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Bacterial fitness shapes the population dynamics of antibiotic-resistant and -susceptible bacteria in a model of combined antibiotic and anti-virulence treatment

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HIGHLIGHTS

- Combined antibiotic & anti-virulence drugs can clear antibiotic-resistant infections.
- Delay between delivery of drug types can render the treatment successful.
- Fitness cost associated with antibiotic-resistance shapes the optimal treatment.

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ABSTRACT

Bacterial resistance to antibiotic treatment is a huge concern: introduction of any new antibiotic is shortly followed by the emergence of resistant bacterial isolates in the clinic. This issue is compounded by a severe lack of new antibiotics reaching the market. The significant rise in clinical resistance to antibiotics is especially problematic in nosocomial infections, where already vulnerable patients may fail to respond to treatment, causing even greater health concern. A recent focus has been on the development of anti-virulence drugs as a second line of defence in the treatment of antibiotic-resistant infections. This treatment, which weakens bacteria by reducing their virulence rather than killing them, should allow infections to be cleared through the body's natural defence mechanisms. In this way there should be little to no selective pressure exerted on the organism and, as such, a predominantly resistant population should be less likely to emerge. However, before the likelihood of resistance to these novel drugs emerging can be predicted, we must first establish whether such drugs can actually be effective. Many believe that anti-virulence drugs would not be powerful enough to clear existing infections, restricting their potential application to prophylaxis. We have developed a mathematical model that provides a theoretical framework to reveal the circumstances under which anti-virulence drugs may or may not be successful. We demonstrate that by harnessing and combining the advantages of antibiotics with those provided by anti-virulence drugs, given infection-specific parameters, it is possible to identify treatment strategies that would efficiently clear bacterial infections, while preventing the emergence of antibiotic-resistant subpopulations. Our findings strongly support the continuation of research into anti-virulence drugs and demonstrate that their applicability may reach beyond infection prevention.

1. Introduction

Bacterial resistance to antibiotic agents is an increasing problem in modern society. The introduction of every new class of antibiotic (from the original β-lactam, penicillin, to the more recent lipopeptides such as daptomycin) has been followed by the emergence of new strains of bacteria resistant to that class, many emerging in the clinic only a few years after the introduction of the drug (Butler and Buss, 2006; Clatworthy et al., 2007; Lewis, 2013). Given that the pace of antibiotic discovery has dramatically slowed down (most classes of antibiotic were discovered in the 1940s to the 1960s, the 'Golden Era' of antibiotics, with the past 40 years giving us only five significant new classes (Butler and Buss, 2006; Lewis, 2013) and pharmaceutical companies devoting less research into discovering

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new antibiotics (Projan and Shlaes, 2004; Mellbye and Schuster, 2011), this poses a huge problem. Development of novel treatments for bacterial infections is of utmost importance.

Traditional antibiotics are classed either as bactericidal or bacteriostatic, working to kill bacteria or inhibit bacterial growth respectively (Clatworthy et al., 2007; Mellbye and Schuster, 2011). While effective in eliminating susceptible bacterial infections, antimicrobials impose selective pressure on the bacteria, leading to the rise of resistant clones within the bacterial population. Resistance can be acquired either through spontaneous chromosomal mutation and then selection by antibiotic use, known as vertical evolution, or through acquiring genetic material from other resistant organisms, known as horizontal evolution (Tenover, 2006). Horizontal evolution occurs via mechanisms such as conjugation, transformation and transduction (Alanis, 2005) and can take place between strains of the same or different bacterial species. Horizontal evolution can lead to multidrug resistance and is a major concern in hospitals where resistant bacteria are able to remain viable despite antibiotic use and are the cause of many serious nosocomial infections in already vulnerable patients (Alanis, 2005; Tenover, 2006).

It has been suggested that the focus of new drug development should be on targeting virulence, the bacteria's ability to cause disease (Clatworthy et al., 2007), but this approach remains controversial both in terms of its potential efficacy and its ability to counter microbial resistance. Anti-virulence drugs would minimise any harm caused by bacteria while they remain in the host until they can be cleared by natural defences. This can occur either by being flushed out of the system or by being eradicated by the host's immune response. This *should* exert