it is probable that genes conferring resistance to antibiotics have existed in nature for as long as bacteria have been producing antibiotics. Plasmids are an ideal vector in the spread of these resistant genes, both vertically and horizontally, and are particularly important in the acquisition of resistance to many antibiotics [39, 30]. These traits do come at a disadvantage and it is widely recognised that there is a fitness cost associated with harbouring these resistant plasmids. However, the exact fitness cost imposed by these plasmids is still debated with evidence that this could be close to zero in some cases [40].

Resistant genes do not pose a problem so long as they are contained within non pathogenic bacteria. However, almost immediate resistance to penicillin was recognised after the introduction of the drug in 1946 [41]. This highlighted the ease at which these genes could spread to other bacteria. The extensive use and misuse of antibiotics in human and animal medicine and agriculture has proliferated the spread of these resistance genes within pathogenic bacteria [42, 43, 44].

1.1.4 *Impact in Healthcare*

After the introduction of antibiotics enormous gains were made in healthcare. With the new found ability to effectively treat bacterial infections, advances in transplantation, chemotherapy and more complex surgeries was possible [45]. However, the continued spread of antibiotic resistant bacteria is threatening to see a return to this pre-antibiotic era [46]. Antibiotic resistance not only

affects the health of humans but it also imposes a significant economic burden. Longer hospital stays are associated with antibiotic-resistant infections, increasing hospital costs and limiting resources such as beds [47]. The presence of multi-drug resistant bacteria often requires the use of second or even third line antibiotics which are more costly.

Unfortunately, ensuring that antibiotics are taken exactly as prescribed cannot be guaranteed. Humans will often fail to finish a course of antibiotics due to the alleviation of symptoms and the inaccurate assumption that the bacteria have been successfully cleared. Antibiotics are also only effective against bacteria and as such any use of these substances to treat a viral or fungal infection further contributes to their misuse. To increase the public knowledge on appropriate antibiotic use, antibiotic awareness campaigns (AAC) have been implemented internationally with mixed results [48, 49].

1.1.5 *Impact in Agriculture and Aquaculture*

Antibiotics are used extensively in agriculture. In the US alone it is estimated that antibiotic use within agriculture accounts for 80% of the total consumption of antibiotics [50]. The vast majority of antibiotic use is within livestock, with crops accounting for less than 0.5% of the total amount used. A large proportion of the antibiotics used within livestock are deemed medically important for human health [51]. Antibiotics are used within animals for the same reason they are used within humans: to help fight bacterial diseases and infections. However, antibiotics have also been used to promote growth within livestock. Antibiotic use can alter gut bacteria and cause more rapid growth by allowing feed to be converted to muscle much faster. By improving feed efficiency, antibiotics allow the same amount of meat to be produced with a

smaller number of animals. This in turn provides economic benefits to both the consumer and producer. When used for growth promotion, antibiotics are given at subtheraputic concentrations. With lower concentrations of antibiotics being linked to increases in resistant bacteria the use of antibiotics for growth promotion has been banned in some countries [12].

Aquatic environments are often more supportive to pathogenic bacteria than terrestrial environments and as such are affected by a large number of bacterial diseases [52]. Antibiotics are often administered prophylactically to try and prevent bacterial diseases from arising. This is due to the most common mode of delivery of antibiotics being within feed. If a bacterial disease is present the diseased fish are less likely to feed resulting in under-dosing and the persistence of the bacterial disease. Bacterial diseases can wipe out entire stocks of fish resulting in massive economic losses [53]. In recent years more vaccinations have been developed as a preferred method of disease control. Vaccines offer a better and long lasting level of protection and allow for a decrease in the use of antibiotics. However, as there are some diseases for which a vaccine is not available the need to prolong the effectiveness of antibiotics is important.

The use of antibiotics in both agriculture and aquaculture creates reservoirs of resistant bacteria. Resistant bacteria can enter aquatic environments due to sewage, hospital waste and agricultural run-off resulting in high levels of antibiotic resistant genes present in the environment [54]. These resistant bacteria can then form biofilms on surfaces creating an environment which lends itself to high rates of gene transfer between bacteria [55]. Concern exists over the possibility that genes which confer resistance in bacteria within the environment and in animals may cross over into bacteria present in the human

microbiome [56]. However, there is limited knowledge on exactly how much transfer there is between these systems.

1.2 MATHEMATICAL BACKGROUND

The use of mathematical modelling within antibiotic resistance research has grown considerably over the past few decades. With the ever increasing presence of resistant bacteria and the lack of new antibiotics being manufactured, the future effectiveness of current antibiotics remains uncertain. The use of antibiotics increases the likelihood of resistance developing, with resistance to new antibiotics detected shortly after their introduction into clinical use. It has been stated time and time again that action must be taken to ensure antibiotics are being used optimally, to reduce the overuse and misuse of these substances and ensure their future effectiveness [13, 57, 58].

Previous modelling studies on the emergence and spread of resistance to antibiotics focus mainly on two settings: within-hospital and within-host. Compartmental models are used predominately throughout the literature for modelling in both these settings. Structured compartmental models lend themselves to this field of research as it is possible to include additional compartments to address the complexity of the biological system. At a basic level antibiotic resistance research focuses on the change in population size of a susceptible and resistant population and the vectors which facilitate these changes. By using a system of coupled ordinary differential equations analysis of the system both analytically and numerically is possible. This can provide qualitative predictions which allow for evaluation of different interventions to reduce the spread of resistance.

1.2.1 *Mathematical Models of Antibiotic Treatment - Within-hospital*

Antibiotic-resistant infections are an increasing threat to society and have become a menace in hospital settings. The prevalence of infections such as, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) continue to rise, increasing hospital stays, morbidity and mortality [59, 60]. By understanding the dynamics of the spread of these infections it is possible to develop and predict strategies to prevent further spread of resistant bacteria. Within-hospital models tend to focus on the spread of antibiotic resistant infections between patients within a single ward setting e.g. ICU, or in a simplified hospital setting. Despite the focus being on a single ward or hospital these studies don't consider them as a closed system. Patients are often admitted from and discharged into a general community. There is an entire subset of within-hospital modelling papers which focus on controlling the spread of resistant infections through different practices. Strategies such as hand-washing and limiting the number of patients each nurse is responsible for are undoubtedly an important aspect in controlling hospital infections. However, this thesis is focused on the impact of differing antibiotic usage patterns.

Bonhoeffer et al. [61] initially presents a general mathematical model to consider the impact a single antibiotic has under various treatment patterns. This study considered three sub-populations of individuals: uninfected, infected with susceptible bacteria and infected with resistant bacteria. They found that when a single antibiotic is used the total reduction of infected hosts is almost independent of the pattern in which the antibiotic is administered. With a slight increase in total reduction of infected hosts only if the antibiotic was used extensively at the beginning of treatment. The model was then exten-

ded to examine the effect treatments with multiple antibiotics would have. The resistant compartment was split into 3 new compartments: resistant to antibiotic A, resistant to antibiotic B and resistant to both antibiotics. Three treatment strategies were compared: cycling, where antibiotics are alternated; 50-50 mix, where equal proportions of the infected host population receive each antibiotic; and combination, where antibiotics are given simultaneously to each infected host. When comparing the total reduction of infected hosts, cycling the antibiotics was always less beneficial than using the antibiotics in a 50-50 mix. Whether a 50-50 mix or combination of antibiotics is superior depends on a relationship between the fraction of patients that acquire resistance in response to single and combination treatment.

Lipsitch et al. [62] considers a model similar to that by Bonhoeffer et al. However, it varies slightly from that used by Bonhoeffer et al. in that resistance is only observed to one of the antibiotics given. The bacteria are completely susceptible to the other antibiotic and remain that way. They consider the impact the rate at which each antibiotic is used has on the prevalence of resistance to antibiotic 1. Unsurprisingly, they predicted that an increase in antibiotic 1 increased the number of individuals colonised with bacteria resistant to that antibiotic. However, increasing the use of antibiotic 2 decreased the number of individuals colonised with bacteria resistant to antibiotic 1, to the point of extinction. Suggesting that by switching to an antibiotic to which no resistance is present can decrease the prevalence of bacteria resistant to another antibiotic. Despite the use of antibiotic 2 reducing the prevalence of resistance to antibiotic 1 at a population level, when individuals were tracked according to the treatment they had received the results differed. At an individual level, patients treated with antibiotic 2 are more likely to be colonised with bacteria resistant to antibiotic 1 compared to those who have not been treated with

antibiotic 2. This suggests that care must be taken when interpreting results of proposed interventions.

With suggestions that cycling antibiotics may slow the emergence and spread of resistant bacteria, Bergstrom et al. [63] takes another look at the potential of this treatment pattern. Patients are considered to either be uncolonised by the bacteria in question or colonised with either susceptible bacteria, bacteria resistant to antibiotic 1 or bacteria resistant to antibiotic 2. The effect antibiotic cycling has on the emergence and spread of the resistant bacterial populations is examined. They find that cyclic use of antibiotics results in a cyclic pattern within the frequency of each bacterial strain. With each change in antibiotic the frequency of resistance to that antibiotic increases with the frequency of resistance to the other antibiotic declining. At each change the new antibiotic is temporarily more effective due to the low rate of resistance present. This results in the number of uncolonised patients briefly surging. By comparing the average fraction of patients carrying resistant bacteria under a cycling treatment protocol to an alternative 50-50 mix protocol, Bergstrom et al. determines if cycling antibiotics is indeed more effective at reducing resistance. Bergstrom et al's findings support the claim from Bonhoeffer et al. that antibiotic cycling is unlikely to reduce the spread of antibiotic resistance with mixing predicted to be more effective.

Obolski and Hadany [64] once again examine the effects of the three prominent antibiotic strategies: cycling, mixing and combining. They note that previous studies assume that patients acquire resistant bacteria at a constant rate. With evidence suggesting that the frequency of horizontal gene transfer and mutation increases when bacteria are under stress, they re-evaluate these treatment protocols under this new assumption. Their findings are in keeping

with combination therapy being more efficient when comparing the decrease in the number of patients colonised with an infection resistant to a single antibiotic. However, they show that stress-induced genetic variation leads to combination therapy performing poorly in inhibiting the emergence of resistance to both antibiotics. In fact, their findings suggest that cycling antibiotics is the preferred protocol when resistance is acquired through stress-induced mutation.

With attempts to compare the different treatment strategies empirically resulting in inconclusive results [65, 66, 67]. The debate over the optimal strategy continues. Tepekule et al. [68] took a slightly different approach to determining the optimal treatment strategy. Using a mathematical model similar to those used by Bonhoeffer et al. and Bergstrom et al., they consider the three multi-drug strategies and two mono-drug treatments. They determine which treatment strategy is the best for a large range of parameter sets by using linear discriminant analysis and particle swarm optimisation. Comparing all five strategies, combination therapy was found to be the best strategy in over half the parameter sets. Where mono-drug therapies were not beneficial, combination therapy performed better than both cycling and mixing 70% of the time. In addition, the results showed that mixing antibiotics tends to perform better than cycling them. Where combination therapy did not perform as well, the parameter regions were generally found to be more biologically unrealistic.

Despite the increase in use of mathematical models to study antibiotic resistance within a hospital setting, the use of these models to examine antibiotic usage strategies is limited. The models have largely focused on the use of two antibiotics used in a cyclic, mixed or combination protocol with the results proving inconclusive. One limitation of modelling at the hospital level is that

there is little information on how the antibiotics affect the dynamics of the bacteria they are targeting. Patients are also assumed to possess only one strain of bacteria at a time. By focusing on the individual level, it is possible to examine the effect different antibiotic treatments have on the dynamics between multiple bacterial strains within the same host. This provides the opportunity to look at improving the rate of clearing an infection and minimising the emergence of resistant strains.

1.2.2 *Mathematical Models of Antibiotic Treatment - Within-host*

The understanding that plasmids play a major role in the spread of resistance genes between bacterial species opened the door for modelling the spread of antibiotic resistance on a bacterial level, inside an individual host [69]. Plasmids have been well studied within literature with several modelling studies examining the conditions under which plasmids can be maintained within a bacterial cell [70, 71, 72]. The spread of plasmid mediated resistance, where all plasmids are assumed to be carrying the resistant gene, can be modelled as a simple SI model (Figure 1.4). The plasmid-free cells are regarded as the susceptible compartment and the plasmid-bearing cells the infected (resistant) compartment. Introducing resistance as a selective advantage within an antibiotic environment, it is possible to examine the effectiveness of antibiotic treatments and the spread of resistance through the population. This simple SI model represents the basis of most of the following studies and the basis upon which the models within this thesis were based.

D'Agata et al. [73] examine a series of models building up from an entirely susceptible bacterial population with an immune response, through a population of susceptible and resistant bacteria where resistance is mediated by

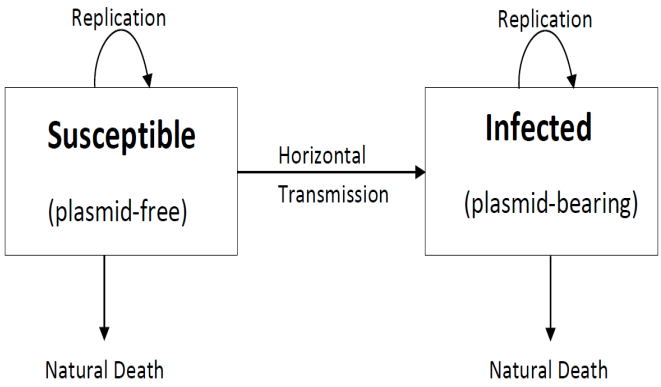

Figure 1.4: Schematic representation of a simple susceptible and infected model of bacteria. Resistance to antibiotics is contained within a plasmid. The susceptible compartment is plasmid-free representing a susceptible bacterial population. The infected compartment is plasmid-bearing representing a resistant bacterial population.

horizontal gene transfer, to a population of susceptible, resistant and multi-drug resistant bacterial strains. By applying various treatment strategies of single and multiple antibiotics they make three main conclusions. Firstly they conclude that shorter duration of antibiotic therapy or an early interruption in therapy result in resistant strains progressing. Secondly they compared the results of using two antibiotics in a sequential regimen to that of a combination regimen. The combination regimen prevents the emergence of the resistant strain compared to the sequential treatment. Finally they concluded that one of the most important factors in preventing the emergence of resistant bacteria is the early initiation of antibiotic treatment.

One assumption which is present in the above study is that antibiotics, when present, are present at a constant rate. This assumption means that while the pattern and length of antibiotic treatment can be altered, there is no information regarding different dosage levels or the clearance of the antibiotic by the

hosts system. Geli et al. [74] incorporate pharmacodynamics into their model of susceptible and resistant bacteria by considering the antibiotic-induced death rate to be a function of the concentration of antibiotic present. This allows for comparison of treatments of different concentrations and durations of antibiotic exposure. Geli et al. examine four different ecological dynamics of bacteria, which they refer to as: unregulated, regulated, opportunistic and self-limiting. They find that all antibiotic use increases the selection of resistance, regardless of the treatment regimen. However, the length of treatment at which selection of resistance is most intense varies depending on the bacterial dynamic. Shorter durations of treatment are found to be optimal in preventing selection of resistant bacteria in most cases but increased concentration and duration see the time with symptoms decrease. The dosing strategies optimal for clinical treatment may not be optimal for preventing the spread of resistance. By considering the bacterial populations as four different dynamics Geli et al. identify that "one-size" does not fit all.

Despite incorporating a concentration function into their model, Geli et al. only considered a constant concentration of antibiotic. While this may be a realistic scenario in the case of intravenous antibiotics, the majority of antibiotic regimens involve taking a set dose at set time intervals. This leads to the fluctuating concentration of the antibiotic within the hosts system and has a potential impact on the treatment of infections and the emergence of resistant bacteria. Ankomah and Levin [75] address the issue of a constant concentration of antibiotic by assuming that when antibiotics are not being added to the system the concentration of antibiotic declines exponentially. Further reduction in the concentration of antibiotic is due to antibiotics flowing away from the site of infection. With the concentration of antibiotic varying throughout treatment, they study the impact different doses and frequencies of

administration affect the time to clearance of the bacteria and the rate of evolution of resistance. Ankomah and Levin predicted that as the concentration of dose increases the time to eradication of the infection and the emergence of resistance decreases. They do highlight that antibiotics can produce unwanted side-effects at higher concentrations and so increases in concentration may not always be possible. In addition, the benefits of increasing the concentration of antibiotic reach a saturation point above which further increases have little to no effect on the time to eradication or emergence of resistance. When analysing the effect of different frequencies of administering the antibiotics, provided the concentration of antibiotic was sufficiently great there was little effect on the rate of clearance of the infection. Their findings support the 'hit hard and hit fast' approach to antibiotic treatments.

The spread of antibiotic resistance continues to threaten the use of antibiotics to treat bacterial diseases. By modelling the dynamics of different bacterial populations, the above studies were able to consider the impact varying the dose, duration or frequency of antibiotic doses had on the eradication of the infection and potential emergence of resistance. While these studies may identify treatments which are more effective, they all limit their search to treatments with a constant concentration of antibiotic in each dose. Treatments of this pattern are the conventional way to administer antibiotics but there is no reason, other than convenience, that this pattern is used. By limiting the pattern of treatment there are potentially better treatment regimens which are not being considered.

1.2.3 *Mathematical Models and Optimising Antibiotic Treatment*

The use of optimisation techniques alongside mathematical modelling allow for treatment regimens to be considered that may otherwise have been overlooked. These techniques have been useful for identifying potential treatment strategies in areas such as cancer chemotherapy and HIV treatment [76, 77, 78, 79]. The following studies use an optimisation technique to identify optimal antibiotic treatment regimens.

Pena-Miller et al. [80] consider a set up where a 'commensal' bacterial strain are forced to compete against a fitter 'pathogenic' bacterial strain. In the absence of antibiotic or the over-deployment of antibiotic, the pathogen would out compete the commensal bacteria. Resistance can only be acquired through mutations at the point of cell division and not via any other mechanisms such as gene transfer. Constructing a mathematical model of this system they find that all fixed-dose antibiotic treatment regimens lead to the eventual loss of the commensal bacteria. However, by using optimal control theory they show, theoretically, that there exists antibiotic pulsing treatment strategies which select against the pathogen while supporting the commensal bacteria. In this paper they do not consider the eradication of pathogens but do propose that single-drug treatments could be successful in eradicating the pathogen. They suggest that such treatments would be dynamic in time and may well consist of pulses of antibiotic.

Imran and Smith [81] use optimal control theory to identify antibiotic treatment regimens which ensure eradication of bacteria in a biofilm and surrounding fluid while minimising the amount of antibiotic applied. Using a numerical example they first examine periodic discrete dosing regimens.

They identify that there exist solutions to their model but these are sensitive to changes in initial bacterial populations. Higher initial populations will result in the bacteria not being entirely eradicated. However, they identify that increasing the concentration of antibiotic or reducing the period between doses can turn some of these failures into successes. Using optimal control theory they identify that cycling between applying and withdrawing the antibiotic in decreasing dosages is the optimal treatment course. Varying initial conditions and parameter values they explore a range of optimal dosing strategies. The optimal dosing strategy is shown to effectively eradicate the bacteria in cases where the periodic discrete dosing was unsuccessful.

The following study was published after the paper by the author of this thesis [82], which showed similar results. Khan and Imran [83] take a similar approach to Imran and Smith but Khan and Imran consider the presence of resistant bacteria. By modelling the dynamics of a susceptible and resistant bacterial population they use this model to identify treatment regimens which eradicate the bacteria while minimising the amount of antibiotic used. Administering antibiotics at periodic intervals with reducing dosage strengths (tapering) is one way to reduce the amount of antibiotic being used compared to discrete constant dosing intervals. However, if the dose strength is reduced too much the bacteria are able to re-emerge and the treatment fails. Using optimal control theory they found that a high initial dose followed by a gradual withdrawal of the antibiotic not only keeps treatment costs down but eliminated both the susceptible and resistant bacteria for a wide range of initial conditions. The concentration profile for the optimal strategy is similar to that obtained from the tapering strategy but ensures the correct dose strength is used. Care must be taken when assigning costs to the terms within the control function. If too high a cost is placed on minimising the amount

of antibiotic used it was found that it may result in the bacteria not being eradicated in favour of reducing the amount of antibiotic used. A finding from their model was that resistant bacteria could not persist without susceptible bacteria, therefore eliminating the susceptible bacteria was sufficient to eradicate both bacterial populations.

The above studies indicate that more effective antibiotic treatment regimens exist but it requires a move away from discrete constant dose treatments. Optimal control theory allows for these alternative treatments to be identified. However, these studies assume that it is possible to control the concentration of antibiotic within the host system at all times. By controlling the concentration of antibiotic Khan and Imran found that a sufficiently high initial dose followed by a decrease in concentration would eradicate an infection while minimising the amount of antibiotic used. With most antibiotic treatments consisting of a discrete dose followed by a period of withdrawal of the antibiotic, the question of how these optimal treatments would be achieved remains to be answered.

The following study was again published after the paper by the author of this thesis but is included as the only other known use of a genetic algorithm (GA) within antibiotic treatment regimen optimisation. Cicchese et al. [84] explore the use of two other optimisation techniques, genetic algorithms and surrogate-assisted optimisation through radial basis function (RBF) networks. Using a model of granulomas in a *Mycobacterium tuberculosis* infection, they optimise a treatment regimen using a single antibiotic (this is repeated for two different antibiotics). The optimisation techniques are used to identify the dose size and the dosing frequency which eradicates the bacteria quickly while keeping antibiotic dosages low. The search space is constrained to five

different dose sizes and seven dose frequencies giving 35 different treatment regimens for each antibiotic. The solutions are known for both antibiotics so comparison between the two optimisation techniques can be made. They find that the GA accurately identifies the optimal treatment regimen every time it is run for one of the antibiotics and in almost all the runs for the second antibiotic. In contrast, the RBF network is unable to accurately predict the optimal solution for either antibiotic but most of the solutions are within the same region as the optimal solution. Cicchese et al. then go on to optimise the treatment regimen when both antibiotics are given simultaneously. For this they only consider the use of the RBF networks. This is due to the GA being more computationally expensive and their overall goal being to identify regions of space rather than unique locations where the treatment design is optimal. They show the potential for RBF networks to be used to guide experimental testing of new antibiotic regimens.

Cicchese et al. highlighted the ability for optimisation techniques to be used to identify realistic dosing regimens which eradicate bacteria while minimising the concentration of antibiotic being used. The genetic algorithm was shown to be effective at identifying the optimal treatment regimen from a range of possible solutions. However, these solutions were constrained to the standard constant dose treatments typically used for antibiotic treatment. The previous studies using optimal control theory highlighted that better dosing regimens can be found by moving away from these constant dose regimens. By allowing a genetic algorithm to search through all possible treatment regimens it may be possible to identify realistic non-constant dosing regimens which optimise the use of antibiotics.

1.2.4 *Genetic Algorithms*

The Genetic algorithm (GA) was first invented by John Holland in the early 1970's. GA's belong to the larger class of evolutionary algorithms which generate solutions to optimisation problems using techniques inspired by natural evolution, such as inheritance, mutation, selection and crossover [85]. Despite being a randomised search GA's are by no means random, instead they use historical information to direct the search into the region of better performance within the search space.

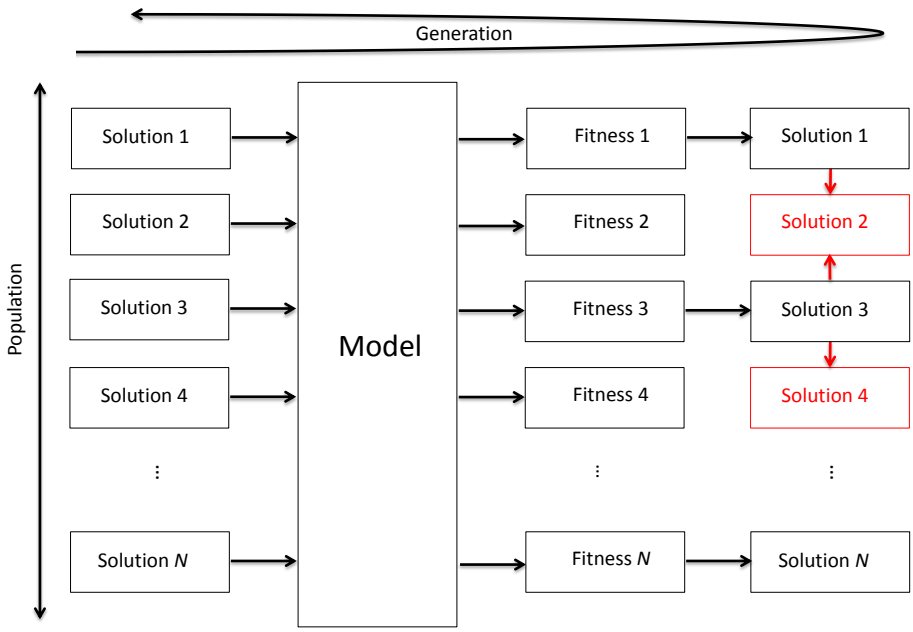

Figure 1.5: Schematic outline of one generation within a genetic algorithm.

Genetic algorithms work by firstly generating a random initial population (or set) of possible solutions. In this thesis, the solutions will be a vector of real numbers or integers. Each solution is put into the (mathematical) model and a fitness score is given based on a defined objective function. The algorithm performs a process of fitness-based selection and recombination to create a successor population [86] (Figure 1.5). To create the next generation of solu-

tions, the algorithm uses a defined fitness value to rate the members of the current population. 'Parents' are selected from the current population to create the next generation. The probability of a solution being chosen as a parent is given by (1.1). Therefore the fitter the solution, the more likely it is to be selected as a parent.

$$p_i = \frac{\text{Fit}(i)}{\sum_i \text{Fit}(i)} \tag{1.1}$$

Children are produced from the parents in one of three ways: by taking the best solutions from the current population (elite child), by combining the vector entries of a pair of parents (crossover child) or by making random changes to a single parent (mutation child). Elite children are produced first. A pre-determined number of the fittest parents are taken forward as children. These solutions will remain unchanged. Next the crossover children are produced. A crossover fraction determines the fraction of the new population, excluding elite children, which will be made from combining the entries of two parent solutions using a random binary vector of length equal to the length of the solution vectors. The crossover child is created by taking the element, 'gene', from parent 1 if the entry in the binary vector is a 1, otherwise the gene from parent 2 is taken. The remaining children are made up of mutation children. To produce a mutation child the GA adds a random number to each element of the parent vector. This random number is chosen from a Normal distribution with mean 0. The standard deviation is not fixed and is reduced linearly in every generation until it reaches 0 in the final generation. This ends the first generation within the GA. This new population of solutions is then rated using the defined objective function and the process repeats to form the next generation of solutions. This process is repeated until a stopping criteria is met, either a set number of generations have been reached or the fitness of

the dominant solution cannot be improved.

Genetic algorithms are a stochastic search algorithm so the GA is run multiple times. Each run takes a randomly chosen initial population of solutions. This ensures that the search space is adequately searched and that it does not get stuck at a single local optimum.

1.3 AIM OF THESIS AND CHAPTER PLAN

One solution to the problem of antibiotic resistance is sought in the discovery of new antibiotics. With resistance to previous antibiotics emerging within a few years of their introduction, new antibiotics would also be destined to failure eventually. However, if a new antibiotic was introduced and used optimally the effectiveness of this antibiotic could be prolonged. Optimal treatment strategies could also halt further emergence of resistance to current antibiotics and prolong their effectiveness.

The use of mathematical models to identify antibiotic treatment regimens is growing. However, one major assumption made within these studies is that antibiotic treatments follow a conventional pattern of X units for N days. When searching for better treatment regimens the constant dose pattern is not challenged.

Previous studies have shown the potential in combining the use of mathematical models with a genetic algorithm to identify optimal treatment regimens in areas such cancer chemotherapy [76, 78]. However, their use within antibiotic research is very limited. Other than the work published by the author of this thesis, the only other known use of a genetic algorithm to optimise

antibiotic treatment strategies constrained the GA to conventional constant dose treatment regimens [84]. So the question remains: what are the optimal doses and duration of antibiotic treatments to minimise the emergence of resistant bacteria?

This thesis therefore aims to use a genetic algorithm approach to identify antibiotic treatment regimens which maximise the success of eradicating infections while minimising the total quantity of antibiotic used.

Chapter 2 develops a mathematical model of the dynamics of a single bacterial strain in the presence of an antibiotic environment. This model is incorporated into a GA to provide a systematic approach to identify optimal treatment regimens which maximise the success of eradicating a bacterial population.

Chapter 3 expands the single strain bacterial model to include the presence of a resistant population. The GA is once again used to optimise antibiotic treatment regimens which maximise the success of eradicating an infection while minimising the amount of antibiotic required. The effect the presence of resistant bacteria has on the optimal treatment pattern identified by the GA is examined.

Chapter 4 takes the work from Chapter 2 and parameterises it to a biological system. A case study consisting of a *Vibrio anguillarum* infection within the larvae of the greater wax moth (*Galleria mellonella*) treated using Tetracycline is studied. The model is parameterised to this system using data from laboratory experiments. The GA is used to identify the optimal treatment regimen to maximise the survival rate of the larvae. Further laboratory experiments are

conducted to test whether the optimal treatment regimen increases larval survival as predicted.

Chapter 5 relaxes the assumption from the previous chapters that antibiotics are given at daily time intervals. Using the model developed in Chapter 3, this chapter explores the effect changing the interval between doses has on the treatment regimens identified by the GA. The GA will initially be used to optimise the time interval between a constant dose treatment regimen to examine whether optimising the dose or the time interval is more effective at eradicating the bacterial infection. The GA will then be extended to identify both the dose and corresponding time vector of the optimal treatment strategy. The results will be analysed to examine whether optimising the time interval between optimal doses can further increase the success of treating a bacterial infection using less antibiotic.

Chapter 6 summarises the results from this thesis and discusses the global context of these results. The limitations of the modelling work carried out and the predictions made are also discussed along with any potential further work.

2

OPTIMISING ANTIBIOTIC TREATMENT REGIMENS TO TREAT BACTERIAL INFECTIONS: A GENETIC ALGORITHM APPROACH

2.1 INTRODUCTION

Bacteria are essential for sustaining both plant and animal life. They are able to thrive in a diverse range of environments, from up in the stratosphere to the depths of the ocean. The human body is colonized with approximately the same number of bacterial cells as it has human cells [87]. Bacteria play an important role in a number of processes, such as, recycling nutrients in the soil, digestion of food and even cleaning oil from aquatic environments [88, 89, 25, 26]. Humans have also managed to harness the properties of bacteria and use them to their advantage. Production of products such as insulin can be genetically engineered by incorporating the human genes into bacteria [90]. However, not all bacteria are beneficial or even harmless. Pathogenic bacteria can invade host cells using them for nutrients, produce toxins which kill cells or even trigger an inappropriate immune response. Either directly, or indirectly, pathogenic bacteria damage the host cells. If an infection of pathogenic bacteria is allowed to multiply the host will begin showing signs of disease. If left untreated the damage done by pathogenic bacteria can result in the death of the host.

Neisseria meningitidis and *Yersinia ruckeri* are just two examples of pathogenic bacteria which not only cause significant health burdens but can also cause economic burdens. *Neisseria meningitidis* is responsible for meningococcus meningitis among other meningococcus diseases in humans. Without treatment the chance of surviving meningococcus meningitis is only 50%, with 10% to 20% of those survivors being left with brain damage, hearing loss or disability [91, 92]. *Yersinia ruckeri* is the causative agent of enteric redmouth disease (ERM) in various species of salmonids worldwide. Despite the use of vaccines, outbreaks of ERM have been identified in vaccinated fish [93, 94, 95]. If left untreated, later stages of the disease see erosion of the jaw and palate, haemorrhaging of internal organs and death. This can lead to the loss of entire stocks of fish. Antibiotics help minimise the impact of pathogenic bacteria by treating the infection, killing the bacteria and saving the host.

When treating bacterial infections guidelines identify which antibiotic is effective against which bacteria. They also indicate the dose and duration of treatment that should be prescribed. However, treatment protocols vary globally and are not routinely updated [96, 97]. Most prescriptions for antibiotics follow the same pattern: X units for N days. The amount of antibiotic taken each day is constant and this is continued for a set number of days. These conventional constant dose regimens are convenient for both patients and manufacturers as all tablets are identical. While these conventional treatment strategies may be effective, the narrow range of possible regimens being considered means that more efficient regimens could be overlooked. The downside to this lack of comparison is that current treatments may be using more antibiotic than necessary, achieving higher concentrations of antibiotic than required or even not achieving adaquate concentrations. While development of resistance to antibiotics may be inevitable, both increased use and sub-optimal

concentrations of antibiotics have been identified as promoters of resistance [98, 99, 42]. Identifying optimal treatments before resistance emerges could help delay this process.

Using conventional treatment regimens as a baseline, this chapter aims to optimise the usage of antibiotics by identifying alternative treatment regimens which are more effective at successfully treating a bacterial infection. A mathematical model of the dynamics of a single bacterial strain in the presence of an antibiotic environment will be developed. Incorporating this mathematical model into a genetic algorithm provides a systematic approach for identifying more effective treatment strategies. Comparison between conventional treatment regimens and these alternative regimens will highlight if more effective treatment strategies can be found by moving away from the current fixed (daily) dose, fixed duration approach. The presence of resistance is not considered until Chapter 3.

2.2 MODEL DEVELOPMENT

Ordinary differential equations (ODE's) have been used extensively within the literature to describe bacteria dynamics [100, 101, 102, 103, 74, 80, 81, 83]. This section starts by building a simple model to describe the behaviour of a population of bacterial cells. The model is then extended to include the presence of an antibiotic, indicated by the addition of an antibiotic-induced death term. In keeping with much of the literature it is initially assumed that the concentration of antibiotic within the system is fixed. The antibiotic-induced death rate is therefore constant and analytical analysis of the system is possible. The model is finally extended to include an additional compartment to model the change in antibiotic concentration over time. This allows for

the antibiotic-induced death rate to vary depending on the concentration of antibiotic present. This section finishes by creating a stochastic framework of the model.

2.2.1 *Bacterial Growth*

Bacteria are known to undergo a process of reproduction known as bacterial fission. Bacterial fission is an asexual process during which a single bacteria cell divides into two identical daughter cells. As asexual reproduction requires energy, it is assumed that the growth of the bacteria is limited by an environmental carrying capacity. When grown in a closed system bacterial growth can be split into 4 distinct stages: lag phase, exponential phase, stationary phase and, eventually, a death phase (Figure 2.1). During the lag phase the bacteria are active and establishing themselves but there is no growth. Once established the bacteria enter the exponential phase where they are dividing by binary fission. As the available resources begin to deplete and waste products begin to accumulate the rate of growth declines. Bacteria then enter the stationary phase where the number of dividing cells equal the number of dying cells. Eventually the lack of resources and increase in waste results in the bacterial cells dying. This shows in the sharp decline in population growth seen in the death phase.

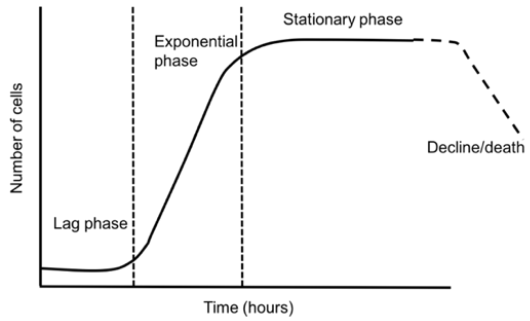

Figure 2.1: Phases of a typical bacteria growth curve.

In a host, it is assumed that the resources present would not deplete to a level that would result in the bacteria reaching the death phase before the death of the host (or treatment begins). Therefore only the first three stages are considered when modelling the growth of the bacteria. The logistic equation has been used in previous modelling studies to model the bacterial growth curve [104, 101, 105, 103]. This study therefore modelled the growth in bacteria number, B, using the standard logistic growth equation, with a growth rate r, environmental carrying capacity K and a density-independent death term (θ). A simple model (2.1) represents the dynamics of a single strain bacterial population.

$$\frac{dB}{dt} = rB\left(1 - \frac{B}{K}\right) - \theta B \tag{2.1}$$

2.2.2 *Introducing Antibiotic-Induced Death*

In keeping with experimental data [106, 107, 108, 109] the relationship between the rate of bacterial death due to the concentration of antibiotic present follows a sigmoid curve (Figure 2.2). For all bacteria the minimum inhibitory concentration (MIC) point is the minimum concentration of antibiotic required to inhibit the growth of the bacteria. The MIC is identified as the concentration of antibiotic where the antibiotic induced death rate is equal to the maximum net growth rate of bacteria, $B_{max} = r - \theta$. Above the MIC point the bacteria are actively killed by the presence of the antibiotic until eradication. The antibiotic induced death rate increases as the concentration of antibiotic increases. However, as it follows a sigmoid curve the antibiotic induced death rate will eventually reach a saturation rate (A_{max}) despite the continued increase in antibiotic concentration.

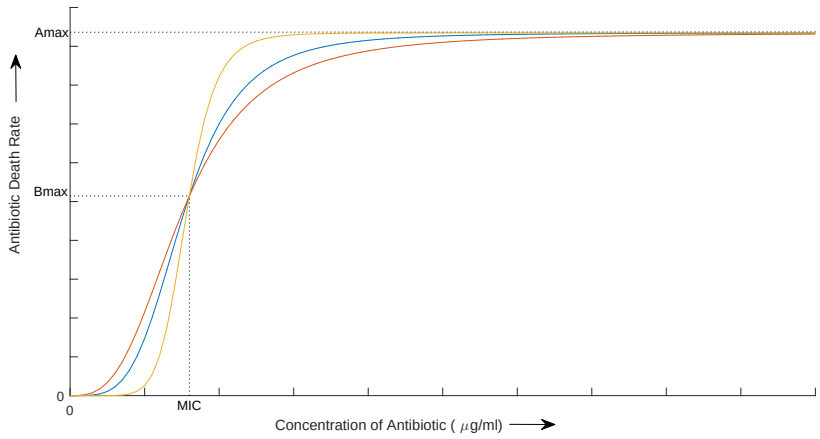

Figure 2.2: Graph showing varying sigmoidal relationships between antibiotic-induced death rate and concentration of antibiotic. A_{max} represents the maximum antibiotic-induced death rate and B_{max} indicates where the antibiotic-induced death rate is equal to the net growth rate of bacteria, this occurs when the concentration is equal to the MIC.

The antibiotic induced death rate for the bacteria, $A(C)$, is therefore a function of the concentration of antibiotic present. To model the relationship between antibiotic concentration and antibiotic induced death rate an extension of the Emax model of antibiotic treatment by Regoes et al [110] was used (2.2).

$$A(C) = \frac{A_{max}\left(\frac{C}{mic}\right)^k}{\left(\frac{C}{mic}\right)^k + \left(\frac{A_{max}}{B_{max}} - 1\right)} \tag{2.2}$$

As $C \to \infty$, $A(C) \to A_{max}$ and if $C = mic$, $A(C) = B_{max}$. Using (2.2) allows for pharmocodynamic information to be used in parameterising the antibiotic-induced death function. The MIC of the bacteria (mic), net growth rate of the bacteria (B_{max}) and maximum antibiotic induced death rate (A_{max}) are all utilised. These are all parameters which could be obtained from experimental results. The hill coefficient, k, is a measure of the steepness of the sigmoid relationship between A and C. This allows for a wide range of concentration death profiles to be modelled. As k varies, the steepness of the curve changes

but the curve still passes through the MIC point at B_{max} and heads towards A_{max}.

2.2.2.1 *Assuming a Fixed Concentration of Antibiotic*

Initially the concentration of antibiotic within the system is assumed to be a fixed rate. Equation (2.1) can then be extended to include the antibiotic-induced death term to give (2.3). With the value of $A(C)$ constant, analytical analysis of the system was carried out.

$$\frac{dB}{dt} = \underbrace{rB\left(1-\frac{B}{K}\right)-\theta B}_{\text{Natural Growth}} - \underbrace{A(C)B}_{\text{AB Death}} \tag{2.3}$$

The steady states of the system can be identified using stability analysis. At equilibrium, $\frac{dB}{dt} = f(B) = 0$, there are two stability points:

$$\begin{aligned} & B\left[r\left(1-\frac{B}{K}\right)-\theta-A\left(C\right)\right]=0 \\ \Longrightarrow\ & B=0 \ \text{ or} \\ & B=B^{*} \ \text{ where } \ B^{*}=K\left(1-\frac{\theta+A\left(C\right)}{r}\right) \end{aligned}$$

Stability of the equilibrium points is found by calculating the derivative of $f(B)$ (2.4) at each of the equilibria.

$$f^{'}(B) = r-\frac{2rB}{K}-\theta-A\left(C\right) \tag{2.4}$$

1. At the extinction equilibrium, $B = 0$, (2.4) is reduced to (2.5).

$$f^{'}(B) = r-\theta-A\left(C\right) \tag{2.5}$$

   The extinction equilibrium is stable when $f^{'}(B) < 0$, so when $r-\theta < A\left(C\right)$. When the death induced by the presence of antibiotics is greater than the natural net growth of the bacteria the system will tend to extinction. This is achieved when the concentration of antibiotic is greater

than the MIC of the bacteria, i.e. $C > mic$.

2. When evaluated at $B = B^*$, (2.4) is reduced to (2.6)

$$f'(B) = r - 2r\left(1 - \frac{\theta + A(C)}{r}\right) - \theta - A(C)$$

$$f'(B) = \theta + A(C) - r \tag{2.6}$$

For B^* to be a stable equilibrium point, $f'(B) < 0$ so $r - \theta > A(C)$. This condition will be satisfied if the concentration of antibiotic is less than the MIC of the bacteria, i.e. $C < mic$. If the concentration of antibiotic is not sufficient that the antibiotic induced death rate is greater than the natural net growth of the bacteria then the bacteria will establish a population even in the presence of an antibiotic.

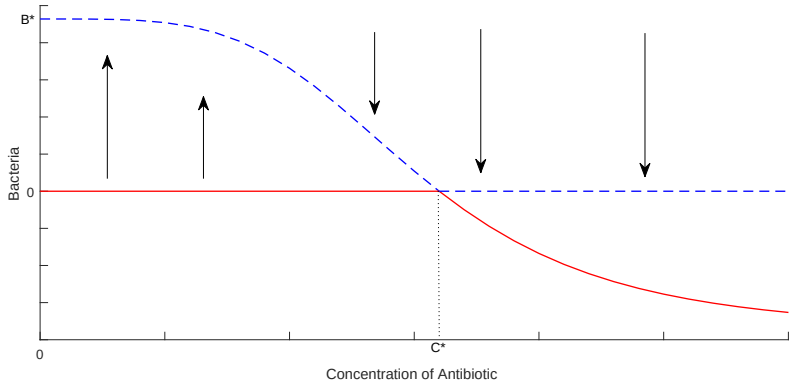

Figure 2.3: Bifurcation diagram for the single bacterial strain model with a fixed concentration of antibiotic. The blue dashed lines represent stable equilibria and the red solid lines the unstable equilibria. At C^* a bifurcation point exists.

Figure 2.3 shows the equilibria as a function of the concentration of antibiotic C. The blue dashed lines represent the stable equilibria and the red solid lines represent the unstable equilibria. At C^* a bifurcation point exists. Below C^* the equilibria at $B = 0$ is unstable, while the equilibria at $B = B^*$ is stable.

Above C^* the equilibria at $B = 0$ is stable, while the equilibria at $B = B^*$ is unstable. C^* represents the concentration of antibitoic at which $A(C) = r - \theta$, also known as the MIC point. For this system to be biologically feasible $r > \theta$ and $r, \theta, A(C) > 0$. Therefore, below the MIC point the bacteria are able to persist even in the presence of antibiotics but above the MIC the bacteria will be eradicated.

2.2.2.2 *Assuming a Varying Concentration of Antibiotic*

Maintaining a constant concentration of antibiotic within a system is only possible in very limited circumstances. Antibiotics are more commonly administered in set doses at certain time intervals. This method of delivery means that the concentration of antibiotic varies throughout the treatment. Figure 2.3 shows the impact different concentrations can have on the outcome of the system. Equation (2.3) is extended to include an additional compartment to model the concentration of antibiotic within the system. Antibiotics are added in set dosages, D_n, at $\hat{t}$ time intervals and degrade according to first order kinetics with a degradation rate g. The half-life of an antibiotic is the length of time it takes for the concentration of the antibiotic to half. Half-lives of antibiotics are well-documented and can be used to obtain the degradation rate, $g = \frac{\ln(2)}{t_{1/2}}$. Combining the bacteria dynamics and antibiotic concentration compartment, the full model can be found in (2.7).

$$\begin{aligned} \frac{dB}{dt} &= \underbrace{rB\left(1 - \frac{B}{K}\right) - \theta B}_{\text{Natural Growth}} - \underbrace{A(C)B}_{\text{AB Death}} \\ \frac{dC}{dt} &= - \underbrace{gC}_{\text{Degredation}} \end{aligned} \tag{2.7}$$

Antibiotics are added to the system in daily doses. When $t = \hat{t}_n$ the concentration of antibiotic $C(t) = C(t) + D_n$ where $\hat{t} = (1, 2, 3, ..., n)$ and D is a vector of doses $D = (D_1, D_2, ..., D_n)$.

2.2.3 *Parameterising the Model*

When the concentration of antibiotic is allowed to vary according to (2.2), there is limited analytical analysis that can be carried out. A numerical approach was taken using a "toy set" of parameter values. Where possible parameter values were based on those found in the literature. (Use of a real-life parameter set can be found in Chapter 4 where the work is used in an experimental set-up.)

The growth rate, r, can be calculated using the doubling time of the bacteria. Doubling times are calculated during the exponential phase of bacterial growth and can range from 12 minutes to 24 hours depending on the bacteria and the medium. In keeping with work by D'Agata et al. [73] the doubling time of the bacteria was assumed to be 6 hours. The degradation rate, g, can be calculated from the half-life of the antibiotic. The half-life of an antibiotic is the time it takes for the concentration of antibiotic within the system to decrease by half. The half-life of antibiotics varies widely, from 68 hours for Azithromycin to 30 minutes for Cloxacillin. It is assumed that antibiotics are delivered in daily doses where $\hat{t} = (1, 2, 3, ...d)$. Due to the assumption of daily dosing the half-life of the antibiotic was assumed to be 35 hours. The MIC point of bacteria can change due to environmental factors or the presence of antibiotics selecting for advantageous mutations. An increase in MIC point is the definition of a resistant bacteria. For the duration of this chapter bacteria are assumed to be susceptible to antibiotics and remain so for the dura-

tion of their lives. A full list of parameters and values can be found in Table 2.1.

Parameter	Description	Value
r	Replication Rate	2.7726
K	Carrying Capacity	1000
θ	Natural Death Rate	0.2
g	Degradation rate of antibiotic	0.48
A_{max}	Max Antibiotic Induced Death Rate	4.67
B_{max}	Max net growth in absence of AB	$r - \theta$
mic	Min inhibitory concentration (MIC)	16
k	Hill coefficient	4

Table 2.1: Full list of parameters and values used within the model.

2.2.4 *Deterministic versus Stochastic Modelling*

Results from deterministic models are determined by parameter values and initial conditions with the impact of stochastic effects not considered. These results will not change unless the initial input is altered. At large population densities these stochastic effects tend to have little impact on the overall system and deterministic models predict the behaviour of the system well. In addition, deterministic models are often cheap to simulate (in terms of run time) and they offer the possibility of some analytical analysis. However, at small population densities the lack of stochastic effects may not be negligible with deterministic models unable to capture typically stochastic phenomena

such as extinction.

At small population sizes stochastic phenomena result in slightly different outcomes from the same system under the exact same conditions. If one hundred people are treated with the same treatment regimen we may not expect all one hundred infections to behave in the same way. Results from a deterministic model would see all one hundred infections behave in an identical manner, for example, all one hundred infections being eradicated or zero infections being eradicated. Stochastic modelling offers a solution to this problem by introducing random noise into the model. Each time the model is simulated, with the same initial conditions, a slightly different result will be produced. Each of the one hundred people being treated will now respond to the treatment in a slightly different way, as would be expected in real-life.

Figure 2.4 highlights the difference in outcome when using a deterministic model compared to a stochastic model. The result from the deterministic model suggests that the treatment regimen implemented is never effective at eradicating the infection. As deterministic models do not reach zero an arbitrary eradication level must be created, e.g. $B < 5$. Below this level it is assumed that the population of bacteria are low enough such that the infection would be eradicated. Despite the population size of bacteria reaching a low number, this treatment regimen would still be dismissed. Using a stochastic model provides a different outcome. In 14% of cases the stochastic model reaches the same conclusion as the deterministic model. However, the remaining 86% of cases would result in the treatment regimen successfully eradicating the infection. With a small population size the random events within the stochastic model have significant impacts on the results.

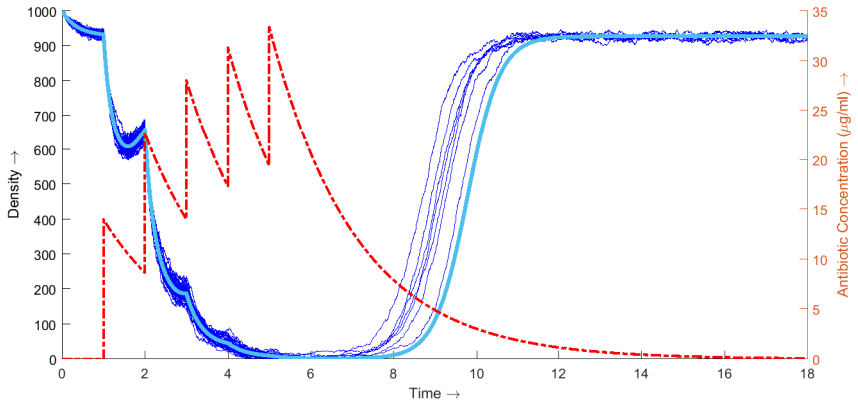

Figure 2.4: Example of the dynamics of the bacterial model using a deterministic (light blue) model and a stochastic (dark blue) model when antibiotics are administered in daily doses. The bacteria persist with the deterministic model but are eradicated 86% of the time with the stochastic model. The red line indicates the concentration profile of the treatment regimen.

There exist different ways in which to include stochasticity in to modelling. One way is to create a stochastic differential equation (SDE). SDE's work by taking a system of ODE's such as (2.7) and including randomness. This can be achieved by using a stochastic process to determine the parameter values e.g. the growth rate of bacteria, or by adding a noise term on to the ODE e.g. Brownian noise. Another well studied way is to use a stochastic simulation algorithm such as Gillespie's algorithm, a form of Markov chain. For the remainder of this thesis the Gillespie algorithm will be used for all stochastic simulation results.

2.2.4.1 *Gillespie Algorithm*

Introduced by Dan Gillespie in 1977 [111], the Gillespie Stochastic Simulation Algorithm (SSA) is a Monte Carlo simulation method. Originally designed to solve an issue regarding chemical reactions where the system size is very small, it is now widely used in areas such as population dynamics and ecology. At large system sizes it is reasonable to use approximations of event outcomes

(such as law of mass action) as it will all balance out in the end. However, with much smaller system sizes the order in which events occur can have significant impact on the outcome (i.e. the death of a single individual might make a large impact on the population).

Gillespie's algorithm uses weighted chance to decide what event happens based on the previous state of the model. Instead of testing in every discrete time-step, the Gillespie algorithm calculates the instant of time at which a new event will take place given the number of possible events and the rate at which these events happen. This reduces the cost of simulating the data as it avoids the need to simulate the time-steps where no events happen. Once an event is selected the model is updated. With only one event happening at each update the problem of events affecting each other within a time-step is avoided. A certain integer value will be added or subtracted from some substance of the model at each update. This new state provides slightly altered chances for each event and the process repeats to calculate the next event to take place. This is repeated until an equilibrium or time limit has been reached.

Event	Outcome	Transition Rate
Birth of Bacteria	$(B \to B+1)$	$: rB(1-\frac{B}{K}) = R(1)$
Death of Bacteria	$(B \to B-1)$	$: \theta B + A(C)B = R(2)$

Table 2.2: Table showing the different events which can occur in the stochastic model, the effect these events have on the population and the rate at which they happen.

Creating the Gillespie algorithm for (2.7) requires the model to be split into the individual events. For this model there are two different events that can

occur (Table 2.2).

Each transition rate is converted into a probability that that event will happen (2.8) by dividing the individual transition rates by the total sum of all transition rates.

$$\text{prob of event } i : P(i) = \frac{R(i)}{\sum_{i=1}^{2} R(i)} \tag{2.8}$$

Using a random number generator, a random number (x) is chosen between 0 and 1. If $x \in [0 : p(1))$ then event 1 occurs and the bacteria population is increased by 1, if $x \in [p(1) : p(1) + p(2))$ then event 2 occurs and the bacteria population is decreased by 1. Using a second random number (z) the time delay (τ) until the next event takes place is calculated from the exponential distribution (2.9) with a rate $\sum_{i=1}^{2} R(i)$. The time is updated and the steps are repeated to identify the next event and time delay. This process can be extended for any number of events and repeated for any length of time. The Gillespie algorithm was coded in MATLAB, example code can be found in Appendix A Section A.1.1.

$$\tau = -\frac{\log(z)}{\sum_{i=1}^{2} R(i)} \tag{2.9}$$

Due to the use of a random number generator each simulation using the Gillespie algorithm will be slightly different. Each treatment regimen is therefore run 5000 times to allow for the variability within the results. A success rate for each treatment regimen is obtained by calculating the percentage of runs which result in eradication of the infection. The 95% confidence interval was calculated in MATLAB using the Clopper-Pearson exact confidence interval. The Clopper-Pearson confidence interval is a common method for calculating binomial confidence intervals [112] where only the number of successful runs and the number of trials are known. It is based on the binomial distribution rather than any approximation to the binomial distribution and can be written as (infimum $S_{\geqslant}$, supremum $S_{\leqslant}$) with

$$S_{\geqslant} = \left\{\theta | P[\texttt{Bin}(n;\theta) \geqslant x] > \frac{\alpha}{2}\right\}$$
$$S_{\leqslant} = \left\{\theta | P[\texttt{Bin}(n;\theta) \leqslant x] > \frac{\alpha}{2}\right\}$$

where $0 \leqslant x \leqslant n$ is the number of successes observed in the sample and $\text{Bin}(n;\theta)$ is a binomial random variable with n trials and probability of success θ.

The Clopper-Pearson exact confidence interval was chosen due to the possibility of success rates close to 0% and 100%. At these values the normal approximation is unreliable. Additionally the Clopper-Pearson confidence interval ensures that the nominal confidence width is covered whereas other confidence methods, such as the normal approximation, may be narrower than the 95% confidence width. The success rate is used throughout this thesis to compare the effectiveness of different treatment regimens.

2.3 CONVENTIONAL TREATMENT REGIMENS

Conventional treatment regimens consist of administering the same dose at set intervals for a set duration of time. With the assumption of daily dosing, the pattern for conventional treatment regimens is X units once a day for N days. Using the parameter values from Table 2.1, the success rate for a range of conventional constant dose treatment regimens was identified. The total amount of antibiotic, X, across the entire regimen was fixed at either 100, 90, 80, 70, 60 or 50 $\mu g/ml$ with the duration of treatment, N, varied from 1 to 10 days. Table 2.3 shows the success rate of the different conventional treatment regimens.

The dose and duration of antibiotic treatment have been identified as important factors in optimising antibiotic use [113, 15]. When considered individually,

Total Dose	Days									
	1	2	3	4	5	6	7	8	9	10
100	90.1	98.2	99.1	99.3	99.4	99.5	9.6	99.0	98.1	94.4
90	85.7	95.4	97.6	98.6	98.1	97.9	97.4	94.7	84.2	45.5
80	76.1	91.8	94.3	95.4	94.9	92.7	82.8	60.8	11.0	0.3
70	60.7	79.9	83.9	84.2	78.0	58.9	22.5	1.3	0	0
60	40.1	59.8	59.7	47.6	21.6	3.6	0	0	0	0
50	13.1	18.5	7.7	1.1	0	0	0	0	0	0

Table 2.3: Success rate of eradicating an infection under various conventional treatment regimens. The total dose is spread evenly across the number of days e.g. if total dose = 90 and days = 5 then the antibiotic is administered in 5 doses of 18.

changing the dose or duration of treatment can result in superior success rates but more effective treatments may be missed. Results from Table 2.3 show that it is possible to increase the success rate by 14% if the amount of antibiotic used is increased from 80 μg/ml over 1 day to 100 μg/ml over 1 day. However, by splitting the 80 μg/ml over 2 days the success rate increases by 15.7% with the added bonus that the environment has been exposed to a lower total antibiotic concentration. Both the dose and duration must be considered when looking for optimal treatments.

By plotting the results from Table 2.3 the presence of a trade-off between dose and duration can be visualised (Figure 2.5). A high dose but over a short duration does not expose the bacteria to the antibiotic for long enough. A long duration means the antibiotic is being spread too thinly resulting in the concentration of antibiotic not remaining above the bacteria's MIC point

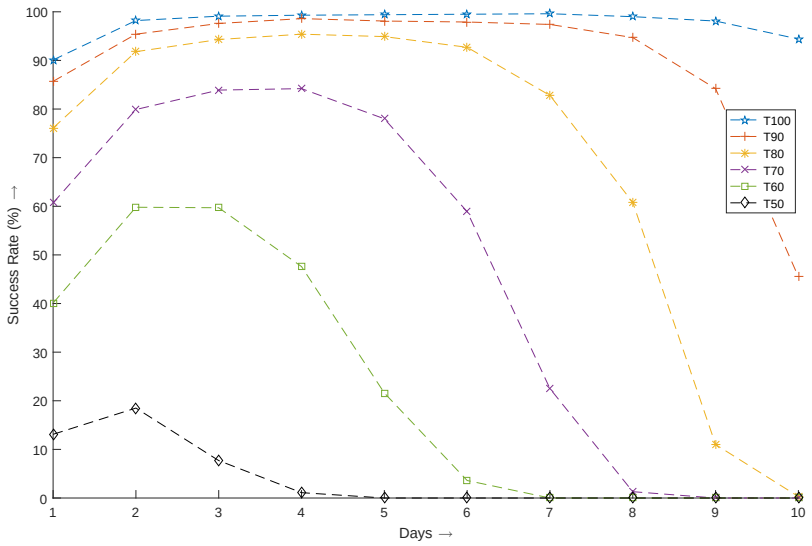

Figure 2.5: Graph representing the data from Table 2.3. Success rate is plotted against number of days of treatment for each of the total doses where T100 represent a total dose of 100.

for long enough. The most effective constant dose regimen lies somewhere between these two extremes.

2.4 Optimising treatment regimens

While Table 2.3 showed that conventional treatment regimens can be very effective in eradicating an infection it gives no indication of the efficiency of other alternative treatment regimens. The overuse of antibiotics is contributing to the increase in antibiotic resistant bacteria. Identifying more effective ways of using less antibiotic is paramount to extending their shelf-life.

This thesis takes the approach of using a Genetic Algorithm (GA) to optimise treatment regimens by maximising the success of eradicating an infection while using the least amount of antibiotic required to do so. The mathematical model of bacterial dynamics (2.7) is incorporated into the GA and used to calculate

the fitness function of varying treatment regimens. The GA searches through possible dosage vectors and identifies the most effective way to distribute the antibiotic across the duration of treatment. Relaxing the assumption of constant dose treatments, the GA identifies the dosage vector $D = (D_1, D_2, ..., D_d)$ where $D_i \in \mathbb{N}_0$, such that the given fitness function, F, is minimised. The advantage to this approach is that if the conventional constant dose regimen is in fact the optimal treatment strategy the GA would identify this. The GA was coded in MATLAB, example code can be found in Appendix A Section A.1.2.

2.4.1 *Setting the Constraints for the Genetic Algorithm*

A conventional treatment regimen is used as a baseline and to set some constraints within the GA to ensure all the treatment vectors are comparable. Conventional treatments using 100, 90 and 80 $\mu g/ml$ of antibiotic in total all contained treatment durations which resulted in success rates above 95% (Table 2.3). While these regimens show that some conventional constant dose treatments can be effective, the high success rates provide little room for improvement. The treatments using a total of 70 $\mu g/ml$ of antibiotic reached a maximum success rate of 84.2% over a 4 day duration. This lower success rate provides a better opportunity to determine if moving away from conventional constant dose treatments may provide more effective treatments. The dosage vector $D = (17.5, 17.5, 17.5, 17.5)$ is therefore taken as the baseline treatment to which all alternative treatments will be compared.

The total amount of antibiotic used by the baseline treatment ($D = (17.5, 17.5, 17.5, 17.5)$) is 70 $\mu g/ml$. Therefore all alternative treatment regimens identified by the GA must use no more than 70 $\mu g/ml$ of antibiotic in total. It has already been shown that it is possible to find greater success rates by using

more antibiotic. Due to the trade-off that exists between the dose and duration, the GA was allowed to explore treatments of longer duration within its search. Treatment lengths of up to 6 days were considered allowing the potential for an increase in duration of up to 50% compared to the baseline treatment. While antibiotics are known to help treat infections the concentration at which they are present is important. Antibiotics can be toxic if the concentration within a hosts system reaches certain levels. The maximum concentration of antibiotic within the system of the baseline treatment was used as an upper bound for any alternative treatments. The maximum concentration within the system must therefore not exceed 40 $\mu g/ml$, in keeping with the baseline treatment. This ensures there are no potential issues with alternative treatment regimens exceeding therapeutic levels. To code this maximum concentration level any treatments exceeding 40 $\mu g/ml$ are penalised with a fitness value of $F = 10^5$.

2.4.2 *Genetic Algorithm with the Deterministic Model*

Due to being less computationally expensive, as each solution only requires one run of the model, the GA was initially run using the deterministic model to simulate the outcomes from the different dosage vectors and inform the fitness function. Using the deterministic model meant that success rates from the various dosage vectors could not be compared within the GA. The fitness function would have to measure and compare some other form of success. The presence of a large population of bacteria within a host worsens the outcome for the host the longer that population is allowed to thrive. The fitness function was therefore designed to minimise the total bacterial load over the duration of the infection. The increase in likelihood of mutations occurring with high bacterial loads also strengthens the need for effective treatment regimens to minimise the total bacterial load. If bacterial load is the only term within

the fitness function the GA would automatically use the maximum amount of antibiotic available. As the overuse of antibiotics is also of major concern, the fitness function was altered to include a term which would minimise the amount of antibiotic being used. Equation (2.10) shows the fitness function used within the GA when using the deterministic model.

$$F = w_1 \underbrace{\sum_{i=1}^{d} D_i}_{\text{Total Antibiotic}} + w_2 \underbrace{\int_0^T B(t)dt}_{\text{Bacterial Load}} \tag{2.10}$$

where, T is the end of the simulation and $w_i \geqslant 0$.

Due to the difference in magnitude of the values for total antibiotic and bacterial load, weights w_1 and w_2 were used. The weights allow for more emphasis to be placed on minimising one term over the other, to ensure a trade-off exists between the two terms $w_1 \geqslant 0.001$ and $w_2 \geqslant 0.001$. The eradication threshold is set to $B < 5$.

2.4.2.1 *Results*

The GA was run with a population size of 100 for 1000 generations. As it is a stochastic method the GA was run 50 times with the most successful dosage vector recorded for each run. The successful dosage vectors were then run through the Gillespie algorithm to generate a success rate of eradicating the infection (i.e. % of runs where the bacteria population dies out). Table 2.4 shows the top 3 results from the GA using the deterministic model when minimising the fitness function (2.10). Each run of the GA produced the same dosage vector. As there is no noise within the deterministic model the results all converge to a single optimum dosage vector very quickly.

			Success Rate (%)
	Dosage Vector	Total Antibiotic	[95% CI, n = 5000]
D1	(39, 12, 3, 0, 0, 0)	54 $\mu g/ml$	42.7 [41.32, 44.08]
	(39, 12, 3, 0, 0, 0)	54 $\mu g/ml$	
	(39, 12, 3, 0, 0, 0)	54 $\mu g/ml$	

Table 2.4: Table comparing the success rates of the dosage vectors produced by the GA with deterministic modelling. The extinction threshold was set to $B < 5$. A success rate was obtained by running the dosage vector using the Gillespie algorithm for 5000 simulations.

The GA identifies a high initial dose followed by a tapering of lower doses to be the most effective way of administering the antibiotics. The regimen identified by the GA does indeed have a smaller fitness function when compared to that obtained from the conventional regimen. The tapered regimen uses less antibiotic and has a lower bacterial burden over the duration of the infection than the conventional regimen. However, the tapered regimen performs considerably worse, 42.7% (95% CI: 41.32, 44.08), when the success rate is compared to that of the conventional regimen, 84.2% (95% CI: 83.16, 85.20). This is due in part to the 'all or nothing' results from the deterministic model. The GA is able to refine the treatment regimen to the exact point where the deterministic result changes from not eradicating the infection to successfully eradicating it. Being so close to this point results in a lot of runs not being successfully eradicated when the regimen is simulated using the Gillespie algorithm.

In addition, population densities in deterministic models never reach zero. An arbitrary point is chosen where the population is assumed to be extinct. In Table 2.4 the extinction point was taken as $B < 5$. To determine if decreasing

the extinction threshold would result in treatment regimens with better success rates the GA was run again. This time the GA was run using the deterministic model but with the extinction point being $B < 2$ (Table 2.5).

	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n = 5000]
D2	(33, 12, 12, 4, 0, 0)	61 μg/ml	72.9 [71.64, 74.13]
	(33, 12, 12, 4, 0, 0)	61 μg/ml	
	(33, 12, 12, 4, 0, 0)	61 μg/ml	

Table 2.5: Table comparing the success rates of the dosage vectors produced by the GA with deterministic modelling. The extinction threshold was set to $B < 2$. A success rate was obtained by running the dosage vector using the Gillespie algorithm for 5000 simulations.

Once again the GA produces the same pattern of a high initial dose followed by tapering doses. Lowering the extinction threshold has resulted in the treatment duration being extended by a day. This duration matches that of the conventional regimen. When compared to the conventional treatment regimen the fitness function value of the new tapered regimen is once again lower. This new tapered regimen still uses less antibiotic than the constant dose regimen and has a lower total bacterial load over the duration of the infection. By lowering the extinction threshold the results obtained from the GA using the deterministic model have increased the success rate from 42.7% (95% CI: 41.32, 44.08) to 72.9% (95% CI: 71.64, 74.13). Despite this increase the success rate of the tapered regimen identified by the GA is still lower than the conventional regimen, 84.2% (95% CI: 83.16, 85.20). The GA is not identifying regimens which are more effective than the baseline conventional treatment. However, if you compare the success rates of the tapered regimens with the

conventional regimens that use a similar amount of antibiotic, the success rate for the tapered regimen is better. This suggests that alternative dosage vectors which increase success rate do exist.

The deterministic model provides no way of evaluating the success rate within the GA. The success or failure of a dosage vector is a binary result and this is causing a problem. To determine whether the GA could indeed identify better treatment regimens the stochastic model was used within the GA instead of the deterministic model. This removes the problem of the success rate of a treatment regimen being obtained only after the GA has finished. Despite knowing this may cause a problem beforehand, the extensive run-time of using the stochastic model within the GA made the deterministic model a reasonable place to start. The extensive run time using the stochastic model is due to each solution being run 1000 times to gain a fitness score.

2.4.3 *Genetic Algorithm with the Stochastic Model*

Despite changing from using the deterministic model to the stochastic model the GA remains largely unchanged. The aim of the GA is still to identify the dosage vector $D = (D_1, D_2, ...D_d)$ where $D_i \in \mathbb{N}_0$, such that the fitness function is minimised. The terms used to calculate the fitness function have been altered but the dose vector format and obvious constraints used previously remain the same.

When using the deterministic model to simulate the effectiveness of a dosage vector there are only two possible outcomes, either it works and eradicates the infection or it does not. Minimising the bacterial load was therefore used as an approximation for the success rate when the deterministic model was used.

By using the stochastic model to calculate the fitness function it is possible to directly identify dosage vectors which have a better success rate than the baseline treatment. If the fitness function is only concerned with maximising the success rate the maximum amount of antibiotic will be used even if it is not required as there is no penalty in doing so. The fitness function for the GA using the stochastic model is therefore a trade-off between maximising success rate while minimising antibiotic use (2.11). As the GA is set to minimise the fitness function, $\sum \hat{B}$ is the sum of the runs where the number of bacteria present at the end of the simulation (T) was greater than zero. By minimising the number of runs which result in bacteria present at the end of the simulation, the number of runs which successfully eradicate the infection is maximised.

$$F = w\,\alpha_1 \underbrace{\sum_{i=1}^{d} D_i}_{\text{Total Antibiotic}} + (1-w)\,\alpha_2 \underbrace{\sum_{i=1}^{N} \hat{B}_i}_{\text{Unsuccessful runs}} \tag{2.11}$$

where

$$\hat{B} = \begin{cases} 1, & \text{if } B(T) > 0. \\ 0, & \text{if } B(T) = 0. \end{cases}$$

Coefficients α_1 and α_2 are used to keep the terms in the range $[0, 1]$. As the maximum amount of antibiotic that can be used is 70 $/mug/ml$, $\alpha_1 = \frac{1}{70}$. Similarly, the maximum number of unsuccessful runs is 1000 and so $\alpha_2 = \frac{1}{1000}$. A weight is also used on each term to vary the trade-off between them. As the value of w increases more emphasis is placed on minimising the amount of antibiotic used. Values of w were chosen such that a set decrease in antibiotic must account for no more than a 1% decrease in success rate to be considered a better treatment regimen. This ensures that minimising the amount of

antibiotic does not cause a considerable decrease in the success rate. Varying the weights on the terms in the fitness function had no qualitative effect on the overall results. A weight of $w = 0$ was therefore chosen.

2.4.3.1 *Results*

Due to the extensive run-time associated with using the stochastic model within the GA, the population size was reduced to 50 and the generations reduced to 100. The GA was repeated 50 times with the identified dosage vector and its success rate recorded for each run. The Gillespie algorithm was run 1000 times for each individual solution to produce the success rate within the GA. Due to the stochastic nature of the GA and the stochasticity of the Gillespie algorithm some noise exists within the dosage vectors. Varying the weights on the terms in the fitness function had no qualitative effect on the overall results. The top 10 dosage vectors identified by the GA using the stochastic model when minimising (5.4) with a weight of $w = 0$ are shown in Table 2.6.

Once again the GA identifies a tapered pattern as the most effective way of administering the antibiotic. By using the stochastic model the GA is able to compare the success rates within the algorithm. This ensures that the dosage vectors identified are indeed more successful than the baseline regimen. By taking the same amount of antibiotic but applying it with a high initial dose followed by lowering doses, the GA increases the success of eradicating the infection from 84.2% (95% CI: 83.16, 85.20) to 91.04% (95% CI: 90.21, 91.82).

Figure 2.6 compares the concentration profile of the conventional baseline regimen with regimen S1, Table 2.6. The concentration of antibiotic from the conventional treatment takes time to build up. By administering a high initial dose the concentration of antibiotic from the tapered regimen is increased

	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n = 5000]
S1	(28, 18, 13, 11, 0, 0)	70 μg/ml	91.04 [90.21, 91.82]
S2	(31, 14, 15, 8, 2, 0)	70 μg/ml	91.04 [90.21, 91.82]
S3	(32, 14, 14, 10, 0, 0)	70 μg/ml	90.76 [89.92, 91.55]
S4	(29, 15, 14, 11, 1, 0)	70 μg/ml	90.66 [89.82, 91.45]
S5	(33, 14, 12, 10, 0, 0)	69 μg/ml	90.64 [89.80, 91.43]
S6	(35, 13, 10, 12, 0, 0)	70 μg/ml	90.54 [89.69, 91.34]
S7	(33, 16, 12, 9, 0, 0)	70 μg/ml	90.48 [89.63, 91.28]
S8	(32, 9, 15, 14, 0, 0)	70 μg/ml	90.10 [89.24, 90.91]
S9	(29, 19, 13, 9, 0, 0)	70 μg/ml	89.84 [88.97, 90.66]
S10	(34, 13, 13, 7, 2, 0)	69 μg/ml	89.16 [88.27, 90.01]

Table 2.6: Table comparing the success rates of the dosage vectors produced by the GA with stochastic modelling where $w = 0$. The top 10 dosage vectors are shown.

above the MIC on the first dose and maintained with smaller doses. By relaxing the constant dose constraint the total concentration of antibiotic within the system can be maintained at a lower level while spending an adequate duration above the MIC.

2.5 SENSITIVITY ANALYSIS

To identify if the pattern of a high initial dose followed by a tapering of lower doses was a consequence of the parameter values chosen, two further parameter sets were analysed (Table 2.7). Parameter Set 1 creates a worse case scenario: the bacteria reproduce quicker, the MIC is higher and the antibiotics

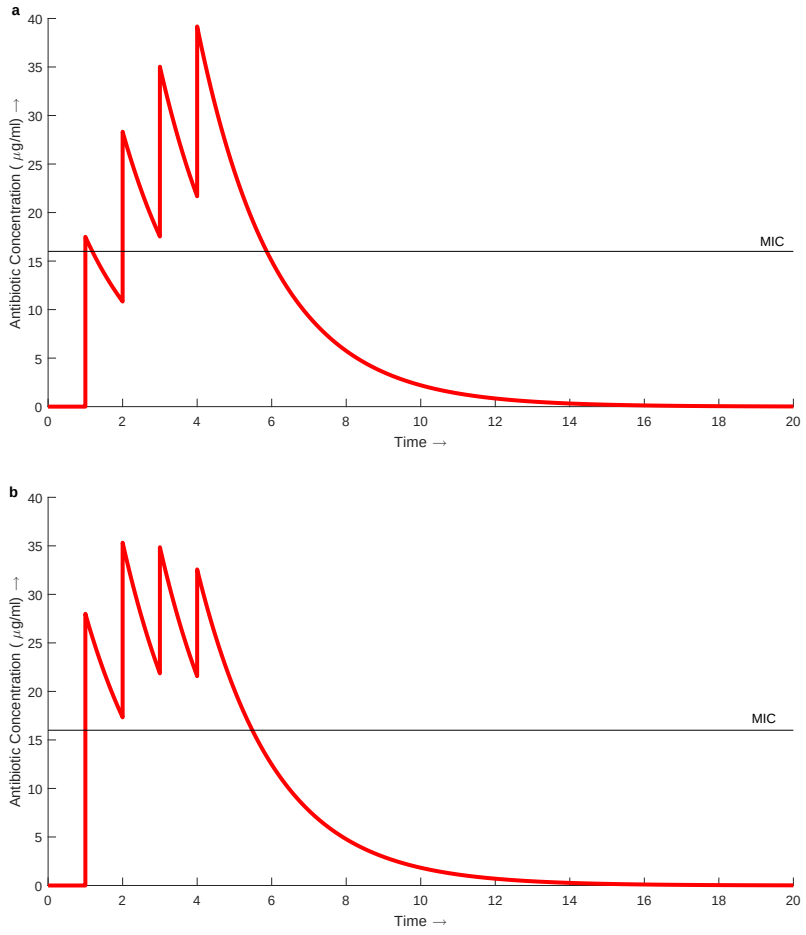

Figure 2.6: Concentration profiles for the baseline conventional treatment regimen, $(17.5, 17.5, 17.5, 17.5)$, and regimen S1 from Table 2.6. (a) The baseline treatment regimen briefly increases the concentration above the MIC after the first dose but quickly drops below again. Future doses increase and maintain the concentration above the MIC reaching a maximum concentration of 40 $\mu g/ml$. (b) S1 increases the concentration above the MIC of the bacteria with further, smaller, doses used to maintain it above the MIC. The maximum concentration reached is 35 $\mu g/ml$.

degrade quicker than the original parameter set. Parameter Set 2 is the opposite with parameters benefiting treatment: the bacteria reproduce slower, the MIC remains the same and the antibiotics degrade slower than the original parameter set.

Parameter	Set 1	Set 2
r	4.176	1.386
K	1000	1000
θ	0.2	0.2
g	0.693	0.347
$Amax$	5.25	2.75
$Bmax$	3.976	1.186
mic	24	16
k	3	4

Table 2.7: Full list of parameters and values for both parameter sets used within the sensitivity analysis.

Parameter Set 1

A baseline conventional treatment regimen was identified for this new parameter set. The dosage vector $D = (34, 34, 34, 34, 34, 0)$ successfully eradicated the infection in 78.10% (95% CI: 76.93, 79.24) of cases. It used a total of 170 $\mu g/ml$ of antibiotic to obtain this and reached a maximum concentration of 65 $\mu g/ml$. Using the GA with the stochastic model constraints were set to ensure alternative treatment regimens did not exceed the maximum amount of antibiotic used or the maximum concentration observed within the conventional regimen.

Results from the GA (Table 2.8) identified treatment regimens which significantly increased the success of eradicating the infection up to 81.6% (95% CI: 80.50, 82.67). The same high initial dose followed by tapering of lower doses is

	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n = 5000]
T1	(34, 34, 34, 34, 34, 0, 0)	170 μg/ml	78.1 [76.93, 79.24]
S1	(55, 33, 29, 27, 24, 0, 0)	168 μg/ml	81.6 [80.50, 82.67]
S2	(54, 34, 32, 24, 20, 0, 0)	164 μg/ml	78.5 [77.33, 79.63]

Table 2.8: Table comparing the success rate of the baseline dosage vector (T1) and the top two dosage vectors (S1, S2) produced by the GA with stochastic modelling using parameter set 1.

seen once again in these results. By administering the antibiotics in a tapered pattern it is also possible to identify treatments which use less antibiotic while maintaining a similar success rate to that of the conventional treatment.

Parameter Set 2

Repeating for the second parameter set, a baseline treatment was identified as $D = (14, 14, 14, 14, 14, 0)$. Using a total of 70 μg/ml of antibiotics with a maximum concentration of 40 μg/ml, this treatment regimen has a success rate of 78.3% (95% CI: 77.13, 79.44). Using the GA with the stochastic model it was possible to increase the success of treating an infection up to 87.40% (95% CI: 86.45, 88.31) (Table 2.9). This was achieved by employing the same tapered pattern with a high initial dose.

Under both parameter sets the GA identifies a high initial dose followed by tapering lower doses as the most effective way to administer antibiotics. Similarly to the original parameter set, dosage vectors identified by the GA have a higher success rate then that of the baseline treatment. In cases where the

	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n = 5000]
T2	(14, 14, 14, 14, 14, 0 0)	70 μg/ml	78.3 [77.13, 79.44]
S3	(30, 14, 8, 9, 9, 0, 0)	70 μg/ml	87.4 [86.45, 88.31]
S4	(31, 12, 11, 9, 7, 0, 0)	70 μg/ml	86.6 [85.62, 87.53]

Table 2.9: Table comparing the success rate of the baseline dosage vector (T2) and the top two dosage vectors (S3, S4) produced by the GA with stochastic modelling using parameter set 2.

success rate is not significantly different less antibiotic was required to achieve the same success. Administering antibiotics in a tapered regimen increases the effectiveness of the treatment compared to a conventional constant dose regimen. However, while the tapered pattern appears to be optimal, the exact doses required are individual to each infection being treated.

2.6 DISCUSSION

Constant dose treatment regimens are the conventional pattern for administering antibiotics to treat bacterial infections. Constant dose regimens have many advantages: it is cost effective to only manufacture tablets in a given strength, there is little room for human error and simpler treatment regimens lead to more reliable compliance. While this pattern of treatment may be effective, different treatment patterns may provide a more efficient use of the antibiotic. With the sub-optimal use of antibiotics contributing to the emergence of antibiotic resistant bacteria [114, 115, 44], standard treatment protocols need to be optimised.

Unfortunately, updates to standard treatment protocols are not timely. Until 2014 the dose of amoxicillin administered to children was based on specific age bands. Due to the increase in average weight of children more than 50% of those receiving the antibiotic were being under-dosed [116]. Many clinical studies have proposed that shorter treatment durations are just as effective as the standard treatment duration for treating a range of bacterial infections [117, 118, 119, 120, 121]. As a result, the duration of treatment for community acquired pneumonia has now been amended to a minimum of 5 days, after which patients should be re-examined. Despite these changes, these may still not be the most effective way of using the antibiotic. There is little consensus on the optimal treatment regimen for antibiotic therapy with many potential regimens not even being considered.

A mathematical model of a susceptible bacterial population was developed. Using a theoretical parameter set, a conventional constant dose treatment regimen was identified which successfully eradicated the infection in 84.2% of cases. This conventional treatment regimen was used as a baseline to compare alternative treatments. A GA was chosen as a method for optimising alternative treatment regimens. By adding constraints to the GA it is possible to ensure that a fair comparison can be made with the baseline regimen. Alternative treatment regimens could use, at most, the same amount of antibiotic as the baseline treatment while not exceeding the maximum concentration within the system at any given time. The GA was run using both a deterministic and a stochastic model to inform the fitness function. Despite being less computationally expensive and providing a similar dosage pattern, the deterministic model did not produce dosage vectors which were significantly better than the baseline treatment. Results were therefore generated using the stochastic

model within the GA.

The GA continually identified a high initial dose followed by a tapering of smaller doses to be the optimal way of administering the antibiotics to increase the success rate. Administering the antibiotic in this tapered pattern increased the success of eradicating the infection from 84.2% to 91.04%, despite using the same amount of antibiotic as the conventional treatment. By administering a high initial dose the concentration of antibiotic is established well above the MIC of the bacteria. The tapered doses maintain the concentration above the MIC for the shortest duration possible. In contrast, the concentration within the conventional regimen has to build up to such a level that it can be maintained above the MIC. This results in the system being exposed to a higher concentration of antibiotic compared to the tapered regimen. Despite increasing the weight placed on minimising the amount of antibiotic being used, the GA was unable to find regimens which used less antibiotic without causing a considerable decrease in success.

Two further parameter sets were analysed and for both systems a tapered treatment regimen was identified as the optimal way to apply the antibiotics. In addition to the tapered pattern increasing the success rate when using an equal amount of antibiotic, the tapered pattern allows for less antibiotic to be used to obtain results significantly similar to that of the conventional treatment. In the case of Parameter Set 1, the GA identified a treatment regimen using 4% less antibiotic than the constant dose treatment. Tapered treatment patterns are not new and have been established in the treatment of *Clostridium difficile* with Vancomycin [122, 123]. However, previous modelling studies have suggested that tapering treatment regimens may result in sub-optimal performance [80, 81]. Despite being qualitatively similar, these treatments are

all quantitatively different. Using the GA identifies tapered regimens which will maximise success rate but there may be tapered regimens which are less effective than the baseline. Care must be taken when generalising alternative treatment patterns.

Despite the conventional treatment regimen being simple to implement and potentially effective at eradicating the infection, there is a push for antibiotics to be used optimally. Antibiotic resistance is an increasing concern with the overuse and misuse of antibiotics a driving factor in increasing resistance. By moving away from constant dose treatments to more tailored treatments it might be possible to prolong the use of the antibiotics we currently have. The work carried out in this chapter suggests that tapered regimens may optimise the use of antibiotics resulting in better success rates and the use of less antibiotic. However, a major limitation to this work was the absence of any resistant bacteria. In cases where infections are not cleared, resistant bacteria have been seen to lead the path in re-colonising an infection [124]. To investigate whether tapered regimens would further facilitate the spread of resistant bacteria the model could be extended to include a sub-population of resistant bacteria.

2.7 SUMMARY

This Chapter aimed to use a genetic algorithm to identify more effective treatment strategies for administering antibiotics. The use of genetic algorithms to optimise treatment regimens enables an extensive number of possibilities to be considered. Previous regimens direct the search for further regimens into regions of better performance. A mathematical model was developed to describe the dynamics of a population of susceptible bacteria in the presence

of an antibiotic. This model was used to calculate the fitness function within the GA by predicting the success of various treatment regimens.

Despite its preferable run-time and similar pattern of results, the deterministic model was found to have limited use in identifying better treatment regimens. Incorporating the stochastic model into the GA produced significantly better results. By redistributing the antibiotic into a tapered pattern with a high initial dose, the GA identified regimens which increased the success of eradicating the infection compared to the conventional baseline treatment. This pattern of treatment was again identified by the GA when two additional parameter sets were examined. Tapered regimens have the potential to increase the success of eradicating an infection, reduce the amount of antibiotic required and expose the system to a lower concentration of antibiotic than conventional treatments.

With antibiotic resistance continuing to pose a threat to future healthcare, the ability to successfully treat a bacterial infection while using the minimum amount of antibiotic required is of global importance. This Chapter highlights the need to consider treatments which vary from the conventional constant dose regimens and the potential for GA's to be a useful tool in exploring alternative treatment strategies. Tapered regimens have already been found to be successful in the treatment of *Clostridium difficile* where they are the standard dosing protocol. However, the majority of treatment regimens for bacterial infections are prescribed in a constant dose pattern.

The impact alternative treatment regimens have on the presence and spread of resistant bacteria was not considered in this Chapter. However, the work

contained in this Chapter creates a framework which can be extended to include the presence of resistance (Chapter 3).

3

OPTIMISING ANTIBOTIC TREATMENT REGIMENS IN THE PRESENCE OF RESISTANT BACTERIA

3.1 INTRODUCTION

In Chapter 2, a mathematical model was developed to predict the behaviour of a single strain bacterial population in the presence of antibiotics. The model was incorporated into a genetic algorithm (GA) which was used to identify optimal antibiotic treatment strategies. A treatment regimen with a high initial dose followed by tapering lower doses was repeatedly identified as the optimal pattern to increase success rates while minimising the amount of antibiotic required. This approach highlighted the practicality of using a GA to identify alternative antibiotic treatment regimens when used in combination with a mathematical model.

An increase in antibiotic resistant bacteria poses a threat to the continued use of antibiotics to treat bacterial infections [125, 126]. The presence of resistant bacteria is already making it extremely difficult to successfully treat certain strains of pneumonia, tuberculosis and gonorrhoea [2, 3, 4, 5]. A significant driver in the emergence of resistant strains is the overuse and misuse of antibitoics [127, 43, 128]. Finding optimal treatment regimens, in the presence of resistant bacteria, is critical in ensuring the prolonged effectiveness of antibiotics.

The aim of this chapter is therefore to extend the work carried out in Chapter 2 by considering the presence of resistant bacteria. The single strain bacterial model will be replaced by a two strain model describing the dynamics of both a susceptible and resistant bacterial population in the presence of antibiotics. The effect the presence of the resistant population has on the outcome of antibiotic treatment patterns will be investigated. Using a conventional constant dose treatment regimen as a baseline, the GA will be used to identify optimal treatment regimens. These regimens will maximise the success of eradicating an infection, where resistant bacteria are present, while minimising the amount of antibiotic required to do so.

This work was published in *Nature Scientific Reports* in the paper by Paterson et al. [82] of which the author of this thesis is the lead author. The paper was written by the author of this thesis who also designed the genetic algorithm and performed the mathematical analysis with some guidance from the co-authors. An edited version of the paper is presented in Section 3.2 with Section 3.3 providing the supporting supplementary information referenced throughout the paper.

3.1.1 *Changes in Notation*

There have been some changes made to the notation used within the function for the antibiotic-induced death term since the work in this chapter was published. As such, there are some discrepancies between the notation used throughout this thesis and that used within the paper. The function for the antibiotic-induced death rate from the paper is:

$$A(C) = \frac{(max - min)\left(\frac{C}{mic}\right)^k}{\left(\frac{C}{mic}\right)^k - \frac{min}{max}}$$

Taking this work forward experimentally, the parameter value for min would be difficult to measure independently. However, $max - min$ represents the maximum antibiotic-mediated death rate and therefore was replaced by $Amax$. The notation for the maximum net growth rate of bacteria in the absence of antibiotics was changed from max to $Bmax$ but still represents the value $(r - \theta)$. Substituting in $Amax = max - min$ and $Bmax = max$ gives:

$$A(C) = \frac{Amax\left(\frac{C}{mic}\right)^k}{\left(\frac{C}{mic}\right)^k - \frac{min}{Bmax}}$$

Replacing min with $Bmax - Amax$ and simplifying gives the notation used throughout this thesis:

$$A(C) = \frac{Amax\left(\frac{C}{mic}\right)^k}{\left(\frac{C}{mic}\right)^k + \frac{Amax}{Bmax} - 1}$$

Despite both functions being equivalent, the change in parameters allows for values to be taken straight from a concentration versus death rate graph such as that in Figure 2.2.

A minor change was also made to the naming of constant dose, set duration treatment regimens. Throughout the paper constant dose treatment regimens were referred to as traditional treatment regimens. However, throughout this thesis these are referred to as conventional treatment regimens.

3.2 Optimising antibiotic usage to treat bacterial infections (Paterson et al. 2016)

Paterson, I.K., Hoyle, A., Ochoa, G., Baker-Austin, C., Taylor, N.G.H., (2016). Optimising Antibiotic Usage to Treat Bacterial Infections. Sci. Rep. 6, 37853

3.2.1 *Introduction*

The discovery of penicillin in 1928 dramatically changed human and animal health and well being. Since then, the discovery of additional antibiotics has further increased survival rates in areas such as surgery and during cancer chemotherapy. However, a lack of new antibiotics and an increase in resistance means these advances are under threat [129]. Resistance to all antibiotics in clinical use has now been observed [36], with the extensive use and misuse of antibiotics being attributed to the spread of these resistant genes [127, 43, 128]. This has caused considerable debate over the future effectiveness in treating bacterial diseases [57, 58]. As such, the World Health Organisation (WHO) has identified antibiotic resistance as one of the major health concerns of the 21st Century.

The apparent ease at which antibiotic resistance spreads is due to the ability of bacteria to acquire additional genes. Genes can be acquired via either mutations or horizontal gene transfer (HGT). While mutations are undoubtedly a source of resistance, HGT is responsible for increased propagation of resistance through bacterial populations [130]. If bacteria acquire resistant genes in an environment where they are beneficial, HGT will facilitate the spread of these genes within the population [69]. Sub-Minimum Inhibitory Concentrations (MIC) of antibiotics and the persistence of high levels of antibiotics within the

environment have been linked to the emergence of resistant genes [131, 54]. Antibiotic treatments must therefore be effective to minimise the influence of an environment which selects for resistance.

Effective antibiotic treatment regimens consist primarily of two variables: the dose and the duration of treatment. For most antibiotics, the manufacturer identifies a conventional treatment regimen which is implemented by doctors and veterinary surgeons when prescribing these antibiotics. These conventional treatment regimens usually consist of a fixed dose administered for a specified duration. Drug efficiency studies are used to determine the dose and duration for these treatment regimens. However, one limitation of this approach is that it only provides information for the regimen being analysed and offers no indication for other potential regimens. AliAbadi and Lees [132] highlighted the importance of rational use of antibiotics and the need to incorporate population pharmacokinetic (PK) and pharmacodynamic (PD) data into dosage scheduling. While conventional treatment regimens may be effective they may not be the optimal duration or dose at which to administer antibiotics.

As the threat of antibiotic resistance spreads the need to optimise antibiotic dosage regimens becomes essential. Mathematical modelling is increasingly being used to investigate optimal treatment regimens for antibiotic therapy [61, 63, 73, 74, 133]. However, these studies either omit pharmacodynamic data, by assuming that the antibiotic induced death rate is constant; or only analyse a very limited number of alternative treatment regimens. With no verification that the duration or doses chosen are optimal, these studies look for an 'optimal' solution from a selection of sub-optimal treatments. This study therefore aims to address these assumptions by considering antibiotic induced

death as a function of the concentration of antibiotic present and by using a genetic algorithm (GA) to identify optimal treatment regimens. The use of a GA allows for the automatic exploration of the vast space of potential treatment regimens, in order to locate the most efficient ones. The effectiveness of conventional treatment regimens in eradicating bacterial infections will be analysed and compared to the alternative treatment regimens identified using the GA. This will be the first study examining the use of a genetic algorithm to optimise antibiotic treatment regimens.

3.2.2 *Methods*

3.2.2.1 *Deterministic Model*

In keeping with previous studies [69, 63, 134, 104] a system of coupled ordinary differential equations are used to describe the dynamics of a population of susceptible (S) and resistant (R) bacteria. As asexual reproduction requires energy it is assumed that the growth rate of bacteria is limited and therefore modelled using the standard logistic growth equation. A cost, α, is associated with carrying the genes which confer resistance to antibiotics [8] and results in a reduced growth rate for the resistant strain. Genes can pass from resistant to previously susceptible bacteria through HGT, β, resulting in the loss of susceptible bacteria and the addition of resistant bacteria. There are 3 main mechanisms of HGT: transformation, transduction and conjugation. This study does not distinguish between the differing modes of HGT. Both susceptible and resistant bacteria die at a natural death rate, θ, and through exposure to antibiotics, $A_i(C)$.

Antibiotics are added to the system in daily doses. When $t = \hat{t}_n$ the concentration of antibiotic $C(t) = C(t) + D_n$, where $\hat{t} = (1, 2, 3, ..., 10)$ and D

is a vector of doses $D = (D_1, D_2, ..., D_{10})$. Conventional treatment regimens assume that D_1=D_2=...=D_{10}, however this study relaxes this constraint. Treatment regimens within this study are limited to a maximum of 10 doses but this could be increased indefinitely. Experimental data [110, 109] suggests that as the concentration of antibiotic increases the death rate increases until it reaches a saturation point. In addition, the concentration of antibiotic naturally decays within a host. The concentration of antibiotic is therefore modelled according to first order kinetics with an elimination constant g. The half-life of the antibiotic is therefore given by $t_{1/2} = \frac{\ln(2)}{g}$

To model the relationship between antibiotic concentration and antibiotic induced death rate the extension of the Emax model of antibiotic treatment by Regoes et al [110] was used (Eq. 3.1).

$$A_i(C) = \frac{Amax_i \left(\frac{C}{mic_i}\right)^{k_i}}{\left(\frac{C}{mic_i}\right)^{k_i} + \frac{Amax_i}{Bmax_i} - 1}, \qquad i \in \{S, R\} \tag{3.1}$$

The full model can therefore be written as (3.2).

$$\begin{aligned}
\frac{dS}{dt} &= \underbrace{rS\left(1 - \frac{S+R}{K}\right) - \theta S}_{\text{Natural Growth}} - \underbrace{\beta SR}_{\text{HGT}} - \underbrace{A_S(C)S}_{\text{AB Death}} \\
\frac{dR}{dt} &= \underbrace{rR\left(1 - \frac{S+R}{K}\right)(1-a) - \theta R}_{\text{Natural Growth}} + \underbrace{\beta SR}_{\text{HGT}} - \underbrace{A_R(C)R}_{\text{AB Death}} \\
\frac{dC}{dt} &= - \underbrace{gC}_{\text{Degredation}}
\end{aligned} \tag{3.2}$$

The parameter values were chosen such that in the absence of antibiotics the resistant strain would not out-compete the susceptible strain. Analytical analysis of the model was performed to identify the conditions which meet this criteria (see Supplementary Equations). This ensures that if resistance

invades it is due to the treatment regimen and not a result of the dynamics of the system. A full list of parameters and values can be found in Table 3.1.

Parameter	Description	Value
r	Replication Rate	2.7726
K	Carrying Capacity	1000
β	Rate of Transmission of Resistant Plasmid	0.00001
θ	Natural Death Rate	0.2
a	Cost of Resistance	0.2
g	Degradation Rate of Antibiotic	0.48
$Amax_S$	Max Antibiotic-induced Death Rate	4.873
$Bmax_S$	Max Net Growth in Absence of Antibiotic	$r - \theta$
mic_S	Min Inhibitory Concentration (MIC)	16
k_S	Hill Coefficient	4
$Amax_R$	Max Antibiotic-induced Death Rate	4.12
$Bmax_R$	Max Net Growth in Absence of Antibiotic	$r(1-a) - \theta$
mic_R	Min Inhibitory Concentration (MIC)	32
k_R	Hill Coefficient	4

Table 3.1: Full list of parameters and values used within the model.

3.2.2.2 *Stochastic Model*

Deterministic modelling contains no randomness and as a result produces the same outcome each time it is run. Provided the population densities are not too small, deterministic models produce good approximations of the system dynamics. However hosts treated with the same treatment regimen will not all

respond in exactly the same way. The small population size of resistant bacteria mean that stochastic events may lead to the emergence or extinction of a resistant strain. A stochastic framework was therefore produced for (3.2). This study uses the well-established Gillespie algorithm [111] to obtain stochastic simulations for the different treatment regimens. By calculating the probability of the individual events occurring, based on rates and parameter values from the deterministic model, the Gillespie algorithm randomly chooses the next event to happen and the time at which it will happen. The population of each bacteria is adjusted accordingly and the process is repeated. As the events are chosen randomly each simulation will be slightly different.

Simulations are run for 30 days to allow for infection to return if treatment regimens are unsuccessful. Each treatment regimen was run 5000 times with the infection either being eradicated or still present at the end of the 30 days. The success rate for each treatment regimen was obtained by calculating the total number of simulations which resulted in the eradication of both susceptible and resistant bacteria. After exposure to an antibiotic treatment regimen, infections which were not eradicated were found to be composed entirely of resistant bacteria. Therefore increasing the success rate decreases the emergence and potential spread of resistant bacteria.

Due to the ability to simulate the model thousands of times the variability within the results is small. However, the 95% confidence interval for each treatment was calculated in MATLAB using the Clopper-Pearson exact confidence interval [135]. This method was chosen due the occurrence of success rates close to 100%. The median time to eradication for all successfully eradicated infections was also calculated. (This data was not normally distributed and therefore the median was used instead of the mean).

3.2.2.3 *Genetic Algorithms*

Genetic algorithms (GA) were proposed by John Holland in the early 1970's [85]. They belong to the larger class of evolutionary algorithms, which generate solutions to optimisation problems using techniques inspired by natural evolution, such as inheritance, mutation, selection and crossover [136]. GAs have previously been used to generate treatment schedules for chemotherapy treatment [76, 78]. Despite being a randomised search GAs are by no means random, instead they use historical information to direct the search into the region of better performance within the search space.

In this study the genetic algorithm was used to identify effective dosage vectors, $D = (D_1, D_2, ..., D_{10})$, which would maximise the success rate of eradicating the infection by minimising the fitness (objective) function (3.3).

$$F = \underbrace{w_1 \alpha_1 \sum_{i=1}^{10} D_i}_{\text{Total Antibiotic}} + \underbrace{w_2 \alpha_2 \int_0^{30} N(t)dt}_{\text{Bacterial Load}} \tag{3.3}$$

Minimising the total amount of antibiotic used, $\sum_i D_i$, exposes the environment to less antibiotic reducing the likelihood of resistance developing. However, using less antibiotic increases the total bacterial burden on the host over the length of the infection, $\int_0^{30} N(t)dt$, where N=S+R. The increased bacterial load not only compromises the health of the host but also offers more opportunity for mutations to arise increasing the risk of further resistance developing. A trade-off exists between the total amount of antibiotic used and the total bacterial load over the course of the infection. Weights w_1 and w_2 allow for more emphasis to be placed on minimising one term over the other. To ensure a trade-off exists, $w_1 \geqslant 0.001$ and $w_2 \geqslant 0.001$ (However, this study later considers the case where $w_1 = 0$, hence the objective is solely to maximise treatment success.) Due to the difference in the magnitude of the

values of each term, correcting factors α_1 and α_2 were used to transform the terms between 0 and 1.

The GA uses the deterministic model to run simulations using the generated dosage vectors. Values from these simulations are then used to compute the fitness function for that dosage vector. The fitness function of each generated dosage vector are compared with the search space moving towards the vector with the smallest fitness function. (The GA was implemented using MATLAB with a population size of 100, for 1000 generations and repeated 50 times with values of 0.01 and 0.99 for Eq. 3.3, w_1 and w_2, respectively). Solutions were then run through the Gillespie algorithm to produce a success rate of eradication for each vector.

An alternative approach is to use the stochastic model as part of the fitness function evaluation within the GA. Limited results were obtained using this approach due to the computational time increasing substantially (in the order of 10^3) compared to using the deterministic model.

3.2.3 *Results*

Numerical simulations were run to analyse the effect different treatment regimens have on the population size of bacteria within an infection. The success rate and time to eradication of the infection were analysed. Treatment regimens are obtained from conventional regimens and from solutions derived using a GA. The results presented were performed with an initial resistant population of 10% of the total bacterial population. When analysed with an initial resistant population of 1% of the total bacterial population the results follow a similar pattern (see Supplementary Table *S1*).

3.2.3.1 *Conventional Treatment Regimens*

Using conventional treatment strategies of a constant dose administered for 10 days the minimum daily dose required to successfully treat the infection is 23 $\mu g/ml$ (Fig. 3.1). Under this regimen the infection is successfully eradicated in 99.8% (95% CI: 99.6, 99.9) of cases (n=5000 for all simulations). Administering 23 $\mu g/ml$ of antibiotics per day increases the concentration of antibiotic within the system over the 10 days, reaching a peak of 60 $\mu g/ml$ on day 10 (Fig. 3.1*b*).

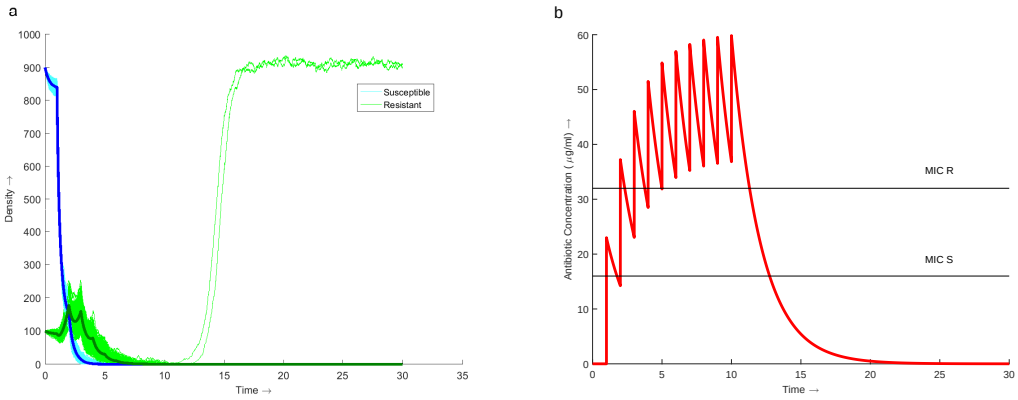

Figure 3.1: Dynamics of the model over 30 days with antibiotic therapy administered at a daily dose of 23 $\mu g/ml$ for the first 10 days. (a) Stochastic simulations of the population dynamics of both susceptible (blue) and resistant (green) bacteria with the deterministic dynamics (bold) overlaid. 5000 simulations were run producing a success rate of eradicating the infection of 99.8% (95% CI: 99.6, 99.9). (b) Simulation of the concentration profile of antibiotic present within the system over the 30 day duration. The MIC lines indicate the concentration of antibiotic required to inhibit the growth of the respective bacterial strain, 16 $\mu g/ml$ for susceptible bacteria and 32 $\mu g/ml$ for resistant bacteria. A maximum antibiotic concentration of 60 $\mu g/ml$ is observed on Day 10.

From Fig. 3.1*b* it is noted that it takes 3 days before the concentration of antibiotic is maintained above the MIC of the resistant strain. During these first

3 days the population of resistant bacteria increases (Fig. 3.1*a*). Once above the MIC of the resistant strain the population begins to decrease. If the infection is not eradicated under the conventional treatment regimen then a resistant infection will emerge.

Until now the study assumed that conventional treatment regimens are administered over 10 days. This assumption was relaxed and the success rate of eradicating the infection over a shorter duration examined (Table 3.2). Shorter treatment duration results in a decrease in the success rate of eradicating the infection. Treatment duration fewer than 8 days experiences a substantial decrease in success rate, to below 90%.

	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n=5000]	Time to Eradication (days) [95% CI]
$T1$	$(23, 23, 23, 23, 23, 23, 23, 23, 23, 23)$	230	99.8 [99.6, 99.9]	7.31 [7.23, 7.39]
$T2$	$(23, 23, 23, 23, 23, 23, 23, 23, 23, 0)$	207	99.0 [98.7, 99.3]	7.29 [7.19, 7.35]
$T3$	$(23, 23, 23, 23, 23, 23, 23, 23, 0, 0)$	184	96.4 [95.8, 96.9]	7.13 [7.04, 7.19]
$T4$	$(23, 23, 23, 23, 23, 23, 23, 0, 0, 0)$	161	87.4 [86.4, 88.3]	7.12 [7.04, 7.20]

Table 3.2: Comparison of success rate and time to eradication for conventional treatment dosage vectors of varying duration. For time to eradication of regimens $T1$, $T2$, $T3$ and $T4$; n = 4990, 4950, 4820 and 4370 respectively.

The time taken to eradicate the bacterial population was also measured. This time was only recorded in the cases where the treatment was successful and the bacterial population completely eradicated. There is a small decrease in the time to eradication as the treatment duration decreases from 10 days to 7 days. However, this is due to the shorter regimen leading to a lower success rate. The 7 day conventional treatment is unable to eradicate infections

which persist beyond 8 days due to the antibiotic continuously degrading beyond the last day of treatment. Due to these persistent infections not being eradicated the median time to eradication lowers in comparison to the longer conventional treatment regimens. As the treatment length increases above 7 days the success rate also increases. The median increase in success rate from 8 days to 10 days is 3.4% but requires 18.7% more antibiotic to achieve this. To maintain a success rate of over 90%, under a conventional treatment regimen, this infection can be treated by administering a minimum of 184 $\mu g/ml$ of antibiotic over 8 days. This regimen results in a success rate of 96.4% and is used as the baseline to look for improved treatments.

3.2.3.2 *Genetic Algorithm with the Deterministic Model*

Due to the toxic nature of antibiotics the total antibiotic concentration within the system at any point in time was constrained to a maximum of 60 $\mu g/ml$ within the GA. This is in keeping with the maximum concentration from the conventional treatment regimen (although this could be relaxed if needed). The GA was run for varying maximum daily dosages of 60, 50 and 40 $\mu g/ml$ per day. The successful dosage vectors were then run through a stochastic model to generate a success rate of eradicating the infection.

The dosage vectors from the GA begin with an increased dose which tapers off as the treatment progresses (Table 3.3). Results from the GA suggests that the duration of therapy could be as little as 4 days (Table 3.3, regimens *D1* and *D3*). However, these treatment regimens have a lower success rate, 91.2% (95% CI: 91.0, 92.5) and 92.3% (95% CI: 91.5, 93.0), than the conventional regimen, 96.4% (95% CI: 95.8, 96.9). For all three maximum daily doses, the longer duration regimens (Table 3.3, regimens *D2*, *D5* and *D8*) are more efficient at treating the infection than the shorter durations with success rates of 94.3% (95% CI: 93.6, 94.9), 94.4% (95% CI: 93.7, 95.0) and 95% (95% CI: 94.4, 95.6)

	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n=5000]	Time to Eradication (days) [95% CI]
D1	(60, 21, 22, 15, 0, 0, 0, 0, 0, 0)	118	91.2 [91.0, 92.5]	3.93[3.88, 3.99]
D2	(60, 22, 18, 17, 11, 0, 0, 0, 0, 0)	128	94.3 93.6, 94.9]	3.98 [3.94, 4.04]
D3	(50, 29, 22, 21, 0, 0, 0, 0, 0, 0)	122	92.3[91.5, 93.0]	4.12[4.06, 4.17]
D4	(50, 28, 20, 20, 10, 0, 0, 0, 0, 0)	128	93.2[92.5, 93.9]	4.17 [4.11, 4.23]
D5	(50, 19, 21, 23, 18, 10, 0, 0, 0, 0)	141	94.4[93.7, 95.0]	4.56[4.50, 4.64]
D6	(40, 35, 23, 21, 13, 0, 0, 0, 0, 0)	132	92.5 [91.7, 93.2]	4.46[4.41, 4.51]
D7	(40, 26, 26, 23, 17, 11, 0, 0, 0, 0)	143	94.0 [93.2, 94.5]	4.77 [4.71, 4.86]
D8	(40, 21, 27, 18, 26, 13, 11, 0, 0, 0)	156	95.0 [94.4, 95.6]	5.33 [5.26, 5.41]

Table 3.3: Table comparing the success rates and time to eradication of dosage vectors produced by the GA with deterministic modelling. Regimens *D1*, *D3* and *D6* represent the best dosage vectors with maximum daily doses of 60, 50 and 40 $\mu g/ml$ respectively. All other runs represent the best dosage vector of increased treatment duration. For Regimens *D1* - *D8*; n = 4560, 4715, 4615, 4660, 4720, 4625, 4700 and 4750 respectively.

respectively. The lack of noise within the deterministic model allows the GA to be very effective in minimising the total antibiotic used. When the shorter dosage vectors from the GA using the deterministic model are analysed using the stochastic model there is too little antibiotic administered over too short a duration leading to the emergence of resistant bacteria. Varying the weights had no significant effect on the results (Table 3.4).

The total concentration of antibiotic in the conventional regimen (Fig. 3.1*b*) increases slowly over the 8 days. The regimens from the GA start with an initial high dose followed by tapering smaller doses which maintain the total concentration of antibiotic above the MIC of the resistant bacteria for the

w_1	w_2	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI]
0.5	0.5	(60, 22, 23, 13, 0, 0, 0, 0, 0, 0)	118	91.9 [91.1, 92.6]
		(60, 22, 22, 14, 11, 0, 0, 0, 0, 0)	129	95.0 [94.4, 95.6]
0.99	0.01	(60, 22, 21, 15, 0, 0, 0, 0, 0, 0)	118	92.3 [91.5, 93.0]
		(60, 22, 18, 17, 11, 0, 0, 0, 0, 0)	128	93.9 [93.2, 94.6]

Table 3.4: Comparison of dosage vectors produced by the GA with deterministic modelling, for varying values of w_1 and w_2.

majority of the duration of treatment (Fig. 3.2). All three regimens *D2*, *D5* and *D8* use less antibiotic in total over a shorter duration than the conventional regimen. Regimen *D2* uses 30% less antibiotic over 5 days instead of 8. Regimen *D5* produces a dosage vector which uses 23% less antibiotic than the conventional regimen and delivers it over 6 days instead of 8. The dosage vector from *D8* uses 15% less antibiotic and is shorter by 1 day in duration.

All the regimens identified by the GA see a reduction in the time to eradication for the infection. The median time to eradication for the 8 day conventional treatment was 7.13 days (95% CI: 7.04, 7.20). By distributing the antibiotic in a high initial dose with tapering smaller doses the median time to eradication for all the the regimens identified by the GA is between 4 and 5.5 days.

3.2.3.3 *Genetic Algorithm with the Stochastic Model*

The GA was run using a stochastic model to maximise the probability of eradication and explore the effectiveness of a longer treatment duration. For the GA using the stochastic model the second term, minimising the bacterial load, in F (3.3) was replaced with a term minimising the number of unsuccessful runs out of the 5000. Due to the increased run time, only a few results could

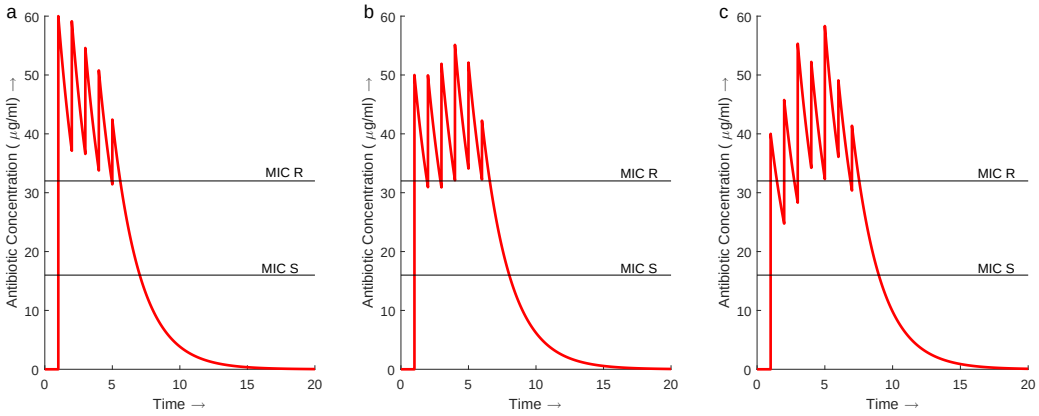

Figure 3.2: Concentration profiles for regimens *D2*, *D5* and *D8* from the dosage vectors identified by the GA with deterministic modelling. (a) Treatment regimen *D2* maintains an antibiotic concentration above the MIC of the resistant strain throughout the 6 day treatment. The maximum total concentration of antibiotic is 60 μg/ml. (b) *D5* also maintains a concentration above the MIC for the resistant bacteria throughout the 6 day treatment reaching a maximum total concentration of 54 μg/ml on day 4. (c) The concentration of antibiotic throughout *D8* increases above the MIC of the resistant bacteria initially but drops back below for the first two days. The concentration is then maintained above the resistant MIC for the remainder of the treatment, reaching a maximum concentration of 58 μg/ml on day 5.

be given (Table 3.5).

The dosage vectors from the stochastic model are noisy due to the randomness in the model. Despite this, the dosage vectors begin to converge to a similar pattern identified using the GA with the deterministic model. A large initial dose followed by an extended period of tapering lower doses is observed. The median time to eradication for the stochastic results are comparable to the deterministic results. However, by using more antibiotic over the longer treatment duration the stochastic regimens have a greater success rate. Despite the increase in total antibiotic these dosage vectors use between 11 and 19% less antibiotic than the conventional regimen with a similar or increased

	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n=5000]	Time to Eradication (days) [95% CI]
S1	(60, 19, 17, 16, 19, 18, 0, 0, 0, 0)	149 μg/ml	96.9 [96.2, 97.2]	4.14 [4.09, 4.20]
S2	(50, 25, 24, 20, 20, 12, 0, 0, 0, 0)	151 μg/ml	98.4 [97.7, 98.5]	4.23 [4.18, 4.31]
S3	(40, 27, 21, 22, 23, 12, 18, 0, 0, 0)	163 μg/ml	97.1 [96.6, 97.5]	5.03 [4.96, 5.11]
S4	(60, 22, 22, 22, 18, 15, 14, 11, 0, 0)	184 μg/ml	99.7 [99.5, 99.8]	3.94 [3.89, 3.99]

Table 3.5: Table comparing the success rates and time to eradication of dosage vectors produced by the GA with stochastic modelling for maximum daily doses of 60, 50 and 40 μg/ml and the case where all 184 μg/ml of antibiotic is used. n = 4845, 4920, 4855 and 4985 for time to eradication of *S1*, *S2*, *S3* and *S4* respectively.

success rate. Dosage regimen *S2* has the greatest success rate, 98.4% (95% CI: 97.7, 98.5), an increase on the conventional 8 day treatment, 96.4% (95% CI: 95.8, 96.9). The GA was able to identify alternative treatment regimens using less antibiotic with a success rate of eradication equal to or better than the conventional treatment. The alternative regimens also successfully treat the infection over a shorter duration than the conventional regimen, around 4 to 5 days, vs. 7 to 7.5 days respectively.

If the priority is not to reduce the total antibiotic used, the GA can be implemented to maximise the effectiveness of current regimens. In this case, how can the 184 μg/ml of antibiotics be distributed to maximise the probability of eradication? (i.e. set $w_1 = 0$ in Eq. 3.3) The GA identifies a high initial dose followed by a tapering of doses (Table 3.5, regimen *S4*) as the optimal distribution of the antibiotics. This regimen resulted in a success rate of 99.7% (95% CI: 99.5, 99.8) compared to 96.4% (95% CI: 95.8, 96.9) obtained from the conventional treatment (Table 3.2). This regimen also eradicates the infection

quicker than the conventional regimen with a median time to eradication of 3.94 days (95% CI: 3.89, 3.99) compared to 7.13 days (95% CI: 7.04, 7.19) for the conventional regimen.

3.2.4 *Sensitivity Analysis*

Due to the difficulty in obtaining exact parameter values for an infection, the effect changes in parameter values have on the success rate of different treatment regimens was analysed. Parameter values relating to the virulence of the bacteria; replication rate (r), transmission rate (β) and cost of resistance (a) were examined. Further sensitivity analysis was performed for parameters concerning the effectiveness of the antibioitcs: degradation rate (g), MIC of susceptible (mic_S) and resistant bacteria (mic_R) and the shape of the antibiotic death function (k). Changes in parameters r, a, g and mic_R show the greatest change and can be found in Figure 3.3. Other results can be found in Supplementary Figure S1. Analysis was performed on the conventional 8 day treatment regimen (Table 3.2, regimen $T3$) and GA generated treatment regimens (Table 3.5, regimens $S2$ and $S4$).

As r, g and mic_R decrease, the success rate for all three treatment regimens converge towards 100%. At these lower parameter values the tapered regimens have no benefit over the conventional regimen. However, as r, g and mic_R increase the success rates for all 3 treatments decrease. As the parameter values continue to increase the benefit of the new tapered regimens increase significantly over the conventional regimen. The cost of resistance follows a similar pattern. As a increases the three treatment regimens are equally as effective with all success rates converging to 100%. However, when a is decreased the success rates for all three treatments also decrease. Despite the decrease in

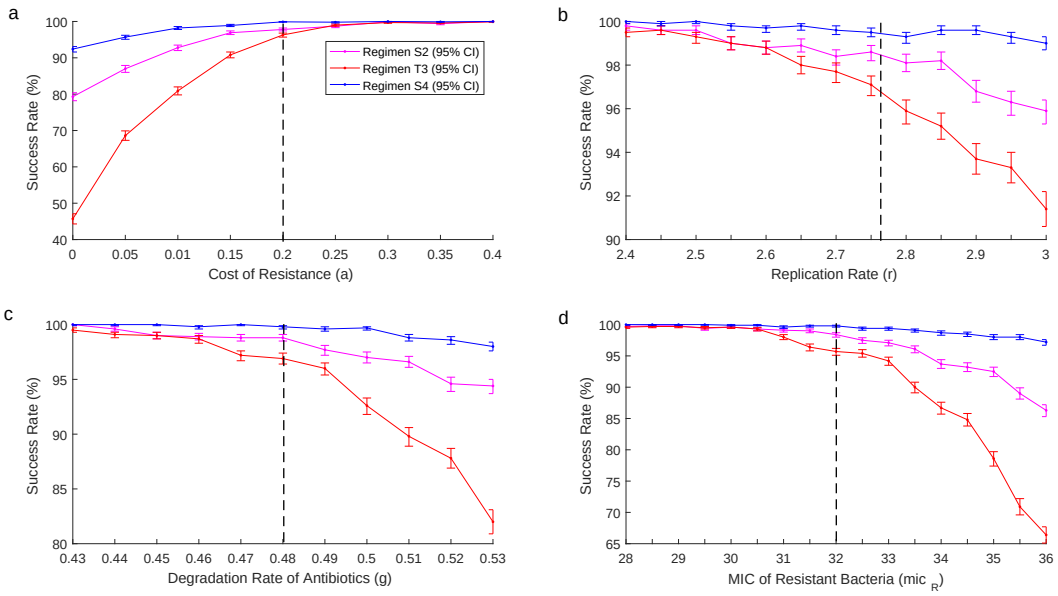

Figure 3.3: Success rates for regimens *S2* (pink), *T3* (red) and *S4* (blue) at varying values for parameters (a) a, (b) r, (c) g and (d) $\mathrm{mic_R}$. Black dashed line shows original parameter values. As parameter values are altered to benefit the infection success rates for all three treatment regimens decrease. With the tapered regimens performing better than the conventional regimen. If parameter values are altered to disadvantage the infection the three regimens converge to a similar success rate.

success rates the tapered regimens obtained from the GA perform better than the conventional regimen. When there is no cost of resistance the success rate of the conventional regimen dropped to below 50% at 45.7% (95% CI: 44.3, 47.1) whereas the tapered regimens remain significantly higher at 79.3% (95% CI: 78.2, 80.4) and 92.4%(95% CI: 91.6, 93.1). Across all the parameter values analysed regimen *S4* consistently maintains a success rate above 90%. Whereas when the same amount of antibiotic is distributed in a conventional manner the success rate can drop to below 50%. Despite regimen *S2* using less antibiotic it also consistently performs better than the conventional regimen.

While the previous tapered regimens perform well when the parameter values are altered, they are not necessarily the optimal dosage vectors for these new parameter sets. To examine whether the tapered effect was a consequence of the parameter values chosen the GA was used to generate optimal dosage vectors for the varied parameter values found in Fig. 3.3. In every run of the GA the optimal solution was an initial high dose followed by tapering doses. Although the optimal solutions do not change qualitatively, i.e. high dose with tapering, the exact doses do vary substantially. An example is shown in Table 3.6 where the growth rate was varied by 10%. Here the same pattern holds qualitatively but there was variation in the exact doses. Tapered regimens may be optimal, however the exact doses need to be personalised across infections.

Parameter	Value	Dosage Vector	Total Antibiotic
r	2.5	(60, 21, 16, 16, 17, 13, 0, 0, 0, 0)	143 $\mu g/ml$
	2.7	(50, 19, 21, 23, 18, 10, 0, 0, 0, 0)	141 $\mu g/ml$
	3	(44, 32, 23, 14, 18, 0, 0, 0, 0, 0)	131 $\mu g/ml$

Table 3.6: Optimal dosage vectors achieved when growth rate is altered by $\pm 10\%$

3.2.5 *Discussion*

Current antibiotic treatment regimens consist of a fixed daily dose administered for a set duration. While these conventional regimens may be easier to administer due to the constant dose, there is little evidence that this is the optimal way of administering antibiotics. Despite the continued increase in antibiotic resistance these conventional treatment regimens remain largely unchanged. More research must be dedicated to ensuring we are using antibi-

otics in an optimal way.

This study considered a conventional treatment regimen of 23 $\mu g/ml$ of antibiotic per day for 10 days. While this regimen successfully eradicated the infection in 99.8% of cases, the daily dose of antibiotic falls between the MIC of the susceptible and resistant bacteria initially facilitating the emergence of resistance. This is due to the time it takes for the total concentration of antibiotic to increase above the MIC of the resistant strain. While between the two MIC points the susceptible bacteria are eradicated allowing the resistant population to increase with little competition. Provided treatment is continued, the concentration of antibiotic will eventually increase above the MIC of the resistant strain.

The GA, using the deterministic and stochastic models, identified that optimal dosage vectors contain an initial high dose followed by tapering lower doses. Initially increasing the concentration of antibiotic above the MIC of the resistant bacteria eradicates the selective advantage observed in the conventional treatment regimen. Smaller doses of antibiotic are then administered to maintain the concentration above the MIC of the resistant strain. In the example shown the tapered regimens reduce the amount of antibiotic required to successfully treat the infection by as much as 23%. In some regimens produced by the GA, the maximum concentration of antibiotic within the system was lower than that observed with the conventional treatment regimen (Fig. 3.2) despite prescribing higher doses. With increased levels of antibiotic selecting for increased resistance, the ability to successfully treat an infection while maintaining a lower total antibiotic concentration over a shorter duration minimises the risk of higher resistance being selected for.

If conventional treatment regimens are adapted to deliver doses above the MIC of the resistant strain then the initial facilitation of resistant bacteria will disappear. However, the total antibiotic concentration within the system will considerably increase. In this scenario increasing the daily dose to above that of the MIC of the resistant strain would increase the total antibiotic concentration to beyond the level determined as toxic, 60 $\mu g/ml$, after 3 days.

The set duration of treatment is often subjective with increased length being used as a precaution. Studies have looked to find the optimal length of therapy [137, 138, 139] but potential treatment durations are based on empirical evidence. This study used a mathematical model as a way to determine the time to eradication of the infection and therefore the minimum duration of treatment required. The 10 day conventional treatment has a median time to eradication of 7.31 days. Additional antibiotic treatment beyond 8 days resulted in a small increase in success rate despite a larger increase in total antibiotic required. Whereas treatment length fewer than 8 days (shorter than the time to eradication) resulted in a much lower success rate. A conventional treatment regimen of 23 $\mu g/ml$ of antibiotic per day for 8 days was therefore taken as a baseline treatment.

The GA can be used to redistribute the antibiotic within the conventional regimen to produce a more efficient treatment regimen. The 8 day conventional treatment used 184 $\mu g/ml$ of antibiotics and achieved a success rate of 96.9% with a time to eradication of 7.13 days. The alternative treatment regimen identified by the GA applied the 184 $\mu g/ml$ of antibiotic in a high dose tapered regimen to achieve a success rate of 99.7% with a time to eradication of 3.94 days. This success rate is comparable with the success rate for the 10 day conventional treatment but the GA generated regimen uses 20% less antibiotic

over fewer days to achieve it. By redistributing the antibiotic in a high dose, tapered pattern the time to eradication of the infection reduces considerably, allowing shorter treatment regimens to be just as effective.

Studies have shown that shorter treatment regimens can be effective in treating bacterial infections [140, 141] with initial loading dose treatments being beneficial in treating patients in critical care medicine [142]. Tapered regimens have been found to be effective when treating *Clostridium difficile* [143, 144]. However, the use of tapered regimens resulted in sub-optimal performance in previous studies using optimal control strategies [80, 81]. From the sensitivity analysis it is shown that as the parameters are altered it is possible for the success rate of a tapered regimen to drop significantly. In the case of reducing the cost of resistance the success rate for the tapered regimen dropped to below 80%. However, when the GA is used to identify an optimal solution for the new parameter set it produces the same tapered pattern but with different dose values. Generic tapering regimens will not always be the most efficient regimen. The sensitivity in the doses required for a successful tapering regimen indicates that personalisation of individual treatments is required. Such personalisation can be achieved with the use of a GA. Despite the need for personalisation the tapered regimens consistently performed better than the conventional regimen when the infection was more virulent or the antibiotic was less effective.

Using a GA to search for an effective treatment regimen allowed for a constrained search of all possible dosage vectors. The lack of noise within the deterministic model allows the GA to converge to a specific minimum antibiotic concentration. However, when this is analysed using the stochastic model, random events mean these treatments are not as efficient. The stochastic model therefore identifies slightly longer treatments with more antibiotic than the

deterministic model, increasing the success rate. The increased computation time of the GA using the stochastic model makes it inefficient. The GA using the deterministic model is much less computationally expensive and produces the same loading dose but with a shorter tapered duration than the results using the stochastic model. The results from the deterministic model still have value and provide a suitable starting point from which to base potential future treatment regimens.

The use of the GA suggests that, in order to optimise antibiotic treatment regimens, the idea of constant doses needs to be addressed. Research indicates that the use of combination or sequential treatments are more effective in preventing resistance [61, 73, 145]. However, these studies use sub-optimal conventional treatments as comparisons and therefore single antibiotic treatments should not be ruled out. Genetic algorithms provide an efficient way of identifying and investigating the potential use of alternative single, and multiple, antibiotic treatment regimens to prolong the effectiveness of current antibiotics.

3.3 SUPPLEMENTARY INFORMATION

This section contains all the supplementary information referred to throughout the paper.

3.3.1 *Supplementary Equations - Analytical Analysis of Antibiotic Free System*

Using stability analysis the steady states of the system, in the absence of antibiotic, can be determined. At equilibrium, $dS/dt = dR/dt = 0$, there are four equilibrium points:

1. Extinction: $(S, R) = (0, 0)$

2. Susceptible Only: $(S, R) = (K(1 - \frac{\theta}{r}), 0)$

3. Resistant Only: $(S, R) = (0, K(1 - \frac{\theta}{r(1-a)})$

4. Co-existence: $(S, R) = (S^*, R^*)$ where

$$S^* = \frac{ar\theta + K\beta\,(ar - r + \theta)}{\beta\,(ar + K\beta)} \tag{3.4}$$

$$R^* = \frac{K\beta\,(r - \theta) - ar\theta}{\beta\,(ar + K\beta)} \tag{3.5}$$

Stability of the equilibrium points are found by calculating the Jacobian (Eq. 3.6) at each of the equilibria and calculating the corresponding eigenvalues.

$$J = \begin{pmatrix} r(1 - \frac{R+2S}{K}) - \beta R - \theta) & -\frac{rS}{K} - \beta S \\ -\frac{rR(1-a)}{K} + \beta R & r(1 - \frac{S+2R}{K})(1-a) + \beta S - \theta \end{pmatrix} \tag{3.6}$$

1. At the extinction equilibrium, $(0, 0)$, the Jacobian is reduced to Eq. 3.7.

$$J = \begin{pmatrix} r - \theta & 0 \\ 0 & r(1-a) - \theta \end{pmatrix} \tag{3.7}$$

The Jacobian matrix (Eq. 3.7) is a diagonal matrix and therefore the eigenvalues can be found on the diagonal. The extinction equilibrium is stable when Eq. 3.8 and 3.9 are satisfied.

$$r < \theta \tag{3.8}$$

$$r(1-a) < \theta \tag{3.9}$$

When the natural death rate is higher than the replication rate, for both the susceptible and resistant strains, the system will tend to extinction.

2. When evaluated at the resistant free equilibrium $(K(1-\frac{\theta}{r}),0)$, the Jacobian is reduced to Eq. 3.10.

$$J = \begin{pmatrix} \theta - r & \theta - \beta K - r - \frac{\beta K \theta}{r} \\ 0 & \beta K\left(1-\frac{\theta}{r}\right) - \theta a \end{pmatrix} \tag{3.10}$$

Eq. 3.10 is upper triangular and therefore the eigenvalues can be found on the diagonal. For the resistant free equilibrium to be stable it must satisfy Eq. 3.11 and 3.12.

$$\theta < r \tag{3.11}$$

$$\beta K\left(1-\frac{\theta}{r}\right) < \theta a \tag{3.12}$$

The replication rate must be greater than the death rate otherwise the susceptible population would die out, therefore Eq. 3.11 must hold true. A lower transmission rate or a higher cost benefits the susceptible population.

3. Evaluating the stability at the susceptible free equilibrium $(0, K(1-\frac{\theta}{r(1-a)})$, the Jacobian is reduced to Eq. 3.13.

$$J = \begin{pmatrix} \frac{\theta}{(1-a)} - \beta K\left(1-\frac{\theta}{r(1-a)}\right) - \theta & 0 \\ \theta - r(1-a) + \beta K\left(1-\frac{\theta}{r(1-a)}\right) & \theta - r(1-a) \end{pmatrix} \tag{3.13}$$

Eq. 3.13 is lower triangular and the eigenvalues can be found on the diagonal. Therefore for the susceptible free equilibrium to be stable it must satisfy Eq. 3.14 and 3.15

$$\frac{\theta}{(1-a)} - \beta K\left(1-\frac{\theta}{r(1-a)}\right) - \theta < 0 \tag{3.14}$$

$$\theta < r(1-a) \tag{3.15}$$

The net replication rate must be greater than the death rate otherwise the resistant population would die out, therefore Eq. 3.14 must hold true. A higher transmission rate or a lower cost benefits the resistant population making it possible for the resistance bacteria to invade and out-compete an entirely susceptible population.

4. Analysis of the stability of the co-existence equilibrium is not possible due to the eigenvalues being analytically intractable. If it is hypothesised that stable co-existence is possible then from the previous equilibrium points it can be concluded that co-existence will occur, assuming a positive net growth rate for both bacteria, if:

$$\beta K\left(1-\frac{\theta}{r}\right)-\theta a>0$$

and

$$\frac{\theta}{(1-a)}-\beta K\left(1-\frac{\theta}{r(1-a)}\right)-\theta>0$$

Using the analytical analysis parameter values were chosen such that they satisfy Eq. 3.11 and 3.12. Therefore the resistant strain would not out-compete the susceptible strain in the absence of antibiotics.

3.3.2 *Supplementary Table S1 - Results from 1% initial resistant population*

Run	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n=5000]
A	(21, 21, 21, 21, 21, 21, 21, 21, 21, 21)	210	99.5 [99.3, 99.7]
B	(21, 21, 21, 21, 21, 21, 21, 21, 21, 0)	189	98.4 [98.0, 98.7]
C	(21, 21, 21, 21, 21, 21, 21, 21, 0, 0)	168	96.7 [96.2, 97.2]
D	(21, 21, 21, 21, 21, 21, 21, 0, 0, 0)	168	85.4 [84.4, 86.4]
E	(60, 22, 4, 0, 0, 0, 0, 0, 0, 0)	86	86.9 [85.9, 87.8]
F	(50, 23, 13, 0, 0, 0, 0, 0, 0, 0)	86	87.2 [86.2, 88.1]
G	(40, 30, 20, 10, 0, 0, 0, 0, 0, 0)	100	91.8 [91.0, 92.5]
H	(60, 21, 10, 10, 2, 0, 0, 0, 0, 0)	103	96.2 [95.6, 96.7]
I	(50, 27, 19, 4, 0, 0, 0, 0, 0, 0)	100	96.4 [95.8, 96.9]
J	(40, 32, 18, 19, 0, 0, 0, 0, 0, 0)	109	96.2 [95.6, 96.7]
K	(60, 22, 22, 21, 15, 14, 10, 4, 0, 0)	168	99.9 [99.8, 100.0]

Table S1: Comparison of conventional dosage vectors (runs A, B, C and D), dosage vectors produced by the GA with deterministic modelling (runs E, F, G and K) and dosage vectors produced by the GA with stochastic modelling (runs H, I and J) for an infection with a resistant population of 1% of the total bacterial population.

3.3.3 *Supplementary Figure S1 - Results from varied parameter values on success rate of regimens T3, S2 and S4*

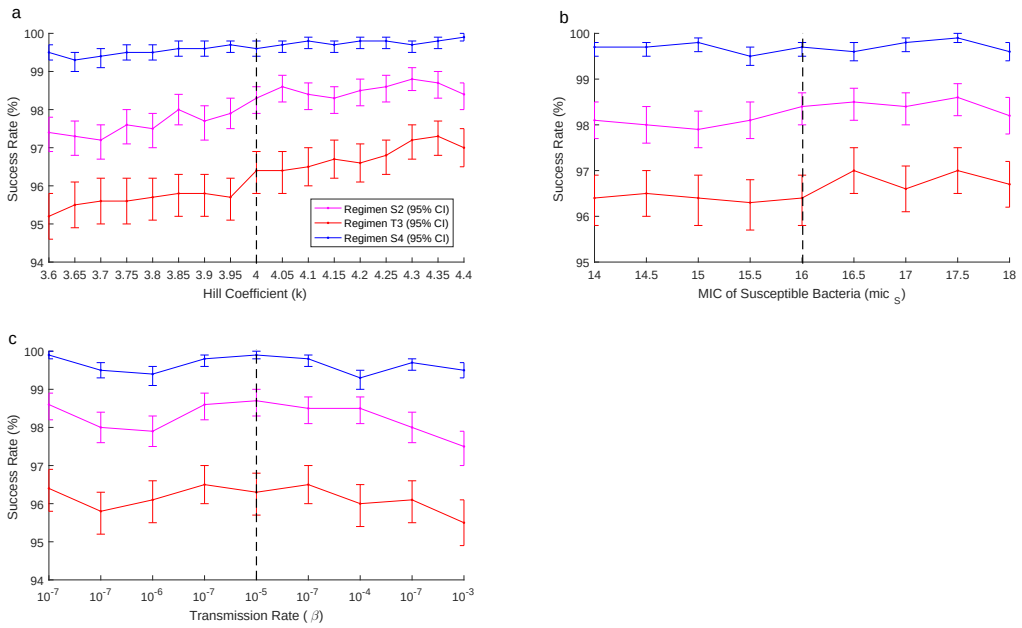

Figure S1: Success rates for regimens $S2$ (pink), $T3$ (red) and $S4$ (blue) at varying values for parameters (a) k, (b) mic_S and (c) β. Black dashed line shows original parameter values. (a) Increasing k results in an increase in success rate for all 3 regimens. The difference in success rate between the 3 regimens remains consistent. (b) Altering the MIC of the susceptible bacteria has little effect on the success rate of the 3 treatment regimens. (c) Increasing the transmission rate of the resistant bacteria begins to decrease the success rate. The difference in success rate between the 3 regimens remains consistent.

3.4 SUMMARY

The aim of this Chapter was to build upon the work of the previous Chapter by introducing a population of resistant bacteria. A two strain model was developed to describe the dynamics of both a susceptible and resistant bacterial population in the presence of antibiotics. Analysis of the two strain model

indicated that the presence of a resistant strain could result in a previously susceptible infection becoming resistant after exposure to antibiotics.

Using a conventional constant dose treatment as a comparison, the GA was combined with the two strain model to identify treatment regimens which reduced the likelihood of resistant infections developing. Although exact treatments are highly dependent on parameter values and initial bacterial load, a significant common trend was identified throughout the results. A treatment regimen consisting of a high initial dose followed by an extended tapering of doses increased the success of eradicating the infection while also minimising the amount of antibiotic used. Antibiotic resistance continues to be a significant global health concern and these results suggest that consideration should be given to revising current antibiotic treatment regimens.

The introduction of a population of resistant bacteria had no effect on the optimal pattern of antibiotic treatment, with the tapered pattern being identified when using both the one and two strain model. Section 3.2.4 highlights a further benefit of a tapered dosage pattern. When compared to the conventional constant dose treatment pattern the tapered pattern is less sensitive to changes in parameter values. Despite being a common pattern the exact doses required varied extensively between the two different models. Therefore a general rule for administering antibiotics in this tapered pattern could not be generated. However, personalisation of the tapered regimens could be achieved by incorporating the GA into the treatment decision making process.

Chapters 2 and 3 both identified tapered treatment regimens as the optimal way to administer the antibiotic. Over a range of parameter values and initial conditions it proved difficult to 'break' this pattern. This leads to a simple

question: is this pattern indeed the best, or is the model fundamentally flawed in a way which favours this result? Chapter 4 aims to address this question.

4

A CASE STUDY: OPTIMISING TETRACYCLINE DOSING STRATEGIES IN THE TREATMENT OF VIBRIO ANGUILLARUM IN GALLERIA MELLONELLA LARVAE

4.1 INTRODUCTION

Results from Chapters 2 and 3 identified that conventional constant dose treatment regimens are not always the optimal way to prescribe antibiotics. Using a genetic algorithm (GA), a personalised regimen consisting of a high initial dose followed by tapering doses was found to be more effective at eradicating the infection. Sensitivity analysis (Section 3.2.4) showed that the tapered regimen was more efficient than the conventional regimen over a wide range of parameter values. However, these results were based on theoretical parameter values and contained no experimental validation. This raises the question of whether these results are biologically realistic or a product of the approach and parameters chosen. Would a tapered treatment regimen still be identified by a GA as the optimal pattern to administer antibiotics when parameterised with real-life data? Is the GA able to identify alternative treatment regimens which result in significantly better success rates when used to inform a biological experiment?

This chapter therefore aims to repeat the work carried out in Chapter 2 by using data obtained from a real-life system. A case study consisting of a susceptible *Vibrio anguillarum* infection within larvae of the greater wax moth

(*Galleria mellonella*) treated using Tetracycline was studied. Experimental data was obtained for this system under various control and conventional antibiotic treatment regimens. Combining this data with previous work, a mathematical model was parameterised to this system.

The parameterised model was incorporated into the GA and used to identify alternative treatment regimens which optimised the success of eradicating the infection. The results from the GA were then used to inform further laboratory experiments. The *G. mellonella* larvae were treated according to the treatment regimen identified by the GA. The results from the experiment were compared with those from the GA to see if the GA was indeed able to identify a better treatment regimen and successfully predict the outcome of the real-life system.

4.2 THE CASE STUDY

4.2.1 *Vibrio Anguillarum*

Gram-negative bacteria present an ever growing problem in the face of antibiotic resistance. Due to the presence of a unique outer membrane, gram-negative bacteria are naturally resistant to many classes of antibiotics. Many species of gram-negative bacteria cause infections such as pneumonia, gonorrhea, cholera and even the plague. If left untreated, gram-negative bacteria can enter the bloodstream causing sepsis and septic shock, a highly fatal condition. With many strains resistant to multiple previously susceptible antibiotics [146, 147], gram-negative bacteria pose a significant threat to human health [148].

V. anguillarum is a well-studied Gram-negative bacterium: it is the causative agent of vibriosis, one of the most prevalent and devastating diseases in marine

aquaculture [149]. Vibriosis is a highly fatal haemorrhagic septicaemia which causes significant economic losses worldwide [150]. Due to the potential losses in species of economic importance, such as salmon, turbot, rainbow trout and cod, a vaccine exists against vibriosis. This vaccine has been widely successful in grown fish [151]. However, these vaccines are not effective in the larval stage due to their immature immune system. Antibiotics are therefore used to treat vibriosis in aquaculture with tetracycline and quinolones being the first drugs of choice. Resistance to V. anguillarum has been reported within aquaculture environments [152]. With limited antibiotics licensed for use in aquaculture, optimal treatment regimens are essential to ensure the prolonged effectiveness of these antibiotics and reduce the spread of further resistance.

4.2.2 *Greater wax moth - Galleria mellonella*

Due to the strict legislation surrounding whole-animal studies [153], experimentation with *V. anguillarum* in animals such as Atlantic salmon (*Salmo salar*) was infeasible for this study. The larvae of the greater wax moth (*G. mellonella*) was considered as an alternative host due to their low maintenance and being inexpensive to purchase. *G. mellonella* larvae are increasingly being used as a host to study infectious diseases [154, 155]. Further to this, a study by McMillan et al [156] examined the virulence of *V. anguillarum* in *G. mellonella* larvae. This provided existing data to parameterise part of the model.

4.2.3 *Tetracycline*

Antibiotics are not specific to either human or animal treatment. As resistance to antibiotics continues to increase, the use of antibiotics within animal sectors has come under greater scrutiny [157, 158]. Aquatic environments can

act as reservoirs for antibiotics and resistance genes [54]. This has led some countries to prohibit the use of certain antibiotics in aquaculture due to their importance in human healthcare [128]. However, legislation and guidelines around antibiotic use vary widely between countries.

The use of tetracycline in aquaculture has been linked to the increase in tetracycline resistant genes within the environment [159]. Tetracycline is a broad spectrum antibiotic produced by the Streptomyces genus of Anctinobacteria. It is widely used in both human and animal medicine. Tetracycline is a predominately bacteriostatic antibiotic and works by inhibiting protein synthesis. It may also alter the cytoplasmic membrane of bacteria causing leakage of intracellular contents, such as nucleotides from the cell. It is a fast acting antibiotic with a half life between 6 and 12 hours.

4.3 EXPERIMENTS USING CONVENTIONAL TREATMENT REGIMENS

This section presents an overview of biological experiments led by Dr Andrew Desbois within the Institute of Aquaculture at the University of Stirling. The author of this thesis was involved in the design of these experiments but did not carry them out. The experiments were designed to examine the effect conventional antibiotic treatment regimens have on the survival rate of the hosts. The data from these experiments would be used to parameterise a model of the dynamics of a *V. anguillarum* infection within *G. mellonella* larvae in the presence of tetracycline. This model will then be used to identify more effective treatment regimens using a genetic algorithm.

G. mellonella larvae were injected with 1×10^7 total colony forming units (CFU) of *V. anguillarum*. The bacteria were left to establish within the host for

2 hours. At 2, 24 and 48 hours the larvae were injected with the antibiotic tetracycline. The antibiotic treatment consisted of a total of 0.5 μg/g of tetracycline across the duration of treatment. Conventional treatment regimens consist of constant doses given in set time intervals. Three different constant dose treatments were administered and the effect on the survival of the larvae recorded.

The 0.5 μg/g of tetracycline was split equally over one, two or three days giving dosage vectors $(0.5, 0, 0)$, $(0.25, 0.25, 0)$ and $(0.166, 0.166, 0.166)$ respectively. At 2, 24, 48, 72, 96, 120, 144 and 168 hours the larvae were checked for signs of life. Any larvae that didn't respond were removed from the experiment and their death noted. Four control groups were also run: unmanipulated, phosphate-buffered saline (PBS)-only, antibiotic-only and *V. anguillarum*-only. The four control groups are used to ensure the results are due to the treatment regimens only. Unmanipulated larvae ensure the larvae survive the duration of treatment with no other interventions. Larvae injected with PBS-only ensure that injecting substances into the larvae does not account for their death. The antibiotic-only controls for toxicity of the treatment regimens. Finally, the larvae injected with *V. anguillarum* ensures that the bacteria do die due to the bacterial infection and therefore any survivors are due to the antibiotic. Results from the control groups and conventional treatment regimens can be found in Table 4.1.

Injecting the larvae with *V. anguillarum* at 0 hours and administering no antibiotic resulted in the bacterial infection killing all larvae by 72 hours. Only 3% of the larvae died within the first 24 hours with the majority of larvae (77%) dying between 24 and 48 hours after being injected with the bacteria. These results, along with additional data, will be used to parameterise the

Group	Infected	Treatment (μg/g)			Relative Survival (%)						
		Day 1	Day 2	Day 3	24h	48h	72h	96h	120h	144h	168h
Unmanipulated	-	-	-	-	97	97	97	97	95	95	95
PBS only	PBS	PBS	PBS	PBS	100	98	60	60	60	60	60
Tet. only	PBS	0.4	0.2	0.133	100	100	100	100	100	100	100
Vib. only	Vib. 79	PBS	PBS	PBS	97	20	0	0	0	0	0
T1	Vib. 79	0.5	PBS	PBS	100	100	100	78	69	61	56
T2	Vib. 79	0.25	0.25	PBS	100	97	83	75	58	56	56
T3	Vib. 79	0.166	0.166	0.166	100	75	58	33	28	28	22

Table 4.1: Table showing survival rates (at 24 hour intervals) of *G. mellonella* larvae injected with 1×10^7 total CFU of *V. anguillarum* and treated using a constant dose treatment regimen, T1, T2 and T3. Unmanipulated, PBS only, Tet. only and Vib. only are control groups. PBS only controls for the method of antibiotic delivery, Tet. only controls for the toxicity of the antibiotic and Vib. only is the infected control group and treated with PBS only.

model of bacterial growth within the larvae in Section 4.4.3.

Administering 0.5 μg/g of tetracycline, in a conventional constant dose, resulted in a survival rate of 55.6% (95% CI: 38.1, 72.1) at 168 hours when administered over 1 or 2 days (Table 4.1, *T1* and *T2*). When administered in one dose all larvae survive for the initial 72 hours. After 72 hours there is a 22% decrease in the number of larvae still alive. When the tetracycline is spread out over two equal doses the larvae begin to die after only 24 hours. Spreading the antibiotic further, over 3 days, also shows that no larvae died within the first 24 hours. However, a considerable decrease in success rate to 22% (95% CI: 10.1, 39.2) is observed at 168 hours. These results are used in Section 4.4.4 to parameterise a model in the presence of tetracycline.

4.4 MODEL DEVELOPMENT

This section begins by outlining the mathematical model of the growth of *V. anguillarum* bacteria within a *G. mellonella* larvae host. Data was obtained which is used to calculate the net growth rate of the bacteria within the larvae. The remainder of this model is parameterised using data collected in Table 4.1. The model is then extended to include the presence of antibitoics within the system. The results from the conventional treatment regimens in Table 4.1 are used to perform a least squares approach to fully parameterise the model.

4.4.1 *Modelling the Growth of V. anguillarum in G. mellonella Larvae*

The growth curve of *V. anguillarum* within the larvae is assumed to follow a logistic growth equation due to limiting resources within the host. The presence of a sub-population of resistant bacteria was not considered due

to the use of a single strain of the V. anguillarum bacteria. The short duration of the experiments make it unlikely that resistance would evolve. The bacteria replicate at a rate r with a carrying capacity K. A simple term, θ, is used to describe the effect of the host's immune response. The bacterial burden within the wax moth larvae (B) is therefore modelled according to (4.1).

$$\frac{dB}{dt} = rB\left(1 - \frac{B}{K}\right) - \theta B \tag{4.1}$$

A study by McMillan et al [156] recorded the bacterial burden within wax moth larvae at various time points after being injected with 1×10^7 CFU of *V. anguillarum*. Data beyond 48 hours could not be obtained due to the death of the larvae. The time series data from this study (Table 4.2) was used to calculate the net growth rate of the bacteria within the larvae.

Hour	$\log_{10}$ CFU/ml	Standard Error
0	6.849726	0
2	5.914942	0.121248
4	6.286829	0.10486
8	6.678288	0.148285
24	7.819691	0.142387
48	8.97435	0.258376

Table 4.2: Time series data from McMillan et al. (2015) showing the bacterial burden of *V. anguillarum* within *G. mellonella* larvae at 2, 4, 8, 24 and 48 hours after inoculation.

When the bacteria are injected into the larvae there is an initial drop in the total bacterial load. This is due to the bacteria establishing within the host

(see lag phase in Section 2.2.1). After a couple of hours the bacteria become established and begin to multiply. The data from 0-2 hours is therefore omitted from the calculation of the net growth rate of the bacteria. In the absence of limiting resources bacteria grow exponentially. Assuming an initial exponential growth, the net growth rate can be calculated by solving the exponential equation (4.2). Calculating the growth rate early in the infection discounts any influence from limited resources. The net growth rate, a, was calculated between hour 2 and hour 4 (4.3).

$$x(t) = x(t_0)e^{a(t-t_0)} \tag{4.2}$$

$$\begin{aligned} 10^{6.287} &= 10^{5.915}e^{2a} \\ a &= \frac{0.372}{2}\ln(10) \qquad \approx 0.43 \end{aligned} \tag{4.3}$$

Larvae injected with 1×10^7 CFU of *V. anguillarum* were observed to have died after 48 hours. The maximum bacterial burden (z) the larvae are capable of harbouring before they die was identified by McMillan et al as 10^9 CFU. With a net growth rate of 0.43 the bacteria multiply rapidly and the bacterial burden in the larvae reached 10^9 too quickly. The net growth rate was re-calculated between hour 2 and hour 8 (4.4)

$$\begin{aligned} 10^{6.678} &= 10^{5.915}e^{6a} \\ a &= \frac{0.736}{6}\ln(10) \qquad \approx 0.29 \end{aligned} \tag{4.4}$$

After discussion with Dr Andrew Desbois, it was deemed that $a = 0.43$ was indeed too high and that $a = 0.29$ was more biologically realistic. To allow the use of an event based stochastic framework to simulate the model the net growth rate a was replaced with the growth term r and death term θ. With a net growth rate of 0.29, i.e. $a = r - \theta = 0.29$, the replication rate was taken

to be $r = 0.35$ and the death rate $\theta = 0.06$ based on stochastic simulations in Figures 4.1 - 4.3.

4.4.2 *Stochastic Modelling Framework*

Previously in this thesis, Gillespie's stochastic simulation algorithm (SSA) was used to introduce randomness into results produced by a mathematical model. Due to the number of bacteria being in the range of 10^9 the SSA method proves to be too computationally expensive for use within this chapter. It is a well documented problem that as a population size increases the number of events increase and, as a result, the time to the next event gets smaller. This slows the algorithm down and requires extensive computational resources to complete the simulation. A solution to this problem exists in the use of an approximation method called τ-leaping [160].

4.4.2.1 *Tau Leaping*

τ-leaping reduces the computation time by calculating all the events which occur within a time interval of length τ before updating the propensity functions. There are two events which can occur from (4.1), (Table 4.3). The number of each event which occur within the time interval τ, is a Poisson distributed random variable where the mean is dependent on the event rate R_i and τ interval. The variable B is therefore updated by (4.5). The propensity functions (R_i) are updated and the process repeats until an end time is reached. The tau-leap method was coded in MATLAB, example code can be found in Appendix A Section A.1.3.

Event	Outcome	Transition Rate
Birth of Bacteria	$(B \rightarrow B+1)$	$: rB(1-\frac{B}{K}) = R_1$
Death of Bacteria	$(B \rightarrow B-1)$	$: \theta B = R_2$

Table 4.3: Table showing the different events which can occur, the effect these events have on the population of *V. anguillarum* and the rate at which they happen.

$$B(t+\tau) = B(t) + (P(\tau R_1) - P(\tau R_2)) \tag{4.5}$$

Each treatment regimen is run 3600 times to allow for variability within the results from the stochastic nature of the tau-leap algorithm. A survival rate is calculated for each treatment regimen by taking the percentage of runs which result in the eradication of the infection. The 95% confidence interval is calculated in MATLAB using the Clopper-Pearson exact confidence interval.

4.4.3 *Parameterising the Model of V. anguillarum Growth in G. mellonella Larvae*

Using the data obtained from McMillan et al. parameter values were established for the replication rate (r), immune-induced death rate (θ) and the death load of bacteria (z). Due to the rapid death of the larvae, a realistic carrying capacity (K) could not be identified from the data in Table 4.2. By comparing the results from the experiments carried out with *V. anguillarum*-only (Table 4.1) to model simulations, a parameter value for K can be established.

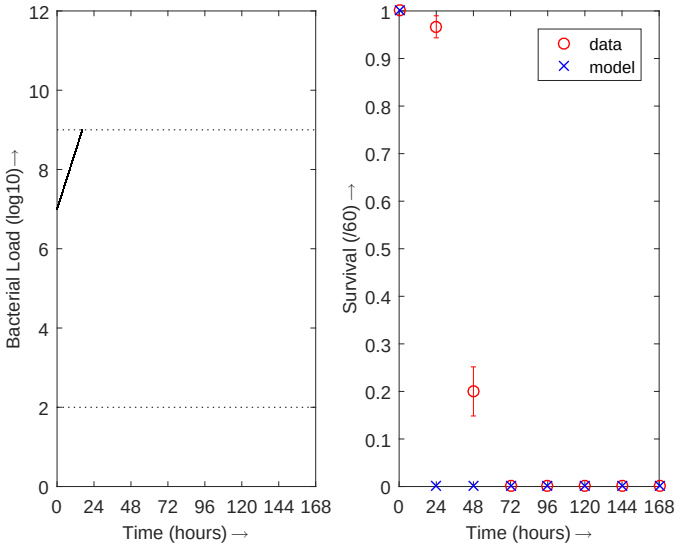

Figure 4.1: Simulation of the dynamics of the *V. anguillarum* bacterial load when no treatment is given (left). A comparison of the fraction of larvae still alive at each 24 hour time point (right).

For all values of K the model predicted the larvae would die much quicker than was observed in the experiments. Despite using a stochastic framework to simulate the growth of the bacteria in the larvae the high population numbers mean behaviour of individual bacteria have little effect on the overall system. All larvae are predicted to die at the same time (Figure 4.1).

To add some variation to the model the replication rate for each run of the simulation was taken from a normal distribution with mean of 0.35 and a standard deviation of 0.07. It is reasonable to assume that the growth rate of bacteria within different larvae may replicate at slightly different rates. Incorporating this variation into the model produces more realistic results due to variations in time of death for hosts. Despite this, the larvae are still predicted to die too quickly (Figure 4.2).

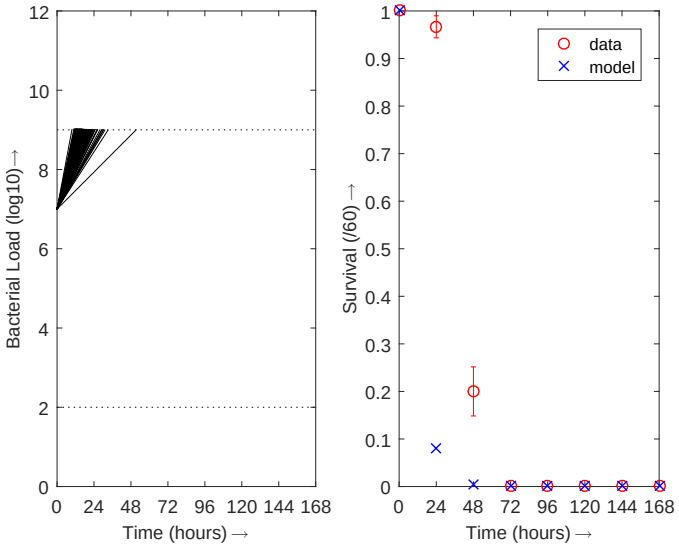

Figure 4.2: Simulation of the dynamics of the *V. anguillarum* bacterial load when no treatment is given. The replication rate of the bacteria is taken from a normal distribution with $\mu = 0.35$ and $\sigma = 0.07$ (left). A comparison of the fraction of larvae still alive at each 24 hour time point (right).

In the data from McMillan et al. the larvae were checked at 48 hours and then again at 72 hours. With no larvae alive at the 72 hour mark the bacterial load at 48 hours (10^9CFU) was deemed to be the maximum that could be sustained. This may have been an underestimate. Increasing the maximum bacterial load the larvae can sustain to $10^{11.5}$ with a carrying capacity of 10^{13} produced a much better fit for the data (Figure 4.3).

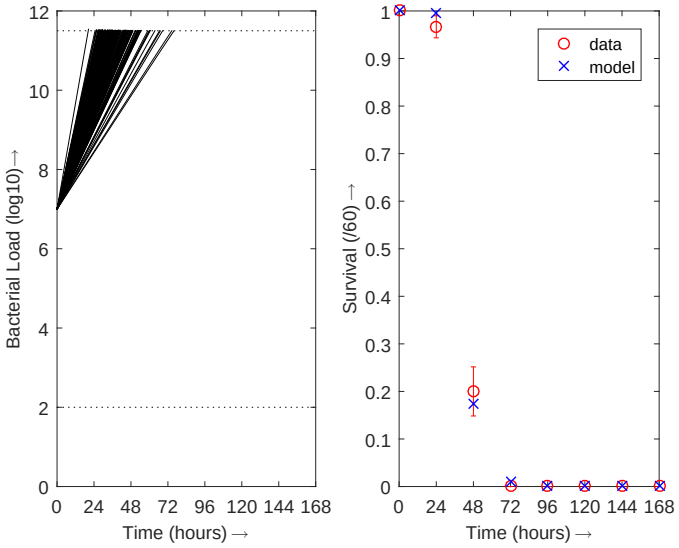

Figure 4.3: Simulation of the dynamics of the *V. anguillarum* bacterial load when no treatment is given. The replication rate of the bacteria is taken from a normal distribution with $\mu = 0.35$ and $\sigma = 0.07$, $z = 10^{11.5}$ and $K = 10^{13}$ (left). A comparison of the fraction of larvae still alive at each 24 hour time point (right).

4.4.4 *Introducing Antibiotic-Induced Death*

The model of a *V. anguillarum* infection in greater wax moth larvae (4.1) was extended to include the presence of antibiotics. Table 4.3 is updated to include the addition of an antibiotic induced death term ($A(C)$) (Table 4.4).

Event	Outcome	Transition Rate
Birth of Bacteria	$(B \rightarrow B+1)$	$: rB(1-\frac{B}{K}) = R_1$
Death of Bacteria	$(B \rightarrow B-1)$	$: \theta B + A(C)B = R_2$

Table 4.4: Table showing the different events which can occur in the presence of antibiotic, the effect these have on the population of *V. anguillarum* and the rate at which they happen.

Antibiotic presence within the system is modelled in keeping with the function outlined in previous chapters (4.6). An additional compartment for concentration of antibiotic (C) is also added to the model. Antibiotics are added, $C(t) = C(t) + D_n$, at time $t = \hat{t}$ hours, where $\hat{t} = (2, 24, 48)$ and D is a vector of doses $D = (D_1, D_2, D_3)$. Degradation of the antibiotics following first order kinetics with an elimination constant g (4.7).

$$A(C) = \frac{A_{max}\left(\frac{C}{mic}\right)^k}{\left(\frac{C}{mic}\right)^k + \left(\frac{A_{max}}{B_{max}} - 1\right)} \tag{4.6}$$

$$\frac{dC}{dt} = -\underbrace{gC}_{\text{Degredation}} \tag{4.7}$$

The parameter values from the previously parameterised model (4.1), r, θ and K, remain the same for the extended model. The values for the remaining parameters, A_{max}, mic, k and g, are identified using a non-linear least squares approach. A genetic algorithm (GA) was used to search through the possible combinations of parameter values. Each combination of potential parameter values is simulated using the stochastic model for each of the three conventional treatment regimens. The stochastic model was run 3600 times for each regimen. The percentage of runs where the bacterial burden has not reached the death threshold is recorded every 24 hours. The aim of the GA was to

identify the vector of unknown parameters, (A_{max}, mic, k, g), which minimises the difference between the simulated data (M_i) and the experimental data (E_i) at each time point (4.8).

$$F = \sum_{i=1}^{6} (E_i - M_i)^2 + w(E_7 - M_7)^2 \tag{4.8}$$

Modelling of pharmacokinetics is outwith the scope of this thesis. Due to the lack of pharmacokinetics, the minimal pharmacodynamic data and the simplified immune response more weight was placed on the data at the final time point. A full list of parameters and values can be found in Table 4.5.

Parameter	Description	Value
r	Replication Rate	0.35
K	Carrying Capacity	10^{13}
θ	Natural Death Rate	0.06
g	Degradation rate of antibiotic	0.05
A_{max}	Max antibiotic-induced death rate	0.83
B_{max}	Max growth in absence of AB	$r - \theta$
mic	Min inhibitory concentration (MIC)	0.1
k	Hill coefficient	3.25
z	Death Load of Bacteria	$10^{11.5}$
c	Eradication Threshold	10^2

Table 4.5: Full list of parameters and values used within the model.

Using the fully parameterised stochastic model the predicted survival rate at 168 hours for each of the conventional treatment regimens was calculated (Table 4.6). The 95% confidence interval for each survival rate was calculated using the Clopper-Pearson exact confidence interval. This method was chosen

to avoid any problems should success rates close to 0% or 100% occur. The variability within the results from the model is small due the ability to run thousands of simulations.

Group	Dosage Vector	Model Survival Rate (%) [95% CI, n=3600]	Exp Survival Rate (%) [95% CI, n=36]
T1	(0.5, 0, 0)	57.7 [56.1, 59.3]	55.6 [38.1, 72.1]
T2	(0.25, 0.25, 0)	61.0 [59.4, 62.6]	55.6 [38.1, 72.1]
T3	(0.166, 0.166, 0.166)	26.3 [24.9, 27.8]	22.2 [10.1, 39.2]

Table 4.6: Comparison of the results obtained from the model and the laboratory experiments for different constant dose treatment regimens.

A two-tailed Fisher's exact test was performed to determine if the results from the model were statistically different to the results from the conventional experiments for Table 4.6 *T1*, *T2* and *T3*. Fisher's exact test was used in place of a chi-squared test due to the sample size being small for the experimental results. The assumptions of random sampling and independent observations are met. The null and alternative hypothesis are as follows:

π_m = survival rate of hosts from model results
π_e = survival rate of larvae from experimental results

$H_0 : \pi_m = \pi_e$
$H_1 : \pi_m \neq \pi_e$

Fisher's exact test doesn't use a mathematical function to estimates the probability of a value of a test statistic; instead, you calculate the probability

of getting the observed data, and all data sets with more extreme deviations, under the null hypothesis that the proportions are the same. The p-value is calculated by adding together the probabilities of all combinations that have lower probabilities than that of the observed data. The cut-off p-value is calculated using (4.9).

$$p_{cutoff} = \frac{((a+b)!\,(c+d)!\,(a+c)!\,(b+d)!)}{a!b!c!d!N!} \tag{4.9}$$

where, a, b, c and d are the individual frequencies of a 2x2 contingency table, and N is the total frequency. Minitab was used to obtain the p-values at the 95% confidence level throughout this chapter. At the 0.05 significance level comparisons between the experimental and model results for *T1*, *T2* and *T3* have p-values 0.866, 0.498 and 0.705 respectively. The success rates from the experiments and the model are not significantly different at the 5% significance level. This was expected as the model was parameterised using the experimental data.

4.5 OPTIMISING ANTIBIOTIC TREATMENT REGIMENS

With the stochastic model parameterised to the *V. anguillarum* infection within the *G. mellonella* larvae it was used to inform the fitness function of a GA. The aim of the GA was to identify the dosage vector, $D = (D_1, D_2, D_3)$, when administered at hours, $\hat{t} = (2, 24, 48)$ that would maximise the success of eradicating the bacterial infection within the wax moth larvae. The assumption that antibiotics are prescribed in constant doses was relaxed. It is assumed that below 10^2 bacteria the host's immune system is able to clear the remaining infection. An eradication threshold was therefore set at $c = 10^2$, below this point the number of bacteria is assumed to be zero. The GA minimises the fitness function (4.10). Minimising the number of runs which result in bac-

teria remaining at the end of the simulation ($B(T)$) maximises the survival rate.

$$F = w \underbrace{\sum_{i=1}^{d} D_i}_{\text{Total Antibiotic}} + (1-w) \underbrace{\sum_{i=1}^{N} \hat{B}_i}_{\text{Unsuccessful runs}} \tag{4.10}$$

where

$$\hat{B} = \begin{cases} 1, & \text{if } B(T) > 0. \\ 0, & \text{otherwise.} \end{cases}$$

To ensure a comparison can be made between the dosage vector identified by the GA and the conventional treatment regimens from the experiments, some constraints were imposed on the GA. The dosage vector could use a maximum of 0.5 $\mu g/g$ of tetracycline over a maximum of 3 days. A weight, $w = 0$, was used to place the emphasis on maximising the success of eradicating the infection. Ensuring the GA used the maximum amount of antibiotic made it easier to directly compare if the GA was able to identify significantly better treatment regimens.

The dosage vectors identified by the GA (Table 4.7) begin with a high initial dose followed by tapering lower doses. Minitab was used to perform a one-tailed Fisher's exact test to determine if at the 0.05 significance level the model success rate for the dosage vector *GA1* was significantly greater than the model success rate of the conventional dosage vector *T1* or *T2*. The tapered dosage vector was found to have a significantly greater success rate than both traditional regimens with $p<0.0001$ for both comparisons.

The third dose of antibiotic was considered to not be significant due to being a very small dose and was attributed to noise within the GA. This suggests that a tapered two day treatment regimen is more effective at eradicating

	Dosage Vector	Model Survival Rate (%) [95% CI, n=3600]
GA1	(0.322, 0.169, 0.009)	69.3 [67.8, 70.8]
GA2	(0.340, 0.135, 0.025)	63.3 [61.7, 64.9]

Table 4.7: Comparison of dosage vectors produced by the GA with a weight $w = 0$.

the *V. anguillarum* bacteria than the conventional constant dose approach. A biological experiment was conducted to investigate this claim.

4.5.1 *Optimal Treatment Regimen Experiments*

A further experiment was led by Dr Andrew Desbois to examine whether the tapered regimen would perform better than the conventional regimens, as predicted by the GA, in a real-life system. *G. mellonella* larvae were injected with 1×10^7 CFU of *V. anguillarum* and treated with tetracycline at 2, 24 and 48 hours. With the assumption that the third dose is noise within the model and for lab simplicity, the dosage vector was cleaned to $D = (0.333, 0.167, 0)$.

Results from the experiment (Table 4.8) obtained a survival rate of 70.6% (95% CI: 53.0, 84.1) when using the tapered regimen. The survival rate for the tapered regimen from the model and the experiment are similar at the 0.05 level, p-value 1.000.

Comparisons between the model results for regimens *T1* and *E1*, and *T2* and *E1* show that the success rate of the tapered regimen is significantly greater than both conventional regimens with p-value < 0.001 at the 0.05 level when using a one-tailed Fisher's exact test for both comparisons. However,

	Dosage Vector	Model Survival Rate (%) [95% CI, n=3600]	Experiment Survival Rate (%) [95% CI, n=37]
E1	(0.333, 0.167, 0.000)	69.5 [68.0, 71.0]	70.6 [53.0, 84.1]

Table 4.8: Comparison of the results obtained from the model and the laboratory experiments when using the tapered treatment regimen identified by the GA.

comparisons between the experiment results for regimens *T1* and *E1*, and *T2* and *E1* show that the survival rate of the tapered regimen is not significantly greater than both conventional regimens with p-value 0.145 at the 0.05 level. While not statistically better, due to the small sample size of larvae a p-value of 0.145 is encouraging.

4.5.2 *Exploring Two Day Treatment Regimens*

The results from the genetic algorithm suggest that a two day treatment regimen is more effective. The general treatment regimen follows the following pattern: $(x, 0.5 - x, 0)$. Figure 4.4 shows the survival rate for a range of two day treatments.

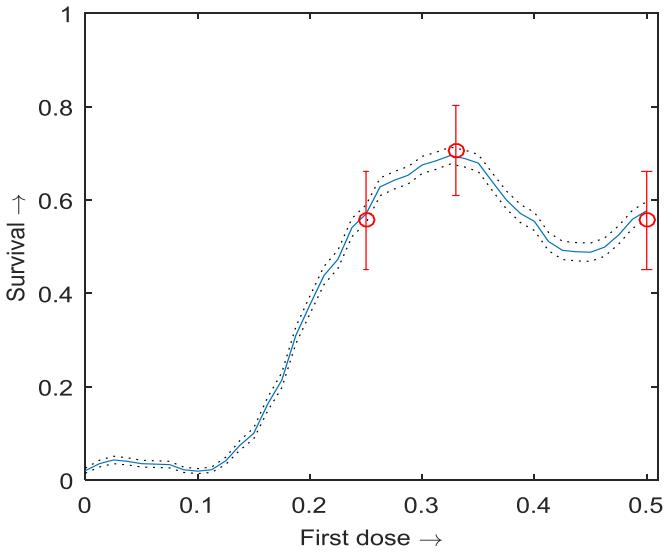

Figure 4.4: Graph showing the relationship between the survival rate of the larvae (obtained using the mathemtaical model) when treated using a two day treatment regimen and the concentration of the initial dose in the regimen. The circles represent the data obtained from laboratory experiments.

Comparing the range of possible two day treatments it can be shown that the GA was able to correctly identify the global maximum. The importance in starting antibiotic therapy early is seen with the low survival rate when the initial antibiotic dose is small. As the initial dose increases the survival rate also increases, to a point. The optimal treatment is found when the initial dose is higher than the subsequent dose. However, some trade-off exists between a high initial dose and maintaining the duration of antibiotic treatment as the survival rate begins to decrease as the initial dose increases further.

4.6 DISCUSSION

Results from the previous chapters repeatedly suggested that tapered treatment regimens are more effective than conventional constant dose treatment regimens at eradicating bacterial infections. However, the doses within these tapered patterns needed to be personalised to the system being treated. In-

corporating a mathematical model into a genetic algorithm allowed for these personalised treatment regimens to be identified. It is recognised that the 'one-size fits all' approach can result in the under-dosing or over-dosing of patients [116]. With a continued increase in multi-drug resistant bacteria, personalised treatment regimens may be a step forward.

A mathematical model of a *V. anguillarum* infection within *G. mellonella* larvae treated with tetracycline was developed. Experimental data was obtained and used to parameterise the model to the case study. The model was then used within a GA to simulate the behaviour of the biological system under various treatment regimens. A tapered treatment regimen was once again identified as the optimal way to administer the antibiotics to maximise the success of eradicating the infection. The dosage vector $D = (0.333, 0.167, 0)$ resulted in a survival rate of 69.5% when simulated using the model. Further biological experiments were carried out to obtain the survival rate for the tapered treatment regimen within the wax moth larvae. A survival rate of 70.6% was achieved, a similar result to that predicted by the model.

Using the GA to identify the personalised tapered regimen produced a statistically significant increase in the survival rate of eradicating the infection over the conventional constant dose regimens when analysed using the model. This is in keeping with the results from previous chapters. The results from the experiments confirmed these findings with an increase in survival rate for the tapered regimen over the conventional regimens from 55.6% to 70.6%. However, these results are not significantly different with a p-value of 0.145. Due to the small sample size of larvae, the confidence intervals are wide. With a p-value of 0.145 and a low sample size, evidence suggests that there would

be merit in repeating this experiment with a larger sample size.

The case study presented here was a very simple set-up in the absence of any resistance. However, when parameterised to the system, the GA was effective at identifying a personalised treatment regimen which increased the success of eradicating the infection.

4.7 SUMMARY

With the optimal use of antibiotics being important for their future effectiveness: Chapters 2 and 3 challenged the idea of conventional constant dose regimens in a theoretical approach. This chapter aimed to examine whether these findings held when applied to a real-life case study. By parameterising a mathematical model to a *V. anguillarum* infection within *G. mellonella* larvae, a GA was used to identify treatment regimens which would maximise the success of eradicating the infection when using tetracycline.

In keeping with the previous results, a tapered pattern was identified as the optimal way to administer the tetracycline. Laboratory experiments were carried out implementing the dosage vector identified by the GA. These results validated the findings of the GA. The tapered pattern did indeed increase the success of eradicating the infection. Due to the small sample size this increase was not statistically significant. However, the p-value was encouraging and warrants more experiments to further investigate these results.

GA's appear to be a useful tool in the search for optimal antibiotic treatment regimens with results accurately predicting the outcome of a real-life system. GA's are useful in their ability to identify regimens which may not have been

considered otherwise. Up to this point the GA has only been used to optimise the dose and duration of treatment given. These are not the only variables that exist within treatment regimens. Chapter 5 therefore explores the effect of changing the time interval between antibiotic doses.

5

INVESTIGATING THE IMPACT OF VARYING TIME INTERVALS BETWEEN ANTIBIOTIC DOSES

5.1 INTRODUCTION

Due to the continued increase in antibiotic resistant bacteria, antimicrobial stewardship continues to be a subject of international importance [161]. In 2015 the WHO Global action plan on antimicrobial resistance outlined five main objectives. Optimising the use of antimicrobial medicines in human and animal health being one of those objectives [162]. In addition the UK Government recently published its 5 year (2019-2024) national action plan for tackling antimicrobial resistance. Once again optimising the use of antimicrobials is highlighted as one of three areas that needs to be focussed on [163]. Strategies to quickly determine if antibiotics are required [164, 165, 166] and which antibiotic is the most appropriate for the bacteria present [167, 168, 169] are important in optimising antibiotic usage. However, this will only go so far if the treatment regimens of antibiotics are not identifying the correct time, quantity and duration of treatment.

Unfortunately, optimising antibiotic treatment regimens is neither straightforward nor simple. With most guidelines for antibiotic therapy based on either expert opinions or anecdotal data. In Chapters 2 and 3, the use of a genetic algorithm (GA) was shown to be effective at identifying alternative dosage vectors for the optimal treatment of a susceptible only and susceptible

and resistant bacterial population respectively. Using a theoretical parameter set, with the interval between doses of antibiotic fixed at 1 day, the GA re-distributed the antibiotic in a high initial dose with tapering lower doses. This pattern of dosage distribution allowed for higher success rates to be achieved while using less antibiotic over a shorter duration when compared to a conventional constant-dose treatment. Chapter 4 further strengthened these results by applying the same method to a simple real-life case study. Parameters obtained from experiments where the larvae of the greater wax moth (*Galleria mellonella*) was injected with *Vibrio anguillarum* were used to inform the model within the GA. Once again a tapered pattern was identified as the optimal way to administer the antibiotics in daily doses. When these results were tested experimentally the outcome was encouraging.

Different antibiotics have different degradation rates and as such the interval between the doses of antibiotics differs depending on which antibiotic is being used. However, studies have shown that longer intervals between doses of amoxicillin and aminoglycosides are just as effective as the standard dosing protocol [170, 171]. Daily dosing with aminoglycosides can result in reduced toxicity and enhanced clinical efficiency. Longer intervals between doses of amoxicillin results in less antibiotic being used. A study of the antibiotic cefazolin found that a single-dose treatment was not as effective as a multi-dose treatment in preventing surgical site infections [172]. These studies suggest that when designing optimal treatment regimens the interval between doses of antibiotics should be considered in addition to the dose and duration. This chapter will therefore relax the assumption present in the previous chapters that antibiotics are given in daily intervals.

By considering a system of susceptible and resistant bacteria, such as that in Chapter 3, this chapter will explore the effect changing the interval between doses has on the treatment regimens identified by the GA. Initially the GA will be used to optimise the time intervals between the doses in a conventional constant-dose treatment regimen to maximise the success of eradicating an infection. These results will be used to examine whether optimising the interval between doses of antibiotics is more effective at successfully eradicating an infection when compared to treatments where the daily doses have been optimised. The GA will then be extended to identify both the dosage vector and corresponding time vector of the optimal treatment strategy. The results will be analysed to examine whether altering the time interval has any effect on the overall success of treatments and the effect this has on the treatment strategies identified by the GA. Will the tapered pattern that has been identified previously still hold as the optimal treatment strategy?

5.2 MODEL OVERVIEW

This chapter uses the two strain model originally developed in Chapter 3 to describe the dynamics of a susceptible and resistant population within a bacterial infection. A brief overview of the model is presented in this section (for full details and equations see Chapter 3, Section 3.2.2).

As highlighted in previous chapters, using a deterministic model within the GA is computationally less expensive but the results can produce low success rates when simulated stochastically. For this chapter the use of a deterministic model within the GA was omitted.

5.2.1 *Stochastic Model*

A stochastic framework was developed for (3.2). All the growth, death and transmission rates are converted into an event. The deterministic rate, such as the growth rate of susceptible bacteria, is replaced by a transition rate for each event. When an event occurs the number of individuals in any particular compartment changes. There are 5 different events that can occur in this model (Table 5.1).

Event	Outcome	Transition Rate
Birth of Susceptible Bacteria	$(S \to S+1)$	$: rS(1-\frac{N}{K}) = R_1$
Death of Susceptible Bacteria	$(S \to S-1)$	$: \theta S + A_S(C)S = R_2$
Transmission of Resistant Gene	$(S \to S-1)$ and $(R \to R+1)$	$: \beta SR = R_3$
Birth of Resistant Bacteria	$(R \to R+1)$	$: rR(1-\frac{N}{K})(1-c) = R_4$
Death of Resistant Bacteria	$(R \to R-1)$	$: \theta R + A_R(C)R = R_5$

Table 5.1: Table showing the different events which can occur in the model, the effect these have on the population of susceptible (S) and resistant (R) bacteria and the rate at which they happen.

The antibiotic-induced death rate for both the susceptible and resistant bacteria is a function of the concentration of antibiotic (C) present within the system (5.1). Antibiotics are added to the system, $C(t) = C(t) + D_n$, when $t = \hat{t}_n$ where $\hat{t}$ is a vector of times, $\hat{t} = (\hat{t}_1, \hat{t}_2, ..., \hat{t}_d)$, and D is a vector of doses $D = (D_1, D_2, ..., D_d)$. The concentration of antibiotics within the system decays

at a rate g and can be modelled according to (5.2).

$$A(C) = \frac{Amax\left(\frac{C}{mic}\right)^k}{\left(\frac{C}{mic}\right)^k + \left(\frac{Amax}{Bmax} - 1\right)} \tag{5.1}$$

$$\frac{dC}{dt} = -\underbrace{\quad gC}_{\text{Degredation}} \tag{5.2}$$

The Gillespie algorithm was used to simulate the stochastic model. By including the events in Table 5.1, the probability of an event being the next event to happen is now (5.3).

$$\text{prob of event } i : P(i) = \frac{R(i)}{\sum_{i=1}^{5} R(i)} \tag{5.3}$$

The Gillespie algorithm gives a slightly different result each time it is run. Each treatment regimen is therefore run 5000 times to show the variability within the result. A success rate for each treatment regimen is obtained by calculating the percentage of runs which resulted in eradication of the infection. This success rate is used to compare the effectiveness of different treatment regimens.

5.2.2 *Parameterising the Model*

Using the parameter values in Chapter 3 the GA was able to identify a treatment regimen which resulted in the eradication of the infection in 99.7% of cases. The 184 $\mu g/ml$ of antibiotic was redistributed into a high initial dose followed by tapering lower daily doses. Due to the high success rate it would be near impossible to identify if non-daily treatment regimens resulted in a significant improvement without running a very large number of repetitions of the model.

The parameter set from Chapter 3 was therefore amended with the MIC of the susceptible and resistant bacteria increasing from 16 and 32 to 20 and 36 respectively. With higher MIC points but the same amount of antibiotic, the success rate of the treatment regimens decreases. With a lower success rate it becomes possible to identify a significant difference between two treatment regimens.

In keeping with Chapter 3 the parameter values ensure that invasion of a resistant infection is the result of the treatment regimen and not due to the dynamics of the system. In the absence of antibiotics the susceptible population will out-compete the resistant strain. A full list of parameters and corresponding values are in Table 5.2.

5.3 OPTIMISING TREATMENT REGIMENS

To identify alternative treatment regimens the GA searches for regimens which produce the smallest value for the given fitness function. Using the stochastic model within the GA allows for the fitness function to be a trade-off between the amount of antibiotic used and the number of infections which are successfully eradicated (5.4). As the GA aims to minimise the value of the fitness function, the second term in (5.4) minimises the number of runs where the infection persists. For the two strain model the number of bacteria present is the sum of susceptible and resistant bacteria, $B(T) = S(T) + R(T)$, where (T) is the time at the end of the simulation. Minimising the number of runs which result in bacteria present at the end of the simulation maximises the success rate of that treatment regimen.

Parameter	Description	Value
r	Replication Rate	2.7726
K	Carrying Capacity	1000
β	Transmission Rate	0.00001
θ	Natural Death Rate	0.2
a	Cost of Resistance	0.2
g	Degradation rate of antibiotic	0.48
$Amax_S$	Max Antibiotic Induced Death Rate	4.873
$Bmax_S$	Max net growth in absence of AB	2.57
mic_S	Min inhibitory concentration (MIC)	20
k_S	Hill coefficient	4
$Amax_R$	Max Antibiotic Induced Death Rate	4.12
$Bmax_R$	Max net growth in absence of AB	2.02
mic_R	Min inhibitory concentration (MIC)	36
k_R	Hill coefficient	4

Table 5.2: Full list of parameters and values used within the model.

$$F = w \underbrace{\sum_{i=1}^{d} D_i}_{\text{Total Antibiotic}} + (1-w) \underbrace{\sum_{i=1}^{N} \hat{B}_i}_{\text{Unsuccessful runs}} \tag{5.4}$$

where

$$\hat{B} = \begin{cases} 1, & \text{if } B(T) > 0. \\ 0, & \text{if } B(T) = 0. \end{cases}$$

As (5.4) is a trade-off between minimising total antibiotic used and maximising success, a weight $w \in (0 : 1]$ is used such that the emphasis placed on each term can be altered. If $w = 0$ the GA will focus only on maximising the success rate, as $w \to 1$ more emphasis is placed on minimising the amount of

antibiotic used.

While minimising the amount of antibiotic being used is preferable, the success rate of eradicating the infection must be prioritised. A value of $w = 0$ ensures treatments are identified that maximise the success rate with no pressure on trying to limit the amount of antibiotic used. To investigate whether the GA could identify alternative treatments which would lower the use of antibiotics but maintain high success rates two further values of w were examined. Values of w were found by equating the two terms within the fitness function and solving (5.5) for varying values of x, where x is the amount of antibiotic.

$$w\left(\frac{x}{184}\right) = (1-w)(0.01) \tag{5.5}$$

Weights were chosen based on the trade-off between the reduction in antibiotic x versus the success rate. A value of $w = 0.109$ and $w = 0.269$ were chosen. A value of $x = 15$ means that a reduction in antibiotic use of 15 $\mu g/ml$ must account for less than a 1% decrease in success to be considered a better treatment regimen. Solving to find w gives a value of 0.109. To increase the emphasis on minimising the antibiotic a value of $x = 5$ was chosen i.e. a reduction in antibiotic use of 5 $\mu g/ml$ must account for less than a 1% decrease in success to be considered a better treatment regimen. Solving to find w gives a value of 0.269.

5.3.1 *Setting Constraints for the Genetic Algorithm*

A conventional constant dose treatment regimen is used as a baseline for comparison with the optimal treatments identified by the GA. This regimen is also used to set some constraints within the GA. These constraints ensure that the treatments identified are comparable in terms of antibiotic usage and maximum concentration. The baseline conventional treatment regimen from

Chapter 3, 23 μg/ml per day for 8 days, is maintained. Due to the changes in the parameter values the success rate is reduced to 69% (67.7, 70.28) with a median time to eradication of the infection of 8.32 days (8.29, 8.35), Table 5.3.

Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n = 5000]	Time to Eradication (days) [95% CI, n = 3450]
(23, 23, 23, 23, 23, 23, 23, 23)	184	69 [67.7, 70.28]	8.32 [8.29, 8.35]

Table 5.3: Success rate of the baseline conventional treatment regimen and median time to eradication of the infection.

Constraints from the conventional treatment regimen are therefore as follows: The maximum amount of antibiotic used within the alternative treatment regimens must not exceed the amount used within the baseline treatment. The maximum amount of antibiotic that can be used across the entire duration of treatment is therefore 184 μg/ml. Antibiotics are toxic and can be lethal in high enough concentrations. To ensure that any regimen identified by the GA does not contain concentrations of antibiotic which might be lethal, regimens identified by the GA do not exceed the maximum concentration of antibiotic present within the conventional regimen. A cap of 60 μg/ml is set as the maximum concentration that can be present within the system at any given time (this constraint is later relaxed).

5.4 RESULTS

5.4.1 *Optimising Time Intervals between Constant Antibiotic Doses*

Up to this point the assumption has been that doses of antibiotics are administered in daily time intervals with the GA identifying the optimum daily

dose. Administering varying doses of antibiotic on a daily interval has the potential to increase success rates and even reduce the amount of antibiotic required to do so when compared to a constant dose regimen (see Chapter 3). However, unless administered in a liquid solution, the practicalities of manufacturing tablets which would allow for varying doses is complex. This raises the question: Is it possible to improve the success rate of constant dose treatments by varying the interval between the doses?

By fixing the dosage vector to that of the conventional treatment regimen $D = (23, 23, 23, 23, 23, 23, 23, 23)$, the GA was adapted to identify the vector of times at which each dose should be administered $\hat{t} = (t_1, t_2, ..., t_8)$ to minimise (5.4). As the amount of antibiotic is fixed only a weight of $w = 0$ was used. The GA will identify the times at which the constant doses should be administered to maximise the success rate of eradicating the infection. The minimum time between doses was set to one hour and the time interval is measured in hourly increments.

The results from the GA (Table 5.4) show a similar pattern in all identified time vectors: the first two doses are given close together, the third is given about half a day later with the remainder of the doses being given in just over daily intervals. Examining the concentration profile of the antibiotic under these treatment regimens (Figure 5.1) it can be seen that the first three doses are given such that the concentration of antibiotic reaches the maximum as quickly as possible. The later doses increase the duration at which the bacteria are exposed to the antibiotic. By taking the constant doses but administering them at different times the success of eradicating the infection increases from 69% (95% CI: 67.7, 70.28) for the conventional treatment up to 97.78% (95% CI:

	Time Vector (hours)	Total Antibiotic	Success Rate (%) [95% CI, n = 5000]	Time to Eradication (days) [95% CI]
T1	(0, 1, 13, 38, 64, 87, 111, 142)	184	97.78 [97.33, 98.17]	4.67 [4.63, 4.71]
T2	(0, 1, 15, 39, 65, 90, 115, 138)	184	97.66 [97.20, 98.06]	4.79 [4.74, 4.83]
T3	(0, 1, 12, 38, 64, 90, 118, 140)	184	97.50 [97.03, 97.91]	4.63 [4.58, 4.67]
T4	(0, 1, 14, 43, 64, 89, 114, 139)	184	97.32 [96.83, 97.75]	4.82 [4.78, 4.86]
T5	(0, 7, 15, 40, 64, 90, 113, 138)	184	97.28 [96.81, 97.73]	4.87 [4.83, 4.91]
T6	(0, 6, 18, 41, 68, 94, 120, 142)	184	96.38 [95.82, 96.88]	5.16 [5.11, 5.20]
T7	(0, 3, 15, 38, 64, 91, 117, 146)	184	96.22 [95.65, 96.73]	4.74 [4.69, 4.79]
T8	(0, 3, 17, 42, 66, 90, 116, 145)	184	96.20 [95.63, 96.71]	4.99 [4.95, 5.02]
T9	(0, 5, 15, 43, 67, 93, 120, 146)	184	96.18 [95.61, 96.69]	5.09 [5.05, 5.13]
T10	(0, 5, 16, 41, 65, 90, 118, 145)	184	96.18 [95.61, 96.69]	4.91 [4.87, 4.95]

Table 5.4: Table comparing the success rates and time to eradication of time vectors produced by the GA which optimise the baseline conventional dosage vector when $w = 0$. The top 10 time vectors are shown. n = 4889, 4883, 4875, 4866, 4864, 4819, 4811, 4810, 4809, 4809 for time to eradication of T1 - T10 respectively.

97.33, 98.17).

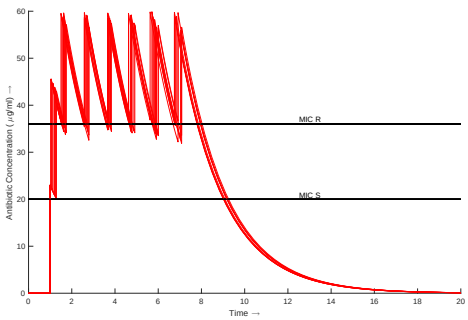

Figure 5.1: Concentration profiles of the top 10 time vectors identified by the GA which optimise the baseline conventional dosage vector with a weight, w=0.

By altering the intervals between the doses, the GA is able to take the constant dose regimen and amend it to create a concentration profile with a high initial dose. This allows the concentration of antibiotic to get above the MIC of the bacteria quickly. In addition to changes in success rates the median time to eradication of the infection reduces for all the regimens identified by the GA when compared to the conventional regimen. The median time to eradication for the base-line constant-dose treatment was 8.32 days (95% CI: 8.29, 8.35). This is reduced to 4.67 days (95% CI: 4.63, 4.71) when the interval between the doses is altered to produce a high initial dose of antibiotic.

5.4.2 *Optimising Antibiotic Doses when administered in Daily Time Intervals*

Returning to the assumption that antibiotics are administered every 24 hours, the GA was used to identify the dosage vector $D = (D_1, D_2, ..., D_d)$ which minimises (5.4) under the new parameter values. Due to the ability to alter the total amount of antibiotic used within these regimens, results were obtained for weights of $w = 0, 0.109$ and 0.269. These results are used as a comparison to identify if there is any benefit in changing the time intervals between constant doses over changing the doses administered in daily intervals.

The results from Table 5.5 where $w = 0$ and Table 5.6 where $w = 0.109$ are similar. Both use almost all the 184 $\mu g/ml$ of antibiotic and achieve a success rate up to 98%. By administering a high initial dose the success rate of eradicating the infection increases from 69% (95% CI: 67.7, 70.28) seen with the conventional constant dose regimen to 98% (95% CI: 97.57, 98.37). Due to the stochastic nature of the GA, noise within the results will always be expected. Dosage vector D10 Table 5.6 has a lower initial dose than all the other identified regimens and results in a lower success rate at 95.36% (95%

	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n = 5000]	Time to Eradication (days) [95% CI]
D1	(58, 23, 23, 23, 23, 20, 11, 3)	184	98.00 [97.57, 98.37]	4.35 [4.32, 4.39]
D2	(58, 24, 20, 22, 21, 19, 18, 2)	184	97.72 [97.27, 98.12]	4.40 [4.37, 4.42]
D3	(59, 21, 22, 23, 22, 22, 13, 2)	184	97.66 [97.20, 98.06]	4.46 [4.42, 4.49]
D4	(55, 22, 23, 23, 22, 21, 15, 2)	183	97.24 [96.75, 97.68]	4.64 [4.61, 4.69]
D5	(60, 20, 20, 25, 20, 21, 14, 4)	184	97.06 [96.55, 97.51]	4.48 [4.45, 4.51]
D6	(55, 22, 25, 20, 21, 23, 11, 0)	177	96.82 [96.30, 97.29]	4.56 [4.53, 4.60]
D7	(56, 24, 20, 18, 27, 23, 10, 4)	182	96.82 [96.30, 97.29]	4.61 [4.57, 4.66]
D8	(56, 25, 23, 19, 22, 11, 26, 1)	183	96.40 [95.85, 96.90]	4.43 [4.40, 4.46]
D9	(58, 16, 27, 21, 22, 23, 11, 4)	182	96.30 [95.74, 96.81]	4.65 [4.30, 5.25]
D10	(55, 24, 24, 22, 17, 25, 4, 8)	179	96.20 [95.63, 96.71]	4.46 [4.43, 4.49]

Table 5.5: Table comparing the success rates and time to eradication of dosage vectors produced by the GA with a weight $w = 0$. The top 10 dosage vectors are shown. n = 4900, 4886, 4883, 4862, 4853, 4841, 4841, 4820, 4815, 4810 for time to eradication of D1 - D10 respectively.

CI: 94.74, 95.93). If D10 was omitted then the remaining dosage vectors are all fairly similar. The median time to eradication of the infections reduces for all the regimens identified by the GA when compared to the conventional treatment regimen. The median time to eradication for the baseline conventional treatment was 8.32 days (95% CI: 8.29, 8.35). This is reduced by 4 days to 4.37 days (95% CI: 4.34, 4.41) when a high initial dose of antibiotic is given.

Increasing the value of w allowed the GA to identify regimens which minimise the total amount of antibiotic without lowering the success rate too far. When run using a value of 0.269 for w (Table 5.7) dosage vectors identified by the GA can obtain success rates above 95% despite using up to 11% less antibiotic. The success rates are slightly lower than those achieved with $w = 0$

	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n = 5000]	Time to Eradication (days) [95% CI]
D1	(58, 24, 22, 21, 24, 20, 13, 0)	182	98.00 [97.57, 98.37]	4.37 [4.34, 4.41]
D2	(58, 24, 20, 23, 23, 22, 9, 4)	183	97.70 [97.25, 98.10]	4.38 [4.35, 4.41]
D3	(57, 22, 22, 24, 22, 20, 10, 4)	181	97.54 [97.07, 97.95]	4.51 [4.48, 4.53]
D4	(57, 22, 24, 22, 19, 25, 11, 0)	180	97.52 [97.05, 97.93]	4.44 [4.42, 4.47]
D5	(56, 19, 26, 22, 23, 22, 13, 2)	183	97.44 [96.96, 97.86]	4.65 [4.62, 4.69]
D6	(59, 22, 23, 22, 23, 9, 23, 2)	183	97.24 [96.75, 97.68]	4.36 [4.33, 4.39]
D7	(57, 24, 21, 23, 21, 21, 4, 11)	182	97.04 [96.53, 97.49]	4.40 [4.37, 4.43]
D8	(57, 24, 21, 23, 22, 22, 0, 0)	169	96.54 [96.00, 97.03]	4.41 [4.38, 4.44]
D9	(60, 22, 22, 23, 15, 25, 7, 5)	179	96.30 [95.74, 96.81]	4.32 [4.29, 4.35]
D10	(48, 30, 18, 22, 23, 20, 15, 6)	182	95.36 [94.74, 95.93]	5.06 [5.01, 5.11]

Table 5.6: Table comparing the success rates and time to eradication of dosage vectors produced by the GA with a weight $w = 0.109$. The top 10 dosage vectors are shown. n = 4900, 4885, 4877, 4876, 4872, 4862, 4852, 4827, 4815, 4768 for time to eradication of D1 - D10 respectively.

and $w = 0.109$, but most are still above 95%.

Comparing the concentration profiles for $w = 0$ and $w = 0.269$ (Figure 5.2) it can be seen that the dosage vectors identified by the GA all have a similar pattern. A high initial dose followed by daily doses which maintain the concentration at its maximum. This creates a similar concentration profile to Figure 5.4. The high initial dose allows the concentration to increase above the MIC of both the susceptible and resistant bacteria immediately with the daily doses maintaining it there for as long as possible. The treatment regimens using less antibiotic maintain the same pattern but over a shorter duration. Due to the decrease in median time to eradication achieved by the pattern of a high initial dose it is possible to reduce the treatment duration by a day with

	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n = 5000]	Time to Eradication (days) [95% CI]
D1	(60, 22, 20, 22, 21, 23, 0, 0)	168	96.26 [95.70, 96.77]	4.37 [4.34, 4.40]
D2	(54, 26, 22, 21, 22, 21, 4, 1)	171	96.24 [95.68, 96.75]	4.49 [4.46, 4.53]
D3	(60, 21, 21, 23, 23, 14, 10, 0)	172	96.14 [95.57, 96.66]	4.37 [4.33, 4.40]
D4	(59, 23, 22, 21, 21, 18, 2, 2)	168	95.92 [95.33, 96.45]	4.31 [4.28, 4.34]
D5	(57, 20, 23, 24, 21, 16, 11, 2)	174	95.84 [95.25, 96.38]	4.57 [4.54, 4.60]
D6	(55, 25, 21, 22, 22, 20, 2, 2)	169	95.80 [95.21, 96.34]	4.54 [4.49, 4.57]
D7	(60, 22, 22, 21, 24, 12, 0, 2)	163	95.54 [94.93, 96.10]	4.29 [4.26, 4.32]
D8	(60, 21, 18, 26, 23, 10, 2, 0)	160	94.26 [93.58, 94.89]	4.46 [4.44, 4.50]
D9	(58, 23, 22, 21, 15, 23, 0, 0)	162	93.82 [93.12, 94.47]	4.33 [4.30, 4.36]
D10	(59, 22, 18, 26, 23, 2, 3, 4)	157	91.96 [91.17, 92.70]	4.47 [4.44, 4.50]

Table 5.7: Table comparing the success rate and time to eradication of dosage vectors produced by the GA with a weight $w = 0.269$. The top 10 dosage vectors are shown. n = 4813, 4812, 4807, 4796, 4792, 4790, 4777, 4713, 4691, 4598 for time to eradication of D1 - D10 respectively.

only a small decrease in success.

When using all 184$\mu g/ml$ of antibiotic there is no significant difference in success rate if you change the doses given daily (Table 5.5) versus changing the time interval between the constant doses (Table 5.4). However, when the time interval between the constant doses is altered the duration of treatment drops from 8 days to just over 6 days.

5.4.3 *Optimising Antibiotic Doses and Corresponding Time Intervals*

It can be seen that while changing the time interval resulted in shorter treatment duration, changing the dosage vector allowed for high success rates

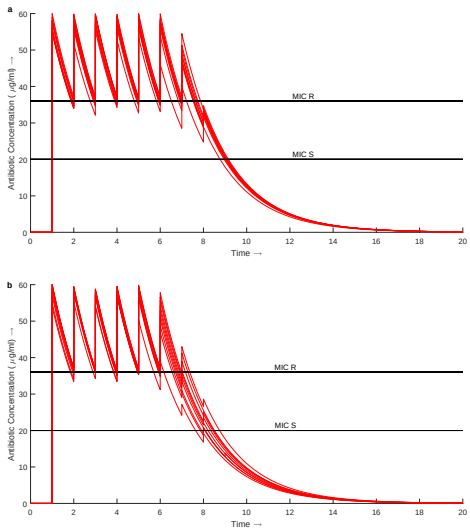

Figure 5.2: Concentration profiles of the top 10 dosage vectors identified by the GA with a weight of w=0 (a) and w=0.269 (b)

while using less antibiotic. Previously the GA has only been programmed to identify either the dosage or time vector and given the other vector. Here the GA is amended to allow it to identify both the doses and the corresponding time at which those doses should be administered.

The aim of the GA is now to identify both the dosage vector and corresponding time vector which minimises (5.4). As both vectors are being searched for the constraints from the previous sections must all be included: the maximum amount of antibiotic used must not exceed 184 $\mu g/ml$, the maximum concentration of antibiotic within the system must not exceed 60 $\mu g/ml$, the minimum time between doses is one hour and the time interval is measured in hourly increments.

	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n = 5000]	Time to Eradication (days) [95% CI]
D1	(60, 14, 22, 20, 19, 15, 15, 18)	183	99.26 [98.98, 99.48]	4.15 [4.11, 4.17]
T1	(0, 16, 38, 58, 78, 94, 108, 126)			
D2	(58, 16, 10, 17, 20, 21, 22, 18)	182	98.98 [98.66, 99.24]	4.04 [4.01, 4.07]
T2	(0, 15, 30, 42, 62, 87, 109, 131)			
D3	(58, 17, 19, 15, 19, 21, 15, 10)	184	98.78 [98.44, 99.07]	4.11 [4.08, 4.14]
T3	(0, 18, 37, 51, 73, 93, 123, 141)			
D4	(58, 12, 12, 22, 18, 16, 22, 19)	179	98.76 [98.41, 99.05]	4.07 [4.05, 4.09]
T4	(0, 10, 26, 45, 64, 84, 104, 127)			
D5	(41, 27, 23, 17, 19, 15, 19, 21)	182	98.68 [98.32, 98.98]	4.42 [4.39, 4.44]
T5	(0, 11, 36, 53, 73, 90, 111, 130)			
D6	(31, 30, 21, 18, 25, 19, 19, 19)	182	98.66 [98.30, 98.96]	4.34 [4.31, 4.37]
T6	(0, 3, 24, 42, 70, 89, 108, 133)			
D7	(58, 15, 23, 20, 22, 16, 10, 10)	174	98.60 [98.23, 98.91]	4.18 [4.15, 4.21]
T7	(0, 13, 38, 60, 84, 100, 112, 122)			
D8	(40, 24, 21, 20, 19, 22, 17, 17)	180	98.56 [98.19, 98.87]	4.40 [4.37, 4.43]
T8	(0, 6, 30, 51, 71, 93, 112, 129)			
D9	(59, 25, 22, 13, 14, 17, 16, 12)	178	98.50 [98.12, 98.82]	4.30 [4.27, 4.33]
T9	(0, 28, 50, 64, 83, 95, 110, 137)			
D10	(29, 32, 17, 24, 20, 21, 21, 20)	184	98.44 [98.06, 98.76]	5.54 [4.50, 4.57]
T10	(0, 5, 23, 47, 71, 90, 114, 137)			

Table 5.8: Table comparing the success rate and time to eradication of dosage vectors and corresponding time vectors produced by the GA with a weight $w = 0$. The top 10 dosage vectors and corresponding time vector are shown. n = 4963, 4949, 4939, 4938, 4934, 4933, 4930, 4928, 4925, 4922 for time to eradication of D1 - D10 respectively.

	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n = 5000]	Time to Eradication (days) [95% CI]
D1	(58, 24, 21, 16, 21, 24, 17)	181	98.54 [98.17, 98.85]	4.43 [4.39, 4.46]
T1	(0, 27, 50, 67, 89, 113, 131, 144)			
D2	(30, 27, 19, 19, 24, 20, 19, 20)	178	98.24 [97.84, 98.59]	4.47 [4.43, 4.51]
T2	(0, 1, 20, 44, 65, 90, 107, 127)			
D3	(48, 19, 16, 20, 16, 16, 24, 13)	172	98.24 [97.84, 98.59]	4.13 [4.11, 4.16]
T3	(0, 8, 25, 47, 63, 81, 106, 123)			
D4	(59, 18, 16, 16, 19, 12, 20, 14)	174	98.02 [97.59, 98.39]	4.17 [4.13, 4.22]
T4	(0, 19, 36, 55, 79, 91, 108, 121)			
D5	(60, 14, 15, 15, 20, 17, 13, 17)	171	97.96 [97.53, 98.33]	4.21 [4.18, 4.25]
T5	(0, 15, 35, 48, 69, 84, 105, 119)			
D6	(54, 18, 14, 22, 23, 16, 14, 17)	178	97.96 [97.53, 98.33]	4.38 [4.32, 4.42]
T6	(0, 17, 29, 53, 81, 96, 112, 130)			
D7	(29, 19, 16, 21, 24, 25, 24, 22)	180	97.28 [96.79, 97.71]	4.71 [4.67, 4.74]
T7	(0, 2, 6, 30, 58, 84, 109, 132)			
D8	(51, 17, 21, 20, 20, 21, 15, 9)	174	97.28 [96.79, 97.71]	4.43 [4.39, 4.46]
T8	(0, 12, 34, 57, 78, 99, 121, 130)			
D9	(31, 19, 23, 22, 17, 21, 24, 20)	177	97.24 [96.75, 97.68]	4.65 [4.62, 4.68]
T9	(0, 7, 20, 43, 59, 81, 108, 129)			
D10	(53, 24, 18, 19, 19, 12, 14, 5)	164	96.38 [95.82, 96.88]	4.48 [4.45, 4.52]
T10	(0, 21, 43, 64, 82, 92, 113, 125)			

Table 5.9: Table comparing the success rate and time to eradication of dosage vectors and corresponding time vectors produced by the GA with a weight $w = 0.109$. The top 10 dosage vectors and corresponding time vector are shown. n = 4927, 4912, 4912, 4901, 4898, 4898, 4864, 4864, 4862, 4819 for time to eradication of D1 - D10 respectively.

	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n = 5000]	Time to Eradication (days) [95% CI]
D1	(58, 13, 17, 21, 15, 13, 15, 9)	161	97.16 [96.66, 97.60]	4.14 [4.11, 4.17]
T1	(0, 11, 31, 51, 71, 85, 99, 105)			
D2	(60, 12, 11, 14, 9, 24, 11, 13)	154	96.96 [96.45, 97.42]	4.02 [3.98, 4.05]
T2	(0, 12, 30, 37, 48, 72, 84, 97)			
D3	(54, 21, 17, 15, 18, 13, 12, 7)	157	96.94 [97.01, 97.90]	4.13 [4.11, 4.16]
T3	(0, 17, 37, 51, 68, 82, 93, 107)			
D4	(58, 21, 17, 17, 8, 9, 19, 9)	158	96.58 [96.04, 97.07]	4.06 [4.03, 4.09]
T4	(0, 21, 38, 55, 69, 73, 91, 124)			
D5	(60, 16, 11, 13, 16, 21, 1, 12)	150	96.28 [95.72, 96.79]	3.98 [3.96, 4.01]
T5	(0, 21, 29, 42, 58, 78, 82, 95)			
D6	(54, 12, 15, 13, 9, 6, 20, 15)	144	95.52 [94.91, 96.08]	3.96 [3.92, 3.98]
T6	(0, 9, 22, 36, 43, 53, 71, 86)			
D7	(56, 19, 1, 20, 17, 12, 13, 13)	151	95.26 [94.63, 95.83]	4.21 [4.18, 4.23]
T7	(0, 18, 22, 41, 60, 73, 86, 103)			
D8	(45, 15, 16, 19, 16, 19, 19, 3)	152	95.12 [94.49, 95.70]	4.07 [4.04, 4.10]
T8	(0, 1, 17, 37, 53, 75, 95, 120)			
D9	(44, 17, 17, 18, 17, 9, 14, 7)	143	94.60 [93.94, 95.21]	4.11 [4.08, 4.13]
T9	(0, 3, 22, 39, 57, 68, 78, 89)			
D10	(58, 14, 12, 12, 16, 15, 9, 3)	139	93.74 [93.03, 94.40]	4.00 [3.89, 4.03]
T10	(0, 19, 25, 38, 56, 71, 82, 91)			

Table 5.10: Table comparing the success rate and time to eradication of dosage vectors and corresponding time vectors produced by the GA with a weight $w = 0.269$. The top 10 dosage vectors and corresponding time vector are shown. n = 4858, 4848, 4847, 4829, 4814, 4776, 4763, 4756, 4730, 4687 for time to eradication of D1 - D10 respectively.

With a weight of $w = 0$ the GA redistributes the 184 $\mu g/ml$ of antibiotic into treatment regimens (Table 5.8) which increase the success rate of eradicating

an infection up to 99.26% (95% CI: 98.98, 99.48). The duration of treatment is reduced to 5.25 days with a median time to eradication of 4.15 days (95% CI: 4.11, 4.17). Allowing the GA to optimise both the doses and the time interval between the doses identifies treatment regimens which are significantly better at eradicating the infection than either altering the dose (Table 5.5) or time interval (Table 5.4) separately.

As the value of w is increased more emphasis is placed on minimising the amount of antibiotic required to eradicate the infection. The confidence intervals for the majority of treatments in Table 5.8 overlap with treatment regimen D1 in Table 5.9. Meaning the results from $w = 0.109$ (Table 5.9) identify treatment regimens which use up to 6% less antibiotic while achieving success rates similar to the majority of treatments where $w = 0$ (Table 5.8). Further increasing the weight on minimising the amount of antibiotic used, $w = 0.269$ (Table 5.10), the GA was able to identify treatment regimens which used almost 22% less antibiotic than the conventional regimen while achieving a success rate of over 95% at 95.52% (95% CI: 94.91, 96.08).

Due to the high variability in doses and time intervals the concentration profiles (Figure 5.3) of the treatment regimens do not appear to converge to a single pattern. However, when the concentration profiles for $w = 0$ and $w = 0.269$ are compared it is possible to see some form of pattern. The high initial dose that was observed in the previous treatment regimens is once again seen in both of these profiles. Later doses are then administered to maintain the concentration at the maximum allowed. When $w = 0$ the GA is not concerned with minimising the antibiotic and so there are many different combinations of times and doses that achieve a high success rate. With $w = 0.269$ the GA tries to minimise the amount of antibiotic being required. Once again there

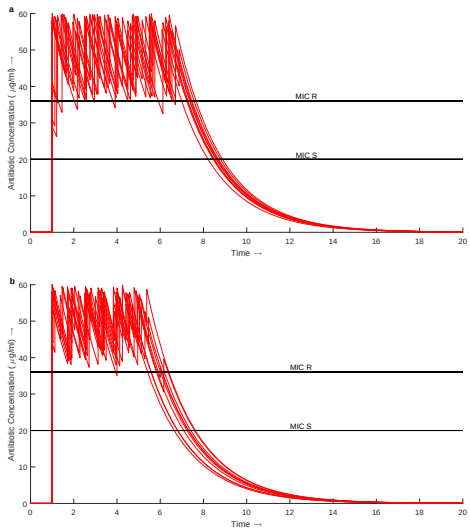

Figure 5.3: Concentration profiles of the top 10 dosage vectors and corresponding time vectors identified by the GA with a weight of w=0 (a) and w=0.269 (b)

is a high initial dose but the interval between the following doses is shorter. This can be seen by the reduction in duration of treatment between the two profiles. By minimising the time between the doses the GA maintains a higher concentration of antibiotic within the system for longer.

5.5 MINI-SUMMARY AND NEXT STEPS

Results obtained from optimising the time interval between constant doses produced similar results to optimising the doses with constant time intervals. By taking current conventional treatment regimens and altering the times at which doses are administered increases the success rate of the conventional treatment regimen from 69% (95% CI: 67.7, 70.28) to 97.78% (95% CI: 97.33, 98.17). The implications for manufacturers are removed if the time between constant doses is altered compared to changing the doses given daily. However, either scenario produces more chance of human error from the patient. Patients either have to remember to take the antibiotic at varying time intervals

or remember to take the correct dose of antibiotic on the correct day.

Despite producing far more complex dosing regimens, the impact altering both the dose and time interval had on the success of eradicating an infection was examined. Significantly better results were obtained when both vectors were optimised. When using all 184 $\mu g/ml$ the success rate increased from 98% (95% CI: 97.57, 98.37) to 99.26% (95% CI: 98.98, 99.48). In addition, incorporating both the time and dose vector into the GA identifies more effective treatment regimens when using less antibiotic. When optimising the doses only, a reduction of 13% of the total antibiotic resulted in a success rate of 94.26% (95% CI: 93.58, 94.89). Optimising the dose and time interval produced similar results of 93.74% (95% CI: 93.03, 94.40) but using 24.5% less of the total antibiotic.

Interestingly, the tapered pattern of treatment no longer holds as the optimal way to administer the antibiotics under the new parameter set. In all results the regimens were converging to maintaining the concentration of antibiotic at the maximum allowed for as long as possible. By increasing the MIC points from those used in Chapter 3 the area between the MIC of the resistant bacteria and the maximum concentration is decreased. In order to be successful a treatment regimen needs to be able to maintain the concentration of antibiotic above the MIC of the bacteria for a suitable duration. If the area between the MIC of the resistant bacteria and the maximum concentration of antibiotic is small then the GA will maximise the antibiotic induced death rate by maintaining the concentration at the maximum allowed for as long as possible. To investigate whether the maximum concentration cap of 60 $\mu g/ml$ of antibiotic was causing the results the cap was increased to 100 $\mu g/ml$ and the results repeated.

With the maximum concentration of antibiotic being increased to 100 $\mu g/ml$ the baseline conventional treatment regimen was amended. The 184 $\mu g/ml$ of antibiotic was split into 4, 5 and 6 equal doses and a success rate calculated for each. The regimen with the maximum success rate was taken as the new baseline conventional treatment. The four day treatment regimen was therefore chosen as the new baseline with a success rate of 98.9% (95% CI: 98.57, 99.17) and a median time to eradication of 4.11 days (95% CI: 4.09, 4.14). The maximum concentration of antibiotic within the four day treatment is slightly higher than the 100 $\mu g/ml$ cap at ~102 $\mu g/ml$ but this was considered to be admissible.

5.6.1 *Optimising Time Intervals between Constant Antibiotic Doses*

The GA is implemented to identify the time vector, $\hat{t} = (t_1, t_2, ..., t_4)$, such that the conventional treatment regimen $D = (46, 46, 46, 46)$ minimises (5.4). Due to the fixed amount of antibiotic, the GA will minimise the number of unsuccessful runs only, $w = 0$.

The treatment regimens identified by the GA in Table 5.11 give the first two doses of antibiotic close together. This creates a high initial dose with the last two doses being given in roughly 36 hour intervals. Extending the interval for the last two doses increases the duration that the antibiotic is maintained within the system. Varying the time interval between doses makes it possible to increase the success rate of the constant dose regimen from 98.9% (95% CI: 98.57, 99.17) up to 99.54% (95% CI: 99.31, 99.71). The treatment duration is reduced from 4 days to 3.21 days and the median time to eradication of the

	Time Vector	Total Antibiotic	Success Rate (%) [95% CI, n = 5000]	Time to Eradication (days) [95% CI]
T1	(0, 6, 39, 77)	184	99.54 [99.31, 99.71]	3.69 [3.67, 3.72]
T2	(0, 8, 42, 75)	184	99.52 [99.29, 99.69]	3.73 [3.72, 3.76]
T3	(0, 6, 46, 75)	184	99.48 [99.24, 99.66]	3.77 [3.75, 3.79]
T4	(0, 4, 46, 73)	184	99.44 [99.19, 99.63]	3.77 [3.75, 3.80]
T5	(0, 13, 50, 83)	184	99.42 [99.17, 99.61]	3.91 [3.89, 3.93]
T6	(0, 8, 45, 87)	184	99.42 [99.17, 99.61]	3.76 [3.74, 3.78]
T7	(0, 8, 37, 72)	184	99.40 [99.14, 99.59]	3.70 [3.68, 3.72]
T8	(0, 17, 50, 84)	184	99.28 [99.00, 99.50]	3.97 [3.95, 3.99]
T9	(0, 3, 39, 86)	184	99.28 [99.00, 99.50]	3.66 [3.64, 3.98]
T10	(0, 7, 51, 92)	184	99.18 [98.89, 99.41]	3.87 [3.85, 3.89]

Table 5.11: Table comparing the success rate and time to eradication of time vectors which optimise the baseline conventional dosage vector with a weight $w = 0$. The top 10 time vectors are shown. n = 4977, 4976, 4974, 4972, 4971, 4971, 4970, 4964, 4964, 4959 for time to eradication of T1 - T10 respectively.

infection is significantly shorter at 3.69 days (95% CI: 3.67, 3.72).

The concentration profile of the treatment regimens, Figure 5.4, show the high initial dose created by administering the first two doses close together. Extending the interval for the last two doses means that the concentration of antibiotic within the system at any given time is also lower than in the conventional treatment. Increasing the concentration cap has allowed the GA to identify alternative treatment regimens which are not trying to maintain the concentration at the maximum allowed for as long as possible as seen in Section 5.4.1.

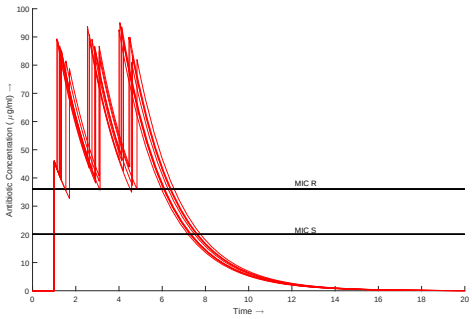

Figure 5.4: Concentration profiles of the top 10 time vectors identified by the GA which optimise the baseline conventional dosage vector with a weight, w=0.

5.6.2 *Optimising Antibiotic Doses when Administered in Daily Time Intervals*

Although the new baseline conventional treatment regimen consists of four doses, the GA was permitted to distribute the antibiotic over a maximum of 8 doses. In a conventional regimen increasing the number of doses beyond four resulted in lower success rates. Various weights were examined with Tables 5.12, 5.13 and 5.14 displaying the results from weights $w = 0, 0.109$ and 0.269 respectively.

Despite some noise within the results, the dosage vectors from Table 5.12 show treatment regimens which consist of four doses with a high initial dose. Increasing the initial dose increases the success of eradicating an infection up to 99.44% (95% CI: 99.31, 99.71), significantly better than the conventional treatment at 98.9% (95% CI: 98.57, 99.17). However, once again there is no significant difference in success rate or median time to eradication when optimising the dosage vector versus optimising the time vector.

	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n = 5000]	Time to Eradication (days) [95% CI]
D1	(78, 31, 47, 26, 0, 0, 0, 0)	182	99.44 [99.19, 99.63]	3.65 [3.63, 3.67]
D2	(76, 50, 27, 17, 7, 0, 0, 0)	177	99.40 [99.14, 99.59]	3.61 [3.59, 3.63]
D3	(70, 41, 29, 32, 8, 0, 0, 2)	182	99.40 [99.14, 99.59]	3.70 [3.68, 3.72]
D4	(69, 41, 38, 35, 0, 0, 0, 0)	183	99.38 [99.12, 99.58]	3.67 [3.65, 3.69]
D5	(79, 22, 25, 56, 1, 0, 0, 0)	183	99.38 [99.12, 99.58]	3.75 [3.72, 3.77]
D6	(72, 41, 36, 30, 0, 0, 0, 4)	183	99.36 [99.10, 99.56]	3.65 [3.63, 3.67]
D7	(60, 48, 32, 33, 1, 0, 0, 0)	174	99.26 [98.98, 99.48]	3.79 [3.76, 3.80]
D8	(76, 32, 15, 51, 0, 0, 0, 0)	174	99.10 [98.80, 99.34]	3.71 [3.69, 3.73]
D9	(56, 47, 41, 31, 0, 6, 0, 0)	181	98.92 [98.59, 99.19]	3.84 [3.82, 3.85]
D10	(61, 44, 24, 34, 0, 5, 0, 0)	168	98.56 [98.19, 98.87]	3.81 [3.79, 3.83]

Table 5.12: Table comparing success rates and time to eradication of dosage vectors produced by the GA with a weight $w = 0$. The top 10 dosage vectors are shown. n = 4972, 4970, 4970, 4969, 4969, 4968, 4963, 4955, 4946, 4928 for time to eradication of D1 - D10 respectively.

Increasing the value of w, Table 5.13, the GA is able to refine the treatment regimens to use less antibiotic while maintaining high success rates. By using a high initial dose followed by a tapering of later doses the GA identifies treatments using 15% less antibiotic while maintaining similar success rates as the baseline.

	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n = 5000]	Time to Eradication (days) [95% CI]
D1	(76, 27, 34, 21, 0, 0, 0, 0)	158	98.62 [98.26, 98.92]	3.67 [3.65, 3.68]
D2	(77, 43, 16, 23, 0, 0, 0, 0)	159	98.54 [98.17, 98.85]	3.63 [3.61, 3.65]
D3	(72, 37, 28, 19, 0, 0, 0, 0)	156	98.52 [98.15, 98.84]	3.69 [3.67, 3.71]
D4	(89, 33, 36, 3, 0, 3, 1, 1)	166	98.02 [97.59, 98.39]	3.60 [3.58, 3.62]
D5	(59, 40, 32, 11, 18, 0, 0, 0)	160	97.82 [97.38, 98.21]	3.85 [3.83, 3.87]
D6	(87, 28, 23, 15, 0, 0, 0, 0)	153	97.72 [97.27, 98.12]	3.62 [3.60, 3.64]
D7	(69, 38, 26, 12, 6, 0, 0, 0)	151	97.64 [97.18, 98.04]	3.71 [3.69, 3.73]
D8	(89, 16, 30, 17, 0, 0, 0, 0)	152	97.58 [97.12, 97.99]	3.66 [3.64, 3.68]
D9	(76, 28, 27, 19, 0, 3, 0, 0)	153	97.58 [97.12, 97.99]	3.68 [3.66, 3.69]
D10	(89, 23, 27, 0, 0, 0, 0, 0)	139	95.24 [94.61, 95.81]	3.60 [3.59, 3.62]

Table 5.13: Table comparing the success rate and time to eradication of dosage vectors produced by the GA with a weight $w = 0.109$. The top 10 dosage vectors are shown. n = 4931, 4927, 4926, 4901, 4891, 4886, 4882, 4879, 4879, 4762 for time to eradication of D1 - D10 respectively.

Comparison of the concentration profiles for all three values of w (Figure 5.5) show that as w increases the GA identifies dosage vectors which have a high initial dose followed by tapering lower doses. This is the same pattern that was observed in previous chapters. By using a tapering pattern an infection can be successfully treated using less antibiotic while exposing the environment to a lower concentration of antibiotic when compared with the conventional treatment.

	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n = 5000]	Time to Eradication (days) [95% CI]
D1	(79, 19, 33, 19, 0, 0, 0, 0)	150	97.62 [97.16, 98.02]	3.73 [3.72, 3.75]
D2	(87, 22, 32, 9, 0, 0, 0, 0)	150	97.22 [96.73, 97.66]	3.64 [3.62, 3.65]
D3	(85, 27, 31, 4, 0, 0, 0, 0)	147	96.58 [96.04, 97.07]	3.62 [3.60, 3.64]
D4	(80, 23, 29, 13, 0, 0, 0, 0)	145	96.26 [95.70, 96.77]	3.67 [3.65, 3.68]
D5	(77, 20, 30, 16, 0, 0, 0, 0)	143	95.96 [95.38, 96.49]	3.74 [3.72, 3.76]
D6	(79, 32, 22, 7, 0, 0, 1, 0)	141	95.68 [95.08, 96.23]	3.64 [3.62, 3.65]
D7	(77, 23, 30, 10, 0, 0, 0, 0)	140	95.50 [94.89, 96.06]	3.70 [3.69, 3.72]
D8	(66, 35, 30, 10, 0, 0, 2, 1)	144	95.46 [94.85, 96.02]	3.73 [3.70, 3.75]
D9	(80, 19, 35, 6, 0, 0, 0, 0)	140	95.20 [94.57, 95.78]	3.70 [3.68, 3.72]
D10	(68, 38, 24, 2, 0, 0, 0, 0)	132	92.54 [91.78, 93.25]	3.67 [3.65, 3.69]

Table 5.14: Table comparing the success rate and time to eradication of dosage vectors produced by the GA with a weight $w = 0.269$. The top 10 dosage vectors are shown. n = 4881, 4861, 4829, 4813, 4798, 4784, 4775, 4773, 4760, 4627 for time to eradication of D1 - D10 respectively.

With $w = 0.269$ the results from the GA reduce the total amount of antibiotic by 24% while still maintaining success rates above 95%. Despite these high success rates the conventional treatment regimen performs better at eradicating the infection for all regimens identified in Table 5.14. Tapering the doses of antibiotic does have its benefits with treatment regimens identified that use 18.5% less antibiotic than the conventional regimen with only a 1.28% reduction in success rate.

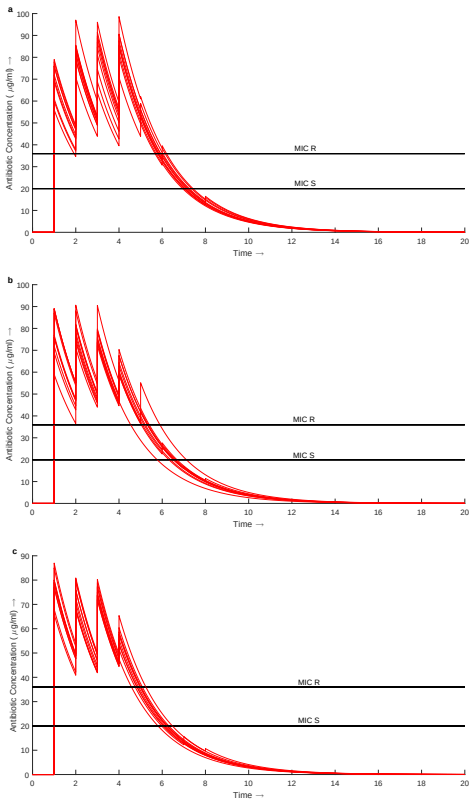

Figure 5.5: Concentration profiles of the top 10 dosage vectors identified by the GA with a weight of w=0 (a), w=0.109 (b) and w=0.269 (c)

5.6.3 *Optimising Antibiotic Doses and Corresponding Time Intervals*

With the promising results seen in Section 5.4.3, the GA was used to identify both the dosage vector and corresponding time vector which minimises (5.4) with the maximum concentration set at 100 $\mu g/ml$.

With the maximum concentration cap increased to 100 $\mu g/ml$ and all emphasis on maximising the success rate (Table 5.15), the GA identified various treatment patterns which have a success of eradicating the infection of up to 99.58% (95% CI: 99.36, 99.74). Incorporating the varied time interval does not improve the success of the treatment regimens when compared to optimising doses only at the higher concentration cap.

	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n = 5000]	Time to Eradication (days) [95% CI]
D1	(65, 24, 28, 12, 25, 3, 24)	181	99.58 [99.36, 99.74]	3.75 [3.73, 3.77]
T1	(0, 23, 27, 54, 77, 80, 88)			
D2	(77, 27, 21, 38, 13, 1)	177	99.54 [99.31, 99.71]	3.65 [3.63, 3.67]
T2	(0, 20, 25, 60, 68, 126)			
D3	(60, 46, 28, 48)	182	99.52 [99.29, 99.69]	3.65 [3.63, 3.67]
T3	(0, 14, 39, 71)			
D4	(65, 34, 54, 25, 3, 3)	184	99.50 [99.26, 99.68]	3.72 [3.71, 3.74]
T4	(0, 19, 48, 79, 100, 123)			
D5	(39, 37, 23, 25, 19, 14, 13, 14)	184	99.44 [99.19, 99.63]	3.75 [3.73, 3.77]
T5	(0, 7, 25, 38, 54, 67, 74, 86)			
D6	(76, 31, 40, 32)	179	99.42 [99.17, 99.61]	3.80 [3.78, 3.82]
T6	(0, 26, 60, 91)			
D7	(69, 40, 53, 22)	184	99.42 [99.17, 99.61]	3.66 [3.65, 3.68]
T7	(0, 14, 51, 65)			
D8	(58, 44, 38, 37)	177	99.30 [99.03, 99.51]	3.69 [3.67, 3.71]
T8	(0, 15, 47, 74)			
D9	(75, 35, 38, 30)	178	99.24 [98.96, 99.46]	3.82 [3.79, 3.84]
T9	(0, 31, 61, 90)			
D10	(60, 53, 25, 31)	169	98.48 [98.10, 98.80]	3.70 [3.68, 3.71]
T10	(0, 22, 41, 58)			

Table 5.15: Table comparing the success rate and time to eradication of dosage vectors and corresponding time vectors produced by the GA with a weight $w = 0$. The top 10 dosage vectors and corresponding time vector are shown. n = 4979, 4977, 4976, 4975, 4972, 4971, 4971, 4965, 4962, 4924 for time to eradication of D1 - D10 respectively.

Increasing the value of w, Tables 5.16 and 5.17, decreases the amount of antibiotic used within the treatment regimens. The success rate of all these

	Dosage Vector		Success Rate (%)	Time to Eradication
	Time Vector (hours)	Total Antibiotic	[95% CI, n = 5000]	(days) [95% CI]
D1	(74, 18, 37, 35)	164	98.74 [98.39, 99.03]	3.61 [3.58, 3.62]
T1	(0, 7, 27, 61)			
D2	(54, 34, 43, 35)	166	98.38 [97.99, 98.71]	3.67 [3.65, 3.68]
T2	(0, 10, 36, 53)			
D3	(91, 23, 21, 23)	158	98.28 [97.88, 98.62]	3.61 [3.59, 3.63]
T3	(0, 18, 43, 71)			
D4	(90, 18, 39, 12)	159	98.24 [97.84, 98.59]	3.64 [3.62, 3.65]
T4	(0, 20, 50, 61)			
D5	(83, 28, 21, 30)	162	97.98 [97.55, 98.35]	3.60 [3.59, 3.63]
T5	(0, 10, 41, 78)			
D6	(92, 25, 23, 11, 5)	156	97.84 [97.40, 98.22]	3.65 [3.62, 3.66]
T6	(0, 31, 52, 76, 100)			
D7	(65, 32, 27, 30)	154	97.80 [97.35, 98.19]	3.70 [3.68, 3.72]
T7	(0, 14, 41, 71)			
D8	(71, 18, 26, 36)	151	97.74 [97.29, 98.13]	3.70 [3.68, 3.73]
T8	(0, 12, 33, 60)			
D9	(88, 21, 12, 29)	150	97.06 [96.55, 97.51]	3.69 [3.67, 3.72]
T9	(0, 27, 49, 63)			
D10	(97, 7, 40, 7)	151	96.60 [96.06, 97.08]	3.62 [3.60, 3.64]
T10	(0, 8, 44, 64)			

Table 5.16: Table comparing the success rate and time to eradication of dosage vectors and corresponding time vectors produced by the GA with a weight $w = 0.109$. The top 10 dosage vectors and corresponding time vector are shown. n = 4937, 4919, 4914, 4912, 4899, 4892, 4890, 4887, 4853, 4830 for time to eradication of D1 - D10 respectively.

	Dosage Vector	Total Antibiotic	Success Rate (%) [95% CI, n = 5000]	Time to Eradication (days) [95% CI]
D1	(60 27 32 25)	144	96.92 [96.40, 97.38]	3.64 [3.62, 3.66]
T1	(0 9 32 53)			
D2	(54 38 21 22 6 4)	145	96.38 [95.82, 96.88]	3.75 [3.73, 3.76]
T2	(0 9 41 57 66 68)			
D3	(81 19 17 25)	142	96.36 [95.80, 96.86]	3.64 [3.62, 3.66]
T3	(0 22 37 53)			
D4	(72 28 30 13)	143	96.36 [95.80, 96.86]	3.67 [3.65, 3.69]
T4	(0 20 44 73)			
D5	(65 25 26 25)	141	96.08 [95.50, 96.60]	3.70 [3.67, 3.72]
T5	(0 12 35 61)			
D6	(75 17 26 24)	142	95.92 [95.33, 96.45]	3.62 [3.60, 3.63]
T6	(0 15 30 55)			
D7	(77 24 12 26 1)	140	95.82 [95.23, 96.36]	3.65 [3.63, 3.66]
T7	(0 21 41 54 65)			
D8	(64 17 26 36)	143	95.64 [95.04, 96.19]	3.64 [3.62, 3.66]
T8	(0 1 22 48)			
D9	(78 10 34 19)	141	95.56 [94.95, 96.11]	3.73 [3.71, 3.75]
T9	(0 9 42 60)			
D10	(59 22 18 21 1 17)	138	95.38 [94.76, 95.95]	3.74 [3.72, 3.76]
T10	(0 13 30 42 47 65)			

Table 5.17: Table comparing the success rate and time to eradication of dosage vectors and corresponding time vectors produced by the GA with a weight $w = 0.269$. The top 10 dosage vectors and corresponding time vector are shown. n = 4846, 4819, 4818, 4804, 4796, 4791, 4782, 4778, 4769 for time to eradication of D1 - D10 respectively.

treatment regimens was maintained above 95% while using up to 25% less antibiotic than the maximum 184 $\mu g/ml$.

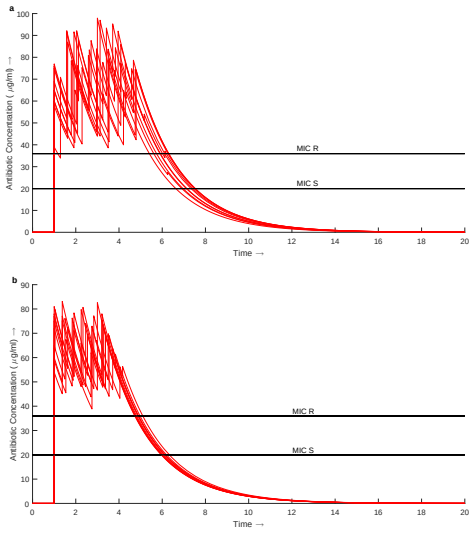

Figure 5.6: Concentration profiles of the top 10 dosage vectors and corresponding time vectors identified by the GA with a weight of w=0 (a) and w=0.269 (b)

Plotting the concentration profiles for the treatment regimens for $w = 0$ and $w = 0.269$ (Figure 5.6) shows the large variability in patterns identified by the GA. However, when comparing the two sets of results it can be seen that the treatments which result in less antibiotic being used lower the maximum antibiotic concentration within the system to ~80 $\mu g/ml$. While there is a large amount of variability, these results have moved away from the pattern of increasing the concentration to the maximum permitted and maintaining it there.

5.7 DISCUSSION

Optimising the use of antibiotics remains at the forefront in the targeted approach to minimising the spread of antibiotic resistance. Ensuring antibi-

otics are only given when necessary and that the appropriate antibiotics are used for the bacterial strain present are important steps. However, if optimal treatment regimens are not identified then the use of antibiotics will continue to propagate resistant bacteria. Bacterial infections which are not completely eradicated by antibiotic therapy increase the likelihood of resistant infections re-establishing [124]. Increasing the success rate of antibiotic treatments helps to minimise the further emergence of resistant bacteria.

Increasing the MIC of the bacterial strains but maintaining the same maximum concentration cap as Chapter 3, 60 $\mu g/ml$, the GA did not identify the tapered pattern to be the optimal way to administer the antibiotics. The GA identified a pattern of increasing the concentration to the maximum allowed for as long as possible. To successfully eradicate an infection the concentration of antibiotic needs to be maintained above the MIC of the resistant strain for a suitable duration. When the area between the MIC of the resistant strain and the maximum concentration is reduced, there are fewer ways in which the duration above the MIC can be obtained. The concentration of antibiotic being administered to a host cannot be infinitely increased. A threshold exists above which the antibiotic becomes toxic to the host. This example highlights the importance of identifying and implementing optimal treatment regimens as early as possible. If antibiotics continue to be used in a way which promotes the increase in resistance, eventually a situation will arise where optimising their use will no longer be possible. The only way to maximise the success of eradicating the infection becomes using as much antibiotic as possible, at the highest concentration possible, for as long as possible.

The maximum concentration cap was increased to 100 $\mu g/ml$, this ensured the concentration cap was not forcing the GA to only maximise the antibiotic-

induced death function. By taking the conventional treatment regimen and relaxing the assumption that doses are delivered in daily time intervals, it was possible to increase the success of eradicating the infection. The GA identified treatment regimens which all followed a similar pattern. Administering the first two doses close together and then extending the interval to 36 hours for the last two doses increased the success rate from 98.9% to 99.54%. However, there was no difference in success when compared to treatment regimens where the daily dose had been optimised, 99.54% versus 99.44%. The duration under which an environment is exposed to antibiotics increases the likelihood of resistance developing [173]. Current treatment durations are arbitrarily chosen with studies indicating that shorter treatment regimens can be just as effective [117, 118, 121]. By altering the interval between the constant doses the GA produced treatment regimens which were shorter.

Optimising the time intervals provides no opportunity to minimise the amount of antibiotic being used. Exposing the environment to larger quantities of antibiotic has also been shown to attribute to the increase in resistant bacteria [98, 42]. When using the GA to identify dosage vectors it is possible to increase the weight on the amount of antibiotic being used. This allows for regimens to be identified which minimise the use of antibiotic while maintaining high success rates. By administering the antibiotic in a tapered pattern, as seen in previous chapters, the GA reduced the total amount of antibiotic by 24% while maintaining success rates above 95%.

The conventional treatment regimen of a constant dose taken at a set time interval is appealing to both manufacturers and patients. However, to increase the effectiveness of antibiotics a move away from this conventional regimen is required. Changing the interval between doses of antibiotics would be

preferable for manufacturers as the doses of antibiotic remain constant. An added advantage for patients is that the treatment duration would be shorter. Although a study by Kardas [174] highlighted that patient compliance with short-term antibiotic therapy for respiratory tract infections is generally quite poor. Altering the doses has the potential to reduce the amount of antibiotic a patient requires. The constant time interval also makes it simpler for the patient to administer. In the case of once daily dosing, compliance rates have been shown to be almost 100% [175, 176]. The downside to this approach is the practicality of manufacturing varying doses would be complex when not in a liquid form.

With both the change in doses and the change in time intervals providing improvements separately, the GA was used to investigate if combining the two would result in even better success rates. Allowing the GA to optimise the dosage vector and corresponding time intervals did not result in significantly better regimens when using all 184 $\mu g/ml$ of antibiotic. The highly variable results also make it difficult to identify a general pattern to the dosing regimen. Incorporating the time interval does produce significantly better results than optimising daily doses alone when using less antibiotic. Optimising both the dose and the time interval results in treatment regimens which are a lot more complex than the conventional treatment. These treatment regimens may be more suited to clinical settings where varying doses and time intervals can be better controlled.

With the increase in antibiotic resistant bacteria, research has begun to examine the effectiveness of using multiple antibiotics in various dosing patterns [177, 178]. The work in this chapter highlights the potential amendments that can be made to single antibiotic treatment regimens to increase their efficiency.

There is a need to ensure single antibiotic are being used in an optimal manner before resorting to multiple antibiotics and using them both in an suboptimal regimen. However, more sensitivity analysis needs to be carried out before more general conclusions can be made on the optimal use of single antibiotics.

5.8 SUMMARY

Mathematical models can be a useful tool in predicting the dynamics of bacterial populations under varying treatment regimens. Previous chapters explored the effectiveness of incorporating a mathematical model into a GA to identify optimal dosage vectors to treat bacterial infections. Extending the GA to allow it to identify the time vector for treatments, this chapter investigated whether optimising the time interval between doses of antibiotic provides any additional improvements in the success of eradicating bacterial infections.

Changing the time intervals between constant doses of antibiotics did not result in a significant difference in success rate when compared to changing the daily doses. However, a shorter duration of treatment was achieved when the time intervals were optimised. In addition, the continued use of constant doses of antibiotic in these regimens remove any difficulties arising from manufacturing and administering the correct dose of antibiotic. A desirable benefit of changing the dose in the conventional treatment regimens is the ability to reduce the amount of antibiotic being used while maintaining high success rates. Whereas changing the time interval between constant doses restricts the GA to using the maximum amount of antibiotic available.

If combined, the benefit from optimising both the dose and time interval differed depending on the parameter values. At the lower concentration cap

a significant improvement in success rate was found when both the doses and the time intervals were optimised when using all 184 $\mu g/ml$ of antibiotic. At the higher concentration cap the addition of optimising the time intervals had no effect. However, optimising the time intervals in addition to the doses produced significantly better treatment regimens for both concentration caps when the amount of antibiotic being used was reduced.

The benefit of optimising the time interval between doses of antibiotics is not clear cut. The increased variability in identified treatment regimens makes identifying a single treatment pattern difficult. Despite this, the potential to increase success rates using less antibiotic warrants additional research.

6

DISCUSSION

Antibiotics are essential for the health and well-being of both humans and animals. However, the continued increase in antibiotic resistant bacteria poses a significant health threat. Genes which confer resistance to antibiotics are not new, but the overuse and misuse of antibiotics is proliferating their spread through bacterial populations [179, 180, 181]. With antibiotic resistance predicted to account for approximately 10 million deaths per year by 2050 [9], there is a continued emphasis on ensuring antibiotics are being used in an optimal manner. Shorter treatment durations have been identified as being as effective as longer durations in treating a number of bacterial diseases [141, 118, 120]. Indicating that current treatment guidelines, while effective, may not be the optimal way in which to administer current antibiotics. 'Optimising' treatment regimens by way of clinical trials only allows comparisons to be made between the finite number of treatments being compared. Clinical trials are also costly and limited by resources. This thesis therefore aimed to combine mathematical modelling with a genetic algorithm approach to identify optimal antibiotic treatment regimens. These alternative regimens maximise the success of eradicating an infection while minimising the amount of antibiotic used. Using a genetic algorithm to optimise treatment regimens allows for a search through potential regimens which may otherwise not have been considered.

This Chapter summarises the results obtained from the work in this thesis and discusses how these results relate to the global context. The limitations of the work and any further work will also be discussed.

6.1 SUMMARY OF RESULTS

It is reasonable to assume that the implementation of new antibiotics would initially produce a scenario where they are being used in the absence of resistance. Chapter 2 began by creating a mathematical model to describe the behaviour of a single bacterial population in the presence of an antibiotic environment. A high initial dose followed by a tapering of lower doses was identified as the optimal way of administering the antibiotic. This optimal treatment used the same amount of antibiotic as the baseline treatment but exposed the bacteria to a lower total antibiotic concentration.

Chapter 3 extended this work by including a resistant population of bacteria. Once again the GA identified a tapered treatment pattern to be the optimal way of administering the antibiotic. The high initial dose increases the concentration above the MIC of the resistant bacteria with decreasing doses maintaining it above this point. The tapered treatment regimen uses less antibiotic than the conventional baseline treatment and decreases the duration of treatment from 8 days to 6 days. Despite using less antibiotic than the conventional constant dose treatment, the tapered regimen increased the success rate. For all treatments which failed to eradicate the infection it was found that the resistant bacteria had re-colonised. Therefore increasing the success rate minimises the emergence of resistant bacteria.

With the GA providing results with a consistent pattern, Chapter 4 aimed to address whether these tapered treatment regimens were indeed a more effective way of administering antibiotics in a real-life system. A case study consisting of a susceptible *Vibrio anguillarum* infection within the larvae of the greater wax moth (*Galleria mellonella*) treated using tetracycline was studied. Using the results from laboratory experiments, the model from Chapter 2 was parameterised to this system. Incorporating this model into the GA, a tapered treatment regimen was once again identified as the optimal way to administer the antibiotic to increase the success of eradicating the infection. Based on model predictions, the tapered treatment regimen showed a significant increase in survival rate from 61% for the constant dose regimen to 69.5% for the tapered regimen. Further experiments confirmed this prediction with a 70% survival rate. The small sample size of larvae meant that the increase was not statistically significant. However, with a p-value of 0.145 and such a small sample size these results are encouraging.

Chapter 5 aimed to investigate whether optimising the time interval between doses could result in further improvements in treatment success or antibiotic usage. When the maximum amount of antibiotic was used it was found that there was no significant difference in success rate if the doses given at daily intervals were optimised or the time intervals between constant doses were optimised. However, when the amount of antibiotic being used is minimised, significantly better results were obtained when both the dose and time interval were optimised compared to optimising the dose only. Optimising the time interval between doses has the potential to increase the success rate of eradicating an infection with less antibiotic when combined with optimal doses. An interesting finding from this chapter showed that when the distance between the MIC of the resistant bacteria and the maximum

concentration cap is sufficiently small, the optimum pattern of administering the antibiotic is always to maintain the concentration at the maximum allowed for as long as possible. Highlighting the need to ensure optimal treatment regimens are identified before resistance increases further.

6.2 GLOBAL CONTEXT

Conventional antibiotic treatment regimens follow a typical pattern of X units of antibiotic for N days. The work from this thesis suggests that these treatment regimes, while effective, may not be the optimal treatment pattern to maximise bacterial eradication while minimising antibiotic usage. The longer a bacterial population exist in the presence of antibiotics the higher the chances are of resistance to the antibiotic developing. Attempts have been made to limit the overuse of antibiotics by identifying treatment protocols where treatment duration could be minimised [121] or where altering the constant dose could result in less antibiotic being administered [170, 171]. Despite being a more efficient use of antibiotic these alternative treatment regimens may still not be the optimal use of current antibiotics.

By administering antibiotics in a tapered pattern it was possible to increase the success rate of eradicating an infection, reduce the treatment duration, reduce the maximum concentration within the system and reduce the amount of antibiotic required to do so when compared to a conventional constant dose treatment regimen. Longer treatment durations and higher concentrations of antibiotics are more likely to select for resistance. Exposing an environment to antibiotics for the shortest time necessary at the lowest dose possible is preferable. Shorter durations of therapy were accompanied by a reduced time to eradication of the infection. With a shorter time to eradication patients

requiring hospitalisation would require shorter stays decreasing the cost associated with these infections. Shorter durations of therapy are also indicated in better patient compliance in an outpatient setting [182].

Further studies indicate the persistence of bacteria, after an infection has been treated, promotes the emergence of resistant strains [183, 184]. By reducing the number of infections which persist, despite antibiotic treatment, it may be possible to prolong the future effectiveness of these antibiotics. Tapered treatment regimens were shown to be less sensitive to changes in parameter values than a conventional treatment regimen (Chapter 3). Tapered treatment regimens remained more effective at eradicating an infection than the conventional treatment regimen when the MIC of the resistant bacteria was increased. Suggesting that even in the presence of resistance, tapered regimens would remain effective for longer and reduce the selection pressure for resistant bacteria.

Despite limited use, tapered regimens are currently used in the treatment of some infections, namely *Clostridium difficile*. Highlighting the feasibility of implementing such treatment regimens into a real-world scenario. Subsequent work by [83], using optimal control theory, supports the findings within this thesis. This study identified that the optimal way to administer antibiotics, in the presence of both susceptible and resistant bacteria, would be with a tapering of the concentration. However, their assumption that the concentration of antibiotic could be controlled at all times makes their findings difficult to implement as a treatment regimen.

6.3 LIMITATIONS AND FURTHER WORK

The results from this thesis indicate that tapered treatment regimens have the potential to increase treatment success while decreasing the amount of antibiotic used. However, further considerations are needed before these results could be translated into clinical use. This section highlights some of the limitations to this work and discusses further work required to address these.

6.3.1 *Modelling Assumptions*

The models presented in Chapters 2 and 3 contain limited in-host dynamics. The eradication of a bacterial infection is the combined effort of both the antibiotic and the host's immune system. The host immune response is a complex system consisting of various chemical and cellular interactions. The parameter θ was taken as a simplification of the host immune response to a bacterial infection. The effect of the host immune response in antibiotic treatment is not well studied. However, a few papers have considered the impact a patient's immune defences have on the outcome of antibiotic treatment [185, 186, 73, 74, 75]. Depending on whether the host immune response is density dependent or density independent affects the improvements predicted by increasing the dose of antibiotic [186]. The models within this thesis could be extended to include a more detailed immune response. However, further experimental studies are required to examine if a correlation exists between the intensity of the host immune response and the bacterial burden.

Pharmacodynamic (PD) and pharmacokinetic (PK) parameters were included within the model with the decay of the antibiotic from the host's system and the rate of antibiotic-induced bacterial death. These parameters

appear to have been sufficient to model the dynamics within the greater wax moth larvae. However, with more complex hosts more PK/PD parameters may be required to accurately predict the dynamics between the antibiotic, the bacteria and the host system. One assumption made within this model was that the concentration of the antibiotic administered reached the bacteria at the same concentration. Antibiotics are often not absorbed 100% by the host's system, with some antibiotic being excreted unchanged. Further work is required to understand the PK/PD parameters which need to be included to accurately predict the dynamics of the antibiotic within a more complex host.

6.3.2 *Patient Compliance*

The results obtained in this thesis assume that the antibiotic treatments are taken exactly as prescribed. Unfortunately, lack of patience compliance in medical treatments is a still a common problem [182, 174]. Conventional treatment regimens require the patient to take the same dose at set intervals. This means they take the same number of tablets for each dose. Tapered treatment regimens require a variation in the dose taken after each time interval. In a hospital setting patient compliance is more reliable with medical professionals ensuring the correct dose is administered at the correct time. While at home, patients would be required to correctly administer the correct dose for the corresponding time period. Further work on the effect of missed doses or early cessation of treatment in tapered regimens is therefore worth considering.

6.3.3 *Parameterisation and Sensitivity Analysis*

Despite identifying a tapered pattern as the optimal way to administer antibiotics, a general rule for designing a tapered treatment regimen could not be generated. Sensitivity analysis on one of the parameter sets showed that tapered treatment regimens are less sensitive to changes in parameter values than conventional treatment regimens. However, results from different parameter sets indicated that the optimal tapered regimen is host specific. Despite maintaining the same pattern, the exact doses changed considerably. This would require prior knowledge about the host, antibiotic and bacterial infection before a suitable tapered regimen could be identified. With some bacterial infections being time critical this may not always be possible. A variety of parameter sets were considered throughout the thesis. However, only one of these was based on biologically realistic values. Further work could be done on identifying the optimal treatment for a range of biologically realistic parameter sets. It may be possible to identify a more general tapered treatment, albeit sub-optimal, when parameters within a biologically realistic range are considered.

6.4 CONCLUDING REMARKS

Antibiotic resistance continues to spread with little hope that new antibiotics will be available soon. It is therefore essential that the antibiotics we currently have are used in an optimal manner that reduces the selection pressure for resistant bacteria. A genetic algorithm provided a systematic approach in the search for alternative treatment regimens. A tapered treatment regimen was identified as the optimal pattern for maximising treatment success while minimising antibiotic usage. The work from this thesis suggests that moving

away from conventional constant dose treatment regimens is required to ensure the future effectiveness of antibiotics.

BIBLIOGRAPHY

[1] S. B. Levy and B. Marshall, "Antibacterial resistance worldwide: causes, challenges and responses." *Nat. Med.*, vol. 10, no. 12 Suppl, pp. S122–9, dec 2004. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/15577930

[2] R. Davidson, R. Cavalcanti, J. L. Brunton, D. J. Bast, J. C. de Azavedo, P. Kibsey, C. Fleming, and D. E. Low, "Resistance to levofloxacin and failure of treatment of pneumococcal pneumonia," *New England Journal of Medicine*, vol. 346, no. 10, pp. 747–750, 2002, pMID: 11882730. [Online]. Available: https://doi.org/10.1056/NEJMoa012122

[3] J. B. Nachega and R. E. Chaisson, "Tuberculosis Drug Resistance: A Global Threat," *Clin. Infect. Dis.*, vol. 36, no. Supplement_1, pp. S24–S30, 2003.

[4] M. Ohnishi, D. Golparian, K. Shimuta, T. Saika, S. Hoshina, K. Iwasaku, S.-i. Nakayama, J. Kitawaki, and M. Unemo, "Is Neisseria gonorrhoeae Initiating a Future Era of Untreatable Gonorrhea?: Detailed Characterization of the First Strain with High-Level Resistance to Ceftriaxone," *Antimicrob. Agents Chemother.*, vol. 55, no. 7, pp. 3538–3545, 2011.

[5] M. Unemo, D. Golparian, and B. Hellmark, "First three neisseria gonorrhoeae isolates with high-Level resistance to azithromycin in sweden: A threat to currently available dual-Antimicrobial regimens for treatment of gonorrhea?" *Antimicrob. Agents Chemother.*, vol. 58, no. 1, pp. 624–625, 2014.

[6] A. A. Salyers, "Why Are Antibiotic Resistance Genes So Resistant to Elimination ?" *Antimicrob. Agents Chemother.*, vol. 41, no. 11, pp. 2321–2325, 1997.

[7] T. M. Barbosa and S. B. Levy, "The impact of antibiotic use on resistance development and persistence." *Drug Resist. Updat.*, vol. 3, no. 5, pp. 303–311, oct 2000. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/11498398

[8] D. I. Andersson and D. Hughes, "Antibiotic resistance and its cost: is it possible to reverse resistance?" *Nat. Rev. Microbiol.*, vol. 8, no. 4, pp. 260–71, apr 2010.

[9] J. O'Neill, "Review on antimicrobial resistance antimicrobial resistance: Tackling a crisis for the health and wealth of nations." 2014. [Online]. Available: https://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf

[10] T. P. Van Boeckel, C. Brower, M. Gilbert, B. T. Grenfell, S. A. Levin, T. P. Robinson, A. Teillant, and R. Laxminarayan, "Global trends in antimicrobial use in food animals." *Proc. Natl. Acad. Sci. U. S. A.*, vol. 112, no. 18, pp. 5649–54, 2015. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/25792457{%}0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4426470

[11] M. K. Chattopadhyay, "Use of antibiotics as feed additives: a burning question," *Front. Microbiol.*, vol. 5, no. July, pp. 1–3, 2014.

[12] C. Cogliani, H. Goossens, and C. Greko, "Restricting Antimicrobial Use in Food Animals: Lessons from Europe," *microbe*, vol. 6, no. 6, pp. 274–279, 2011.

[13] "The microbial threat: The copenhagen recommendation," *Microbial Ecology in Health and Disease*, vol. 10, no. 2, pp. 65–67, 1998.

[14] G. Emilien, W. Van Meurs, and J. M. Maloteaux, "The dose-response relationship in Phase I clinical trials and beyonduse, meaning, and assessment," *Pharmacol. Ther.*, vol. 88, no. 1, pp. 33–58, 2000.

[15] K. B. Pouwels, M. Yin, C. C. Butler, B. S. Cooper, S. Wordsworth, A. S. Walker, and J. V. Robotham, "Optimising trial designs to identify appropriate antibiotic treatment durations," *BMC Medicine*, vol. 17, no. 1, p. 115, Jun 2019. [Online]. Available: https://doi.org/10.1186/s12916-019-1348-z

[16] M. J. Keeling, "Models of foot-and-mouth disease," *Proc. R. Soc. B Biol. Sci.*, vol. 272, no. 1569, pp. 1195–1202, 2005.

[17] E. Prestel, S. Salamitou, and M. S. DuBow, "An examination of the bacteriophages and bacteria of the namib desert," *The Journal of Microbiology*, vol. 46, no. 4, p. 364, Aug 2008.

[18] K. Junge, H. Eicken, and J. W. Deming, "Bacterial activity at -2 to -20°c in arctic wintertime sea ice," *Applied and Environmental Microbiology*, vol. 70, no. 1, pp. 550–557, 2004.

[19] C. A. Lozupone and R. Knight, "Global patterns in bacterial diversity," *Proceedings of the National Academy of Sciences*, vol. 104, no. 27, pp. 11 436–11 440, 2007.

[20] M. McFall-Ngai, "Care for the community," *Nature*, vol. 445, p. 153, jan 2007. [Online]. Available: https://doi.org/10.1038/445153ahttp://10.0.4.14/445153a

[21] M. Candela, F. Perna, P. Carnevali, B. Vitali, R. Ciati, P. Gionchetti, F. Rizzello, M. Campieri, and P. Brigidi, "Interaction of probiotic Lacto-

bacillus and Bifidobacterium strains with human intestinal epithelial cells: Adhesion properties, competition against enteropathogens and modulation of IL-8 production," *Int. J. Food Microbiol.*, vol. 125, no. 3, pp. 286–292, 2008.

[22] S. Fukuda, H. Toh, K. Hase, K. Oshima, Y. Nakanishi, K. Yoshimura, T. Tobe, J. M. Clarke, D. L. Topping, T. Suzuki, T. D. Taylor, K. Itoh, J. Kikuchi, H. Morita, M. Hattori, and H. Ohno, "Bifidobacteria can protect from enteropathogenic infection through production of acetate," *Nature*, vol. 469, no. 7331, pp. 543–549, 2011.

[23] T. Olszak, D. An, S. Zeissig, M. P. Vera, J. Richter, A. Franke, J. N. Glickman, R. Siebert, R. M. Baron, D. L. Kasper, and R. S. Blumberg, "Microbial exposure during early life has persistent effects on natural killer T cell function," *Science*, vol. 336, no. 6080, pp. 489–493, 2012.

[24] M. Kobayashi and Y. Tchan, "Treatment of industrial waste solutions and production of useful by-products using a photosynthetic bacterial method," *Water Research*, vol. 7, no. 8, pp. 1219 – 1224, 1973.

[25] R. Jacoby, M. Peukert, A. Succurro, A. Koprivova, and S. Kopriva, "The role of soil microorganisms in plant mineral nutrition - current knowledge and future directions," *Front. Plant Sci.*, vol. 8, no. September, pp. 1–19, 2017.

[26] A. M. Valdes, J. Walter, E. Segal, and T. D. Spector, "Role of the gut microbiota in nutrition and health," *BMJ*, vol. 361, pp. 36–44, 2018.

[27] K. Chiller, B. A. Selkin, and G. J. Murakawa, "Skin microflora and bacterial infections of the skin," *J. Investig. Dermatology Symp. Proc.*, vol. 6, no. 3, pp. 170–174, 2001. [Online]. Available: http://dx.doi.org/10.1046/j.0022-202x.2001.00043.x

[28] B. Kara Rogers Senior Editor, *Bacteria and Viruses*, ser. Biochemistry, Cells, and Life. Britannica Educational Pub., 2011.

[29] G. R. Cornelis, A. Boland, A. P. Boyd, C. Geuijen, M. Iriarte, C. Neyt, M. P. Sory, and I. Stainier, "The virulence plasmid of Yersinia, an antihost genome." *Microbiol. Mol. Biol. Rev.*, vol. 62, no. 4, pp. 1315–52, 1998. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/9841674{%}0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC98948

[30] P. M. Bennett, "Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria." *Br. J. Pharmacol.*, vol. 153 Suppl, no. January, pp. S347–57, mar 2008.

[31] G. S. R. Reddy and M. Rafi, "Isolation and characterization of a bacterial strain for benzene degradation," *Glob. J. bio-science Biotechnol.*, vol. 1, no. 2, pp. 178–181, 2012.

[32] N. G. H. Taylor, D. W. Verner-Jeffreys, and C. Baker-Austin, "Aquatic systems: maintaining, mixing and mobilising antimicrobial resistance?" *Trends Ecol. Evol.*, vol. 26, no. 6, pp. 278–84, jun 2011.

[33] J. L. Hobman and L. C. Crossman, "Bacterial antimicrobial metal ion resistance," *J. Med. Microbiol.*, vol. 64, no. 2014, pp. 471–497, 2014.

[34] L. M. Wilson, "Etiology of Bacterial Endocarditis: Before and Since the Introduction of Antibiotics," *Annals of Internal Medicine*, vol. 58, no. 6, pp. 946–952, 06 1963.

[35] R. E. Hancock, "Mechanisms of action of newer antibiotics for gram-positive pathogens," *The Lancet Infectious Diseases*, vol. 5, no. 4, pp. 209 – 218, 2005.

[36] H. W. Boucher, G. H. Talbot, J. S. Bradley, J. E. Edwards, D. Gilbert, L. B. Rice, M. Scheld, B. Spellberg, and J. Bartlett, "Bad bugs, no drugs: no

ESKAPE! An update from the Infectious Diseases Society of America." *Clin. Infect. Dis.*, vol. 48, no. 1, pp. 1–12, 2009.

[37] C. Baker-Austin, M. S. Wright, R. Stepanauskas, and J. V. McArthur, "Co-selection of antibiotic and metal resistance." *Trends Microbiol.*, vol. 14, no. 4, pp. 176–82, apr 2006. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/16537105

[38] J. Davies, "Inactivation of antibiotics and the dissemination of resistance genes," *Science*, vol. 264, no. 5157, pp. 375–382, 1994. [Online]. Available: https://science.sciencemag.org/content/264/5157/375

[39] H. Kruse and H. Sørum, "Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments." *Appl. Environ. Microbiol.*, vol. 60, no. 11 PG - 4015-21, pp. 4015–4021, 1994.

[40] C. Dahlberg and L. Chao, "Amelioration of the cost of conjugative plasmid carriage in Eschericha coli K12." *Genetics*, vol. 165, no. 4, pp. 1641–9, dec 2003.

[41] A. Clatworthy, E. Pierson, and D. T Hung, "Targeting virulence: A new paradigm for antimicrobial therapy," *Nature chemical biology*, vol. 3, pp. 541–8, 10 2007.

[42] H. Ariffin, P. Navaratnam, T. K. Kee, and G. Balan, "Antibiotic resistance patterns in nosocomial gram-negative bacterial infections in units with heavy antibiotic usage," *J. Trop. Pediatr.*, vol. 50, no. 1, pp. 26–31, 2004.

[43] F. M. Aarestrup, "Veterinary drug usage and antimicrobial resistance in bacteria of animal origin." *Basic Clin. Pharmacol. Toxicol.*, vol. 96, no. 4, pp. 271–281, 2005.

[44] H. J. Adam, D. J. Hoban, A. S. Gin, and G. G. Zhanel, "Association between fluoroquinolone usage and a dramatic rise in ciprofloxacin-

resistant Streptococcus pneumoniae in Canada, 1997-2006," *Int. J. Antimicrob. Agents*, vol. 34, no. 1, pp. 82–85, 2009.

[45] B. Spellberg, M. Blaser, R. J. Guidos, H. W. Boucher, J. S. Bradley, B. I. Eisenstein, D. Gerding, R. Lynfield, L. B. Reller, J. Rex, D. Schwartz, E. Septimus, F. C. Tenover, and D. N. Gilbert, "Combating antimicrobial resistance: Policy recommendations to save lives," *Clin. Infect. Dis.*, vol. 52, no. SUPPL. 5, pp. 397–428, 2011.

[46] G. M. Rossolini, F. Arena, P. Pecile, and S. Pollini, "Update on the antibiotic resistance crisis," *Curr. Opin. Pharmacol.*, vol. 18, pp. 56–60, 2014. [Online]. Available: http://dx.doi.org/10.1016/j.coph.2014.09.006

[47] N. D. Friedman, E. Temkin, and Y. Carmeli, "The negative impact of antibiotic resistance," *Clin. Microbiol. Infect.*, vol. 22, no. 5, pp. 416–422, 2015. [Online]. Available: http://dx.doi.org/10.1016/j.cmi.2015.12.002

[48] C. A. McNulty, T. Nichols, P. J. Boyle, M. Woodhead, and P. Davey, "The English antibiotic awareness campaigns: Did they change the public's knowledge of and attitudes to antibiotic use?" *J. Antimicrob. Chemother.*, vol. 65, no. 7, pp. 1526–1533, 2010.

[49] B. Huttner, M. Saam, L. Moja, K. Mah, M. Sprenger, S. Harbarth, and N. Magrini, "How to improve antibiotic awareness campaigns: Findings of a WHO global survey," *BMJ Glob. Heal.*, vol. 4, no. 3, pp. 1–9, 2019.

[50] *Antimicrobials Sold or Distributed for Use in Food-Producing Animals*. Food and Drug Administration, September 2014. [Online]. Available: http://www.fda.gov/downloads/ForIndustry/UserFees/AnimalDrugUserFeeActADUFA/UCM338170.pdf

[51] J. A. Paulson and T. E. Zaoutis, "Nontherapeutic use of antimicrobial agents in animal agriculture: Implications for pediatrics," *Pediatrics*,

vol. 136, no. 6, pp. e1670–e1677, 2015. [Online]. Available: https://pediatrics.aappublications.org/content/136/6/e1670

[52] T. Defoirdt, P. Sorgeloos, and P. Bossier, "Alternatives to antibiotics for the control of bacterial disease in aquaculture." *Curr. Opin. Microbiol.*, vol. 14, no. 3, pp. 251–8, jun 2011. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/21489864

[53] T. L. Leung and A. E. Bates, "More rapid and severe disease outbreaks for aquaculture at the tropics: Implications for food security," *J. Appl. Ecol.*, vol. 50, no. 1, pp. 215–222, 2013.

[54] F. Baquero, J.-L. Martínez, and R. Cantón, "Antibiotics and antibiotic resistance in water environments." *Curr. Opin. Biotechnol.*, vol. 19, no. 3, pp. 260–5, jun 2008.

[55] S. J. Sørensen, M. Bailey, L. H. Hansen, N. Kroer, and S. Wuertz, "Studying plasmid horizontal transfer in situ: A critical review," *Nat. Rev. Microbiol.*, vol. 3, no. 9, pp. 700–710, 2005.

[56] H. Radhouani, N. Silva, P. Poeta, C. Torres, S. Correia, and G. Igrejas, "Potential impact of antimicrobial resistance in wildlife, environment and human health," *Frontiers in Microbiology*, vol. 5, p. 23, 2014.

[57] European Commission, "Communication from the Commission to the European Parliament and the Council action plan against the rising threats from antimicrobial resistance," p. 748, 2011.

[58] Department of Health and Department for Environment Food and Rural Affairs, "UK Five Year Antimicrobial Resistance Strategy 2013 to 2018," *Dep. Heal. Dep. Environ. Food Rural Aff.*, p. 43, 2013.

[59] H. Hanberger, S. Walther, M. Leone, P. S. Barie, J. Rello, J. Lipman, J. C. Marshall, A. Anzueto, Y. Sakr, P. Pickkers, P. Felleiter, M. Engoren, and

J. L. Vincent, "Increased mortality associated with meticillin-resistant Staphylococcus aureus (MRSA) infection in the Intensive Care Unit: Results from the EPIC II study," *Int. J. Antimicrob. Agents*, vol. 38, no. 4, pp. 331–335, 2011.

[60] C. Prematunge, C. MacDougall, J. Johnstone, K. Adomako, F. Lam, J. Robertson, and G. Garber, "Vre and vse bacteremia outcomes in the era of effective vre therapy: A systematic review and meta-analysis," *Infection Control and; Hospital Epidemiology*, vol. 37, no. 1, p. 26Ű35, 2016.

[61] S. Bonhoeffer, M. Lipsitch, and B. R. Levin, "Evaluating treatment protocols to prevent antibiotic resistance." *Proc Natl Acad Sci USA*, vol. 94, no. 22, pp. 12 106–11, 1997.

[62] M. Lipsitch, C. T. Bergstrom, and B. R. Levin, "The epidemiology of antibiotic resistance in hospitals: paradoxes and prescriptions." *Proc. Natl. Acad. Sci. U. S. A.*, vol. 97, no. 4, pp. 1938–43, 2000. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/10677558{%}0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC26540

[63] C. T. Bergstrom, M. Lo, and M. Lipsitch, "Ecological theory suggests that antimicrobial cycling will not reduce antimicrobial resistance in hospitals." *Proc. Natl. Acad. Sci. U. S. A.*, vol. 101, no. 36, pp. 13 285–13 290, 2004.

[64] U. Obolski and L. Hadany, "Implications of stress-induced genetic variation for minimizing multidrug resistance in bacteria," *BMC Med.*, vol. 10, no. 1, p. 89, 2012. [Online]. Available: http://www.biomedcentral.com/1741-7015/10/89

[65] D. Raymond, S. Pelletier, T. Crabtree, T. Gleason, L. Hamm, and T. Pruett, "Impact of a rotating empiric antibiotic schedule on infectious mortality

in an intensive care unit," *Crit. Care Med.*, vol. 29, no. 6, pp. 1101–1108, 2001. [Online]. Available: http://ovidsp.ovid.com/ovidweb.cgi?T=JS{&}PAGE=reference{&}D=emed8{&}NEWS=N{&}AN=32554319

[66] J. A. Martinez, J.-M. Nicolas, F. Marco, J. Horcajada, G. Garcia-Segarra, A. Trilla, C. Codina, A. Torres, and J. Mensa, "Comparison of antimicrobial cycling and mixing strategies in two medical intensive care units," *Critical care medicine*, vol. 34, pp. 329–36, 03 2006.

[67] P. J. van Duijn, W. Verbrugghe, P. G. Jorens, F. Spöhr, D. Schedler, M. Deja, A. Rothbart, D. Annane, C. Lawrence, J. C. Nguyen Van, B. Misset, M. Jereb, K. Seme, F. Šifrer, V. Tomiç, F. Estevez, J. Carneiro, S. Harbarth, M. J. C. Eijkemans, M. Bonten, H. Goossens, S. Malhotra-Kumar, C. Lammens, J. Vila, and I. Roca, "The effects of antibiotic cycling and mixing on antibiotic resistance in intensive care units: a cluster-randomised crossover trial," *Lancet Infect. Dis.*, vol. 18, no. 4, pp. 401–409, 2018.

[68] B. Tepekule, H. Uecker, I. Derungs, A. Frenoy, and S. Bonhoeffer, "Modeling antibiotic treatment in hospitals: A systematic approach shows benefits of combination therapy over cycling, mixing, and mono-drug therapies," *PLoS Comput. Biol.*, vol. 13, no. 9, pp. 1–22, 2017.

[69] F. Svara and D. J. Rankin, "The evolution of plasmid-carried antibiotic resistance." *BMC Evol. Biol.*, vol. 11, no. 1, p. 130, jan 2011.

[70] F. M. Stewart and B. R. Levin, "a Przorz Conditions for the Existence of Plasmids," *Genetics*, vol. 87, no. 2, pp. 209–228, 1977.

[71] C. T. Bergstrom, M. Lipsitch, and B. R. Levin, "Natural selection, infectious transfer and the existence conditions for bacterial plasmids," *Genetics*, vol. 155, no. 4, pp. 1505–1519, 2000.

[72] L. N. Lili, N. F. Britton, and E. J. Feil, "The persistence of parasitic plasmids," *Genetics*, vol. 177, no. 1, pp. 399–405, 2007.

[73] E. M. C. D'Agata, M. Dupont-Rouzeyrol, P. Magal, D. Olivier, and S. Ruan, "The impact of different antibiotic regimens on the emergence of antimicrobial-resistant bacteria." *PLoS One*, vol. 3, no. 12, 2008.

[74] P. Geli, R. Laxminarayan, M. Dunne, and D. L. Smith, ""One-Size-Fits-All"? Optimizing Treatment Duration for Bacterial Infections," *PLoS One*, vol. 7, no. 1, 2012.

[75] P. Ankomah and B. R. Levin, "Exploring the collaboration between antibiotics and the immune response in the treatment of acute, self-limiting infections," *Proc. Natl. Acad. Sci.*, vol. 111, no. 23, pp. 8331–8338, 2014.

[76] G. Ochoa, M. Villasana, and E. K. Burke, "An evolutionary approach to cancer chemotherapy scheduling," *Genet. Program. Evolvable Mach.*, vol. 8, no. 4, pp. 301–318, 2007.

[77] R. Luo, M. J. Piovoso, J. Martinez-Picado, and R. Zurakowski, "Optimal antiviral switching to minimize resistance risk in HIV therapy," *PLoS One*, vol. 6, no. 11, 2011.

[78] G. Ochoa and M. Villasana, "Population-based optimization of cytostatic/cytotoxic combination cancer chemotherapy," *Soft Comput.*, vol. 17, no. 6, pp. 913–924, 2013.

[79] H. Moore, L. Strauss, and U. Ledzewicz, "Optimization of combination therapy for chronic myeloid leukemia with dosing constraints," *J. Math. Biol.*, vol. 77, no. 5, pp. 1533–1561, 2018. [Online]. Available: https://doi.org/10.1007/s00285-018-1262-6

[80] R. Peña-Miller, D. Lähnemann, H. Schulenburg, M. Ackermann, and R. Beardmore, "Selecting Against Antibiotic-Resistant Pathogens: Optimal Treatments in the Presence of Commensal Bacteria," *Bull. Math. Biol.*, vol. 74, no. 4, pp. 908–934, 2012.

[81] M. Imran and H. Smith, "A Model of optimal dosing of antibiotic treatment in biofilm," *Math. Biosci. Eng.*, vol. 11, no. 3, pp. 547–571, 2014.

[82] I. K. Paterson, A. Hoyle, G. Ochoa, C. Baker-Austin, and N. G. Taylor, "Optimising antibiotic usage to treat bacterial infections," *Sci. Rep.*, vol. 6, no. November, pp. 1–10, 2016. [Online]. Available: http://dx.doi.org/10.1038/srep37853

[83] A. Khan and M. Imran, "Optimal Dosing Strategies Against Susceptible and Resistant Bacteria," *J. Biol. Syst.*, vol. 26, no. 01, pp. 41–58, 2018.

[84] J. M. Cicchese, E. Pienaar, D. E. Kirschner, and J. J. Linderman, "Applying Optimization Algorithms to Tuberculosis Antibiotic Treatment Regimens," *Cell. Mol. Bioeng.*, vol. 10, no. 6, pp. 523–535, 2017.

[85] J. Holland, *Adaptation in natural and artificial systems: an introductory analysis with applications to biology, control, and artificial intelligence*. University of Michigan Press, 1975.

[86] J. McCall, "Genetic algorithms for modelling and optimisation," *J. Comput. Appl. Math.*, vol. 184, no. 1, pp. 205–222, 2005.

[87] R. Sender, S. Fuchs, and R. Milo, "Revised Estimates for the Number of Human and Bacteria Cells in the Body." *PLoS Biol.*, vol. 14, no. 8, 2016.

[88] J. G. Leahy and R. R. Colwell, "Microbial degradation of hydrocarbons in the environment." *Microbiol. Rev.*, vol. 54, no. 3, pp. 305–15, 1990.

[89] B. A. McKew, F. Coulon, A. M. Osborn, K. N. Timmis, and T. J. McGenity, "Determining the identity and roles of oil-metabolizing marine bacteria

from the Thames estuary, UK," *Environ. Microbiol.*, vol. 9, no. 1, pp. 165–176, 2007.

[90] G. Walsh, "Therapeutic insulins and their large-scale manufacture," *Appl. Microbiol. Biotechnol.*, vol. 67, no. 2, pp. 151–159, 2005.

[91] R. G. Branco and R. C. Tasker, "Meningococcal meningitis," *Curr. Treat. Options Neurol.*, vol. 12, no. 5, pp. 464–474, 2010.

[92] J. Hamborsky, A. Kroger, and C. Wolfe, *Epidemiology and Prevention of Vaccine-preventable Diseases*, ser. CDC, Epidemiology and Prevention of Vaccine-Preventable Disease Series. Centers for Disease Control and Prevention, 2015.

[93] D. A. Austin, P. A. W. Robertson, and B. Austin, "Recovery of a new biogroup of Yersinia ruckeri from diseased rainbow trout (Oncorhynchus mykiss, Walbaum) examination of diseased fish and isolation." *Syst. Appl. Microbiol.*, vol. 26, no. 2003, pp. 127–131, 2003.

[94] B. Fouz, C. Zarza, and C. Amaro, "First description of non-motile Yersinia ruckeri serovar I strains causing disease in rainbow trout, Oncorhynchus mykiss (Walbaum), cultured in Spain," *J. Fish Dis.*, vol. 29, no. 6, pp. 339–346, 2006.

[95] J. W. Tinsley, A. R. Lyndon, and B. Austin, "Antigenic and cross-protection studies of biotype 1 and biotype 2 isolates of Yersinia ruckeri in rainbow trout, Oncorhynchus mykiss (Walbaum)," *J. Appl. Microbiol.*, vol. 111, no. 1, pp. 8–16, 2011.

[96] C. Kerrison and F. A. I. Riordan, "How long should we treat this infection for?" *Archives of Disease in Childhood - Education and Practice*, vol. 98, no. 4, pp. 136–140, 2013. [Online]. Available: https://ep.bmj.com/content/98/4/136

[97] W. S. Lim, D. L. Smith, M. P. Wise, and S. A. Welham, "British Thoracic Society community acquired pneumonia guideline and the NICE pneumonia guideline: How they fit together," *BMJ Open Respir. Res.*, vol. 2, no. 1, pp. 1–3, 2015.

[98] J. E. McGowan, "Antimicrobial resistance in hospital organisms and its relation to antibiotic use." *Rev. Infect. Dis.*, vol. 5, no. 6, pp. 1033–48, 1983. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/6318289

[99] I. Gustafsson, M. Sjölund, E. Torell, M. Johannesson, L. Engstrand, O. Cars, and D. I. Andersson, "Bacteria with increased mutation frequency and antibiotic resistance are enriched in the commensal flora of patients with high antibiotic usage," *J. Antimicrob. Chemother.*, vol. 52, no. 4, pp. 645–650, 2003.

[100] G. F. Webb, E. M. C. D'Agata, P. Magal, and S. Ruan, "A model of antibiotic-resistant bacterial epidemics in hospitals," *Proc. Natl. Acad. Sci.*, vol. 102, no. 37, pp. 13 343–13 348, 2005.

[101] D. J. Rankin, M. Bichsel, and a. Wagner, "Mobile DNA can drive lineage extinction in prokaryotic populations." *J. Evol. Biol.*, vol. 23, no. 11, pp. 2422–31, nov 2010. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/20860700

[102] K. Markov, R. Tsanev, and S. M. Markov, "on the Mathematical Modelling of Microbial Growth: Some Computational Aspects *," *Serdica J. Comput.*, vol. 5, pp. 153–168, 2011.

[103] V. V. Volkova, C. Lanzas, Z. Lu, and Y. T. Gröhn, "Mathematical model of plasmid-mediated resistance to ceftiofur in commensal enteric escherichia coli of cattle," *PLoS One*, vol. 7, no. 5, 2012.

[104] L. N. Lili, N. F. Britton, and E. J. Feil, "The persistence of parasitic plasmids." *Genetics*, vol. 177, no. 1, pp. 399–405, sep 2007.

[105] J. P. Torella, R. Chait, and R. Kishony, "Optimal drug synergy in Antimicrobial Treatments," *PLoS Comput. Biol.*, vol. 6, no. 6, pp. 1–9, 2010.

[106] J. M. Hyatt, D. E. Nix, C. W. Stratton, and J. J. Schentag, "In vitro pharmacodynamics of piperacillin, piperacillin-tazobactam, and ciprofloxacin alone and in combination against Staphylococcus aureus, Klebsiella pneumoniae, Enterobacter cloacae, and Pseudomonas aeruginosa," *Antimicrob. Agents Chemother.*, vol. 39, no. 8, pp. 1711–1716, 1995.

[107] M. Alin, A. Björkman, A. Landberg-Lindgren, and M. Ashton, "The effect of artemisinin, its derivatives and mefloquine against chloroquine-resistant strains of Plasmodium falciparum in vitro," *Transactions of The Royal Society of Tropical Medicine and Hygiene*, vol. 86, no. 4, pp. 365–367, 07 1992. [Online]. Available: https://doi.org/10.1016/0035-9203(92)90220-7

[108] A. Louie, P. Kaw, W. Liu, N. Jumbe, M. H. Miller, and G. L. Drusano, "Pharmacodynamics of daptomycin in a murine thigh model of Staphylococcus aureus infection," *Antimicrob. Agents Chemother.*, vol. 45, no. 3, pp. 845–851, 2001.

[109] Y. Q. Liu, Y. Z. Zhang, and P. J. Gao, "Novel concentration-killing curve method for estimation of bactericidal potency of antibiotics in an in vitro dynamic model," *Antimicrob. Agents Chemother.*, vol. 48, no. 10, pp. 3884–3891, 2004.

[110] R. R. Regoes, C. Wiuff, R. M. Zappala, N. Kim, F. Baquero, B. R. Levin, and K. N. Garner, "Pharmacodynamic Functions : a Multiparameter Approach to the Design of Antibiotic Treatment Regimens Pharmacodynamic Functions : a Multiparameter Approach to the Design of Antibiotic

Treatment Regimens," *Antimicrob. Agents Chemother.*, vol. 48, no. 10, pp. 3670–3676, 2004.

[111] D. T. Gillespie, "Exact stochastic simulation of coupled chemical reactions," *The Journal of Physical Chemistry*, vol. 81, no. 25, pp. 2340–2361, 1977.

[112] C. J. Clooper and Pearson E. S., "Biometrika Trust The Use of Confidence or Fiducial Limits Illustrated in the Case of the Binomial Author (s): C . J . Clopper and E . S . Pearson Published by : Oxford University Press on behalf of Biometrika Trust Stable URL : http://www.jstor.org/stab," *Biometrika*, vol. 26, no. 4, pp. 404–413, 1934.

[113] S. K. Olofsson and O. Cars, "Optimizing Drug Exposure to Minimize Selection of Antibiotic Resistance," *Clin. Infect. Dis.*, vol. 45, no. Supplement_2, pp. S129–S136, 2007.

[114] N. Jumbe, A. Louie, R. Leary, W. Liu, M. R. Deziel, V. H. Tam, R. Bachhawat, C. Freeman, J. B. Kahn, K. Bush, M. N. Dudley, M. H. Miller, and G. L. Drusano, "Application of a mathematical model to prevent in vivo amplification of antibiotic-resistant bacterial populations during therapy," *J. Clin. Invest.*, vol. 112, no. 2, pp. 275–285, 2003.

[115] V. H. Tam, A. Louie, M. R. Deziel, W. Liu, and G. L. Drusano, "The relationship between quinolone exposures and resistance amplification is characterized by an inverted U: A new paradigm for optimizing pharmacodynamics to counterselect resistance," *Antimicrob. Agents Chemother.*, vol. 51, no. 2, pp. 744–747, 2007.

[116] O. Rann, M. Sharland, P. Long, I. C. Wong, A. A. Laverty, A. Bottle, C. I. Barker, J. Bielicki, and S. Saxena, "Did the accuracy of oral amoxicillin dosing of children improve after British National Formulary dose revi-

sions in 2014? National cross-sectional survey in England," *BMJ Open*, vol. 7, no. 9, pp. 1–6, 2017.

[117] G. Agarwal, S. Awasthi, S. K. Kabra, A. Kaul, S. Singhi, S. D. Walter, and I. S. Group, "Three day versus five day treatment with amoxicillin for non-severe pneumonia in young children: a multicentre randomised controlled trial," *Bmj*, vol. 328, no. 7443, p. 791, 2004. [Online]. Available: http://www.bmj.com/lookup/doi/10.1136/bmj.38049.490255.DE

[118] R. el Moussaoui, C. A. J. M. de Borgie, P. van den Broek, W. N. Hustinx, P. Bresser, G. E. L. van den Berk, J.-W. Poley, B. van den Berg, F. H. Krouwels, M. J. M. Bonten, C. Weenink, P. M. M. Bossuyt, P. Speelman, B. C. Opmeer, and J. M. Prins, "Effectiveness of discontinuing antibiotic treatment after three days versus eight days in mild to moderate-severe community acquired pneumonia: randomised, double blind study," *Bmj*, vol. 332, no. 7554, p. 1355, 2006.

[119] K. G. Kyriakidou, P. Rafailidis, D. K. Matthaiou, S. Athanasiou, and M. E. Falagas, "Short- versus long-course antibiotic therapy for acute pyelonephritis in adolescents and adults: A meta-analysis of randomized controlled trials," *Clin. Ther.*, vol. 30, no. 10, pp. 1859–1868, 2008. [Online]. Available: http://dx.doi.org/10.1016/S0149-2918(08)00328-7

[120] D. E. Karageorgopoulos, P. E. Valkimadi, A. Kapaskelis, P. I. Rafailidis, and M. E. Falagas, "Short versus long duration of antibiotic therapy for bacterial meningitis: a meta-analysis of randomised controlled trials in children," *Archives of Disease in Childhood*, vol. 94, no. 8, pp. 607–614, 2009. [Online]. Available: https://adc.bmj.com/content/94/8/607

[121] A. Uranga, P. P. España, A. Bilbao, J. M. Quintana, I. Arriaga, M. Intxausti, J. L. Lobo, L. Tomás, J. Camino, J. Nuñez, and A. Capelastegui, "Duration of Antibiotic Treatment in Community-

Acquired Pneumonia: A Multicenter Randomized Clinical," *JAMA Internal Medicine*, vol. 176, no. 9, pp. 1257–1265, 09 2016. [Online]. Available: https://doi.org/10.1001/jamainternmed.2016.3633

[122] S. H. Cohen, D. N. Gerding, S. Johnson, C. P. Kelly, V. G. Loo, L. C. McDonald, J. Pepin, and M. H. Wilcox, "Clinical practice guidelines for clostridium difficile infection in adults: 2010 update by the society for healthcare epidemiology of america (shea) and the infectious diseases society of america (idsa)," *Infection Control and Hospital Epidemiology*, vol. 31, no. 5, pp. 431–455, 2010.

[123] B. D. Sirbu, M. M. Soriano, C. Manzo, J. Lum, D. N. Gerding, and S. Johnson, "Vancomycin taper and pulse regimen with careful follow-up for patients with recurrent clostridium dificile infection," *Clin. Infect. Dis.*, vol. 65, no. 8, pp. 1396–1399, 2017.

[124] R. Dagan, "Evidence to support the rationale that bacterial eradication in respiratory tract infection is an important aim of antimicrobial therapy," *J. Antimicrob. Chemother.*, vol. 47, no. 2, pp. 129–140, 2001.

[125] B. A. Jindal, M. K. Pandya, and M. I. Khan, "Antimicrobial resistance: A public health challenge," *Med. J. Armed Forces India*, vol. 71, no. 2, pp. 178–181, 2015.

[126] C. L. Ventola, "The Antibiotic Resistance Crisis Part One: Causes and Threats," *P&T*, vol. 40, no. 4, pp. 278–283, 2015. [Online]. Available: https://www.jstage.jst.go.jp/article/electrochemistry/82/9/82{_}14-5-E2639/{_}pdf

[127] D. Ferber, "Superbugs on the Hoof?" *Science (80-.).*, vol. 288, no. 5467, pp. 792–794, may 2000.

[128] F. C. Cabello, "Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment." *Environ. Microbiol.*, vol. 8, no. 7, pp. 1137–44, jul 2006.

[129] A. Teillant, S. Gandra, D. Barter, D. J. Morgan, and R. Laxminarayan, "Potential burden of antibiotic resistance on surgery and cancer chemotherapy antibiotic prophylaxis in the USA: A literature review and modelling study," *Lancet Infect. Dis.*, vol. 15, no. 12, pp. 1429–1437, 2015.

[130] Y. Nakamura, T. Itoh, H. Matsuda, and T. Gojobori, "Biased biological functions of horizontally transferred genes in prokaryotic genomes." *Nat. Genet.*, vol. 36, no. 7, pp. 760–766, 2004.

[131] E. Gullberg, S. Cao, O. G. Berg, C. Ilbäck, L. Sandegren, D. Hughes, and D. I. Andersson, "Selection of resistant bacteria at very low antibiotic concentrations," *PLoS Pathog.*, vol. 7, no. 7, pp. 1–9, 2011.

[132] F. S. Aliabadi and P. Lees, "Antibiotic treatment for animals: Effect on bacterial population and dosage regimen optimisation," *Int. J. Antimicrob. Agents*, vol. 14, no. 4, pp. 307–313, 2000.

[133] R. Peña-Miller, A. Fuentes-Hernandez, C. Reding, I. Gudelj, and R. Beardmore, "Testing the optimality properties of a dual antibiotic treatment in a two-locus, two-allele model." *J. R. Soc. Interface*, vol. 11, no. 96, p. 20131035, 2014.

[134] C. T. Bergstrom, M. Lipsitch, and B. R. Levin, "Natural selection, infectious transfer and the existence conditions for bacterial plasmids." *Genetics*, vol. 155, no. 4, pp. 1505–19, aug 2000.

[135] C. J. Clopper and E. S. Pearson, "The use of confidence or fiducial limits illustrated in the case of the binomial," *Biometrika*, vol. 26, no. 4, pp. 404–413, 1934. [Online]. Available: http://www.jstor.org/stable/2331986

[136] A. Eiben and J. Smith, *Introduction to Evolutionary Computing*, ser. Natural Computing Series. Springer Berlin Heidelberg, 2015.

[137] M. Lutters and N. B. Vogt-Ferrier, "Antibiotic duration for treating uncomplicated, symptomatic lower urinary tract infections in elderly women," *Cochrane Database of Systematic Reviews*, no. 3, 2008. [Online]. Available: https://doi.org//10.1002/14651858.CD001535.pub2

[138] A. Plummer, M. Wildman, and T. Gleeson, "Duration of intravenous antibiotic therapy in people with cystic fibrosis," *Cochrane Database of Systematic Reviews*, no. 9, 2016. [Online]. Available: https://doi.org//10.1002/14651858.CD006682.pub5

[139] Y. Yuan, A. C. Ford, K. J. Khan, J. P. Gisbert, D. Forman, G. I. Leontiadis, F. Tse, X. Calvet, C. Fallone, L. Fischbach, G. Oderda, F. Bazzoli, and P. Moayyedi, "Optimum duration of regimens for helicobacter pylori eradication," *Cochrane Database of Systematic Reviews*, no. 12, 2013. [Online]. Available: https://doi.org//10.1002/14651858.CD008337.pub2

[140] C. M. Parry, V. A. Ho, L. T. Phuong, P. Van Be Bay, M. N. Lanh, L. T. Tung, N. T. H. Tham, J. Wain, T. T. Hien, and J. J. Farrar, "Randomized controlled comparison of ofloxacin, azithromycin, and an ofloxacin-azithromycin combination for treatment of multidrug-resistant and nalidixic acid-resistant typhoid fever," *Antimicrob. Agents Chemother.*, vol. 51, no. 3, pp. 819–825, 2007.

[141] S. Briggs, R. Ellis-Pegler, S. Roberts, M. Thomas, and A. Woodhouse, "Short course intravenous benzylpenicillin treatment of adults with meningococcal disease," *Intern. Med. J.*, vol. 34, no. 7, pp. 383–387, 2004.

[142] C. McKenzie, "Antibiotic dosing in critical illness," *J. Antimicrob. Chemother.*, vol. 66, no. SUPPL. 2, pp. 25–31, 2011.

[143] G. McFarland, Lynne; Surawicz, Christina; Elmer, "Breaking the cycle: strategies for 163 cases of recurrent Clostridium difficile," *Am. J. Gastroenterol.*, vol. 97, no. 7, p. S157, 2002.

[144] L. V. McFarland, "Alternative treatments for Clostridium difficile disease: What really works?" *J. Med. Microbiol.*, vol. 54, no. 2, pp. 101–111, 2005.

[145] S. Kim, T. D. Lieberman, and R. Kishony, "Alternating antibiotic treatments constrain evolutionary paths to multidrug resistance," *Proc. Natl. Acad. Sci.*, vol. 111, no. 40, pp. 14 494–14 499, 2014.

[146] M. M. Butler, S. L. Waidyarachchi, K. L. Connolly, A. E. Jerse, W. Chai, R. E. Lee, S. A. Kohlhoff, D. L. Shinabarger, and T. L. Bowlin, "Aminomethyl Spectinomycins as Therapeutics for Drug-Resistant Gonorrhea and Chlamydia Coinfections," *Antimicrob. Agents Chemother.*, vol. 62, no. 5, pp. 1–10, 2018.

[147] P. M. Hawkey, R. E. Warren, D. M. Livermore, C. A. McNulty, D. A. Enoch, J. A. Otter, and A. P. R. Wilson, "Treatment of infections caused by multidrug-resistant gram-negative bacteria: Report of the British society for antimicrobial chemotherapy/healthcare infection society/british infection association joint working party," *J. Antimicrob. Chemother.*, vol. 73, no. March 2015, pp. iii2–iii78, 2018.

[148] A. Pop-Vicas and S. M. Opal, "The clinical impact of multidrug-resistant gram-negative bacilli in the management of septic shock," *Virulence*, vol. 5, no. 1, pp. 189–195, 2014.

[149] M. Y. Ina-Salwany, N. Al-saari, A. Mohamad, F. A. Mursidi, A. Mohd-Aris, M. N. Amal, H. Kasai, S. Mino, T. Sawabe, and M. Zamri-Saad, "Vibriosis in Fish: A Review on Disease Development and Prevention," *J. Aquat. Anim. Health*, vol. 31, no. 1, pp. 3–22, 2019.

[150] S. C. Soumya Haldar, "Vibrio Related Diseases in Aquaculture and Development of Rapid and Accurate Identification Methods," *J. Mar. Sci. Res. Dev.*, vol. s1, 2014.

[151] D. J. Colquhoun and A. Lillehaug, *Vaccination against Vibriosis*. John Wiley and Sons, Ltd, 2014, ch. 15, pp. 172–184. [Online]. Available: https://onlinelibrary.wiley.com/doi/abs/10.1002/9781118806913.ch15

[152] C. García-Aljaro, J. Riera-Heredia, and A. R. Blanch, "Antimicrobial resistance and presence of the SXT mobile element in Vibrio spp. isolated from aquaculture facilities," *New Microbiol.*, vol. 37, no. 3, pp. 339–346, 2014.

[153] *Animals (Scientific Procedures) Act*, 1986. [Online]. Available: http://www.legislation.gov.uk/ukpga/1986/14/contents

[154] C. J. Y. Tsai, J. M. S. Loh, and T. Proft, "Galleria mellonella infection models for the study of bacterial diseases and for antimicrobial drug testing," *Virulence*, vol. 7, no. 3, pp. 214–229, 2016. [Online]. Available: http://dx.doi.org/10.1080/21505594.2015.1135289

[155] N. Killiny, "Generous hosts: Why the larvae of greater wax moth, Galleria mellonella is a perfect infectious host model?" *Virulence*, vol. 9, no. 1, pp. 860–865, 2018. [Online]. Available: https://doi.org/10.1080/21505594.2018.1454172

[156] S. McMillan, D. Verner-Jeffreys, J. Weeks, B. Austin, and A. P. Desbois, "Larva of the greater wax moth, Galleria mellonella, is a suitable alternative host for studying virulence of fish pathogenic Vibrio anguillarum," *BMC Microbiol.*, vol. 15, no. 1, pp. 1–10, 2015. [Online]. Available: http://dx.doi.org/10.1186/s12866-015-0466-9

[157] M. Wierup, "The control of microbial diseases in animals: Alternatives to the use of antibiotics," *Int. J. Antimicrob. Agents*, vol. 14, no. 4, pp. 315–319, 2000.

[158] K. L. Tang, N. P. Caffrey, D. B. Nóbrega, S. C. Cork, P. E. Ronksley, H. W. Barkema, A. J. Polachek, H. Ganshorn, N. Sharma, J. D. Kellner, and W. A. Ghali, "Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis," *Lancet Planet. Heal.*, vol. 1, no. 8, pp. e316–e327, 2017.

[159] M. Tamminen, A. Karkman, A. Lõhmus, W. I. Muziasari, H. Takasu, S. Wada, S. Suzuki, and M. Virta, "Tetracycline resistance genes persist at aquaculture farms in the absence of selection pressure," *Environ. Sci. Technol.*, vol. 45, no. 2, pp. 386–391, 2011.

[160] D. T. Gillespie, "Approximate accelerated stochastic simulation of chemically reacting systems," *The Journal of Chemical Physics*, vol. 115, no. 4, pp. 1716–1733, 2001.

[161] ECDC, *Proposals for EU guidelines on the prudent use of antimicrobials in humans*. European Centre for Disease Prevention and Control, 2017.

[162] WHO, *Global Action Plan on Antimicrobial Resistance*. World Health Organisation, 2015.

[163] U. K. Government, "Tackling antibmicrobial resistance 2019-2014," 2019.

[164] N. T. Do, N. T. Ta, N. T. Tran, H. M. Than, B. T. Vu, L. B. Hoang, H. R. van Doorn, D. T. Vu, J. W. Cals, A. Chandna, Y. Lubell, B. Nadjm, G. Thwaites, M. Wolbers, K. V. Nguyen, and H. F. Wertheim, "Point-of-care C-reactive protein testing to reduce inappropriate use of antibiotics for non-severe acute respiratory infections in Vietnamese primary health

care: a randomised controlled trial," *Lancet Glob. Heal.*, vol. 4, no. 9, pp. e633–e641, 2016.

[165] T. E. Sweeney, H. R. Wong, and P. Khatri, "Robust classification of bacterial and viral infections via integrated host gene expression diagnostics," *Sci. Transl. Med.*, vol. 8, no. 346, pp. 1–25, 2016.

[166] S. Bhattacharya, A. F. Rosenberg, D. R. Peterson, K. Grzesik, A. M. Baran, J. M. Ashton, S. R. Gill, A. M. Corbett, J. Holden-Wiltse, D. J. Topham, E. E. Walsh, T. J. Mariani, and A. R. Falsey, "Transcriptomic Biomarkers to Discriminate Bacterial from Nonbacterial Infection in Adults Hospitalized with Respiratory Illness," *Sci. Rep.*, vol. 7, no. 1, pp. 1–12, 2017. [Online]. Available: http://dx.doi.org/10.1038/s41598-017-06738-3

[167] A. Machen, T. Drake, and Y. F. Wang, "Same day identification and full panel antimicrobial susceptibility testing of bacteria from positive blood culture bottles made possible by a combined lysis-filtration method with MALDI-TOF VITEK mass spectrometry and the VITEK2 system," *PLoS One*, vol. 9, no. 2, pp. 7–11, 2014.

[168] Y. Charretier, O. Dauwalder, C. Franceschi, E. Degout-Charmette, G. Zambardi, T. Cecchini, C. Bardet, X. Lacoux, P. Dufour, L. Veron, H. Rostaing, V. Lanet, T. Fortin, C. Beaulieu, N. Perrot, D. Dechaume, S. Pons, V. Girard, A. Salvador, G. Durand, F. Mallard, A. Theretz, P. Broyer, S. Chatellier, G. Gervasi, M. Van Nuenen, C. Ann Roitsch, A. Van Belkum, J. Lemoine, F. Vandenesch, and J. P. Charrier, "Rapid bacterial identification, resistance, virulence and type profiling using selected reaction monitoring mass spectrometry," *Sci. Rep.*, vol. 5, no. August, pp. 1–11, 2015. [Online]. Available: http://dx.doi.org/10.1038/srep13944

[169] F. Jalali, F. Ellett, and D. Irimia, "Rapid antibiotic sensitivity testing in microwell arrays," *Technology*, vol. 05, no. 02, pp. 107–114, 2017.

[170] M. G. Tauber, S. Kunz, O. Zak, and M. A. Sande, "Influence of antibiotic dose, dosing interval, and duration of therapy on outcome in experimental pneumococcal meningitis in rabbits," *Antimicrob. Agents Chemother.*, vol. 33, no. 4, pp. 418–423, 1989.

[171] M. S. Stankowicz, J. Ibrahim, and D. L. Brown, "Once-daily aminoglycoside dosing: An update on current literature," *Am. J. Heal. Pharm.*, vol. 72, no. 16, pp. 1357–1364, 2015.

[172] E. Tamayo, J. Gualis, S. Flórez, J. Castrodeza, J. M. Eiros Bouza, and F. J. Álvarez, "Comparative study of single-dose and 24-hour multiple-dose antibiotic prophylaxis for cardiac surgery," *J. Thorac. Cardiovasc. Surg.*, vol. 136, no. 6, pp. 1522–1527, 2008.

[173] T. Gouliouris, B. Warne, E. J. P. Cartwright, L. Bedford, C. K. Weerasuriya, K. E. Raven, N. M. Brown, M. E. Török, D. Limmathurotsakul, and S. J. Peacock, "Duration of exposure to multiple antibiotics is associated with increased risk of VRE bacteraemia: a nested case-control study," *Journal of Antimicrobial Chemotherapy*, vol. 73, no. 6, pp. 1692–1699, 03 2018. [Online]. Available: https://doi.org/10.1093/jac/dky075

[174] P. Kardas, "Patient compliance with antibiotic treatment for respiratory tract infections," *J. Antimicrob. Chemother.*, vol. 49, no. 6, pp. 897–903, 2002.

[175] M. P Alvarez, M. Simón, S. Sánchez, I. Apaloaza, and J. Prieto, "Pharmacovigilance study of azithromycin tablets (500 mg) in the treatment of adult patients with respiratory tract infections," *Revista española de quimioterapia : publicación oficial de la Sociedad Española de Quimioterapia*, vol. 13, pp. 297–305, 10 2000.

[176] P. Kardas and E. Ratajczyk-Pakalska, "Patient adherence in respiratory tract infections: ceftibuten versus other antibiotics (particular study)," *Polski merkuriusz lekarski : organ Polskiego Towarzystwa Lekarskiego*, vol. 10, pp. 445–9, 07 2001.

[177] E. M. Brown and D. Nathwani, "Antibiotic cycling or rotation: a systematic review of the evidence of efficacy," *Journal of Antimicrobial Chemotherapy*, vol. 55, no. 1, pp. 6–9, 01 2005. [Online]. Available: https://doi.org/10.1093/jac/dkh482

[178] P. J. van Duijn and M. J. Bonten, "Antibiotic rotation strategies to reduce antimicrobial resistance in gram-negative bacteria in european intensive care units: study protocol for a cluster-randomized crossover controlled trial," *Trials*, vol. 15, no. 1, p. 277, Jul 2014. [Online]. Available: https://doi.org/10.1186/1745-6215-15-277

[179] F. Siavoshi, P. Saniee, S. Latifi-Navid, S. Massarrat, and A. Sheykholeslami, "Increase in Resistance Rates of H.pylori Isolates to Metronidazole and Tetracycline - Comparison of Three 3-Year Studies," *Arch. Iran. Med.*, vol. 13, no. 3, 2010.

[180] C. Llor and L. Bjerrum, "Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem," *Therapeutic Advances in Drug Safety*, vol. 5, no. 6, pp. 229–241, 2014, pMID: 25436105.

[181] L. J. Shallcross and D. S. C. Davies, "Antibiotic overuse: a key driver of antimicrobial resistance," *British Journal of General Practice*, vol. 64, no. 629, pp. 604–605, 2014. [Online]. Available: https://bjgp.org/content/64/629/604

[182] S. C. Li, "Factors affecting therapeutic compliance: A review from the patient's perspective," *Therapeutics and Clinical Risk Management*, vol. Volume 4, pp. 269–286, 02 2008.

[183] H. Dabernat, P. Geslin, F. Megraud, P. BÃl'guÃl', J. Boulesteix, C. Dubreuil, F. de La Roque, A. Trinh, and A. Scheimberg, "Effects of cefixime or co-amoxiclav treatment on nasopharyngeal carriage of Streptococcus pneumoniae and Haemophilus influenzae in children with acute otitis media." *Journal of Antimicrobial Chemotherapy*, vol. 41, no. 2, pp. 253–258, 02 1998.

[184] R. Dagan, K. P. Klugman, W. A. Craig, and F. Baquero, "Evidence to support the rationale that bacterial eradication in respiratory tract infection is an important aim of antimicrobial therapy," *Journal of Antimicrobial Chemotherapy*, vol. 47, no. 2, pp. 129–140, 02 2001.

[185] M. Imran and H. Smith, "The dynamics of bacterial infection, innate immune response, and antibiotic treatment," *Discrete and Continuous Dynamical Systems. Series B*, vol. 1, 07 2007.

[186] A. Handel, E. Margolis, and B. R. Levin, "Exploring the role of the immune response in preventing antibiotic resistance," *Journal of Theoretical Biology*, vol. 256, no. 4, pp. 655 – 662, 2009.

Part I

APPENDICES

APPENDIX A

A.1 MATLAB CODE

This appendix provides the code executed in 'MATLAB' which has been referenced throughout the main body of this thesis.

A.1.1 *Gillespie Algorithm*

```
clear all;
runs=5000; %Number of runs
tf=20; %End Time
TimetoDeath=[];
Cured=0; %Initial co-exist
EndPop=[];

  %parameter values
r = 2.7726; %Reproduction rate of Susceptible
K = 1000; %Carrying Capacity
ms = 0.2; %Mortality Rate of Susceptibles
Bmax= r-ms; %Max net growth rate in absence of AB for susceptibles
Amax= 4.67; %Min net growth rate at high AB concentrations for susceptibles
MICS= 16; %Pharmocodynamic MIC for Suceptible
kS= 4; %Hill Coefficient
```

```
a = 0.48; %Degradation rate of AB

   tint=[0,1,2,3,4,5,6,tf]; %Time AB applied (vector)
cint=[0 29 14 17 10 7 0]; %Concentration of AB applied (vector)

   for n=1:runs %For each run do the following...
%Initial Conditions
S1 = 1000; %Strain 1 least resistant
C = 0; %Concentration of AB
time=0; %Start Time

   for i=1:(length(tint)-1)
if S1<1
break
end
C=C+cint(i);
C0=C;
timeAB=time;
while time<=tint(i+1)
rate = zeros(3,1);
rate(1,:) = r*S1*(1-((S1)/K)); %Strain 1 reproduction rate
rate(2,:) = ms*S1+((Amax)*((C/MICS)^kS))/(((C/MICS)^kS)+((Amax/Bmax)-
1))*S1; %Strain 1 death rate incl AB death
rate(3,:) = rate(1,:)+rate(2,:); %Total rates

   x1=rand; %Generate random number between 0 and 1
```

```
   %Events
if x1<= rate(1,:)/rate(3,:)
S1=S1+1; %Strain 1 reproduces
elseif x1<= (rate(1,:)+rate(2,:))/rate(3,:)
S1=S1-1; %Strain 1 dies naturally and AB
end

   time=time-log(rand)/rate(3); %Generate time step for event
C = Co*exp(-a*(time-timeAB)); %Concentration of AB

   if S1<1
TimetoDeath=[TimetoDeath;time];
Cured=Cured+1;
break
end
end
end
end
if S1>0
EndPop=[EndPop;S1];
end
end
   disp(Cured/runs);
```

A.1.2 *Genetic Algorithm*

```
%% GA for Integer representation.
% Use of the Genetic Algorithm Function 'ga' from the Matlab Global Optim-
ization toolbox.
```

```
% The ga function allows us to constrain values to be integers

   OptDosage=[];
ObjFunVal=[];
for i=200:4:400
disp(i); %indicates iteration number
%% Number of Variables
nvars = 6;
%% Bounds for the treatment cycles
LB = [ 0 0 0 0 0 0 ]; % Setting the bounds. They can be different
UB = [ 70 70 70 70 70 70 ]; % for every variable

   %% Constrain All Variables to be Integers
intCon = 1:nvars;

   %% Set GA parameters
   options = gaoptimset('CrossoverFrac',0.7,'PopulationSize',50,'Generations',100,
'InitialPopulation',[17.5 17.5 17.5 17.5 0 0 ],'PlotFcns',@gaplotbestfalt);

   rng(i,'twister') % for reproducibility. Fist parameter is the random seed

   [xOpt,fVal] = ga(@(x)FitnessFunction(x),nvars,[],[],[],[],LB,UB,[],intCon,options);

   disp('Integer Solution Returned by GA:')
disp(xOpt)
disp('Value of Objective Function:')
disp(fVal)
OptDosage=[OptDosage; xOpt];
```

```
ObjFunVal=[ObjFunVal; fVal];
end
```

A.1.2.1 *Genetic Algorithm Fitness Function*

```
function F = FitnessFunction(x)

   if sum(x)>70
F=10^5;
else
maxconc = 40;
runs=300; %Number of runs
tf=20; %End Time
v_t0t=sum(x);
maxAB=70;

   %parameter values
rs = 2.7726; %Reproduction rate of Susceptible
K = 1000; %Carrying Capacity
ms = 0.2; %Mortality Rate of Susceptibles
Bmax= rs-ms; %Max net growth rate in absence of AB for susceptibles
Amax= 4.6; %Min net growth rate at high AB concentrations for susceptibles
MICS= 16; %Pharmocodynamic MIC for Suceptible
kS= 4; %Hill Coefficient
a = 0.48; %Degradation rate of AB

   tint=[0,1,2,3,4,5,6,tf]; %Time AB applied (vector)
cint=[0,x(1),x(2),x(3),x(4),x(5),x(6)]; %Concentration of AB applied (vector)
```

```
   %%%Deterministic for Concentration

   P=6;
yo(1)=0;
Conc=[]; TimeFull=[];
for j=1:P,
[t, y] = ode45('ode', [j − 1j], y0, []);

   Conc=[Conc;y(:,1)];
TimeFull=[TimeFull;t(:)];
tlen=length(y(:,1));
yo(1)=y(tlen,1)+x(j);
end

   [t,y]=ode45('ode',[P tf],yo,[]);

   Conc=[Conc;y(:,1)];%Conc
TimeFull=[TimeFull;t(:)];

   if any(Conc>maxconc)
F=10^5;
else

   %%%Stochastic
Cured=0; %Initial co-exist

   for n=1:runs %For each run do the following...
```

```
   %Initial Conditions
S = 1000; %Susceptible
C = 0; %Concentration of AB
time=0; %Start Time

   for j=1:(length(tint)-1)
if S<1
break
end
C=C+cint(j);
C0=C;
timeAB=time;
while time<=tint(j+1)
%Rates
rate = zeros(3,1);
rate(1,:) = rs*S*(1-((S)/K)); %Susceptible reproduction rate
rate(2,:) = ms*S + ((Amax)*((C/MICS)^kS))/(((C/MICS)^kS)+((Amax/Bmax)-
1))*S; %Susceptible death rate incl AB
rate(3,:) = rate(1,:)+rate(2,:); %Total rates

   x1=rand; %Generate random number between 0 and 1

   %Events
if x1<= rate(1,:)/rate(3,:)
S=S+1; %Susceptible reproduces
elseif x1<= (rate(1,:)+rate(2,:))/rate(3,:)
S=S-1; %Susceptible dies
```

```
end

   time=time-log(rand)/rate(3); %Generate time step for event
C = Co*exp(-a*(time-timeAB)); %Concentration of AB

   if S<1
Cured=Cured+1;
break
end
end
end
end

   Curedpen=runs-Cured;

   %%%Fitness Functions
w = 0.269;

   F = w*(vtot/maxAB) + (1-w)*(Curedpen/runs);

   end
end
```

A.1.2.2 *Ordinary Differential Equation for Calculating Concentration*

```
function yd=ode(t,y,flag,a);
%parameter values
a = 0.48;
```

```
yd = zeros(1,1);
yd(1)=-a*y(1);
```

A.1.3 *Tau Leaping Method*

```
clear all;
runs=5000; %Number of runs
tf=168; %End Time
TimetoCure=[];
TimetoDeath=[];
Cured=0; %Initial co-exist
Dead=0;
EndPop=[];
step = 0.25; %Length of tau leap
deathload = 10^11.5;
immunity = 10^2;

   tint = [0 2 24 48 tf]; %Time AB applied
cint = [0 0.340 0.135 0.025];

   for n=1:runs %For each run do the following...
S = 1.28 * 10^7; C = 0; time=0; %Initial Conditions

   %parameter values rr = 0.35;
r = 0.35+0.07*randn(1); %Reproduction rate
m = 0.06; %Mortality Rate
K = 10^13; %Carrying Capacity
```

```
a = 0.05; %Degradation rate of AB
max= (rr-m)+0.05*randn(1); %Max net growth rate in absence of AB for susceptibles
min= -0.54; %Min net growth rate at high AB concentrations for susceptibles
mic= 0.1; %Pharmocodynamic MIC for Suceptible
k= 3.25; %Hill Coefficient

   for j=1:(length(tint)-1)
if S<immunity || S>deathload
break
end
C=C+cint(j);
C0=C;
timeAB=time;
while time<=tint(j+1)
%Rates
rate = zeros(3,1);
rate(1,:) = r*S*(1-((S)/K))*step; %Reproduction rate
rate(2,:) = m*S*step; %Death rate
rate(3,:) = (max-min)*C^k/(C^k-min*mic^k/max)*S*step; %Susceptible AB death rate

   if rate(1,:)<0
rate(1,:)=0;
end
```

```
% calculate the number of events that have occured over the time step:
randomly chosen from Poisson Distribution

numSbirths = poissrnd(rate(1,:)); %susceptible births
numSdeaths = poissrnd(rate(2,:)); %susceptible natural deaths
numABSdeaths = poissrnd(rate(3,:)); %susceptible AB deaths

S = S + numSbirths - numSdeaths - numABSdeaths;
C = Co*exp(-a*(time-timeAB)); %Concentration of AB
time = time+step;

if S<immunity || S>deathload
break
end
end
end

if S<immunity
Cured=Cured+1; %Extinction probabiltiy for Infection
TimetoCure=[TimetoCure;time];
elseif S>deathload
Dead=Dead+1; %Extinction probabiltiy for Host
TimetoDeath=[TimetoDeath;time];
%%StoreD(n,1)=time; %Time to death
end
end

disp(Cured/runs);
```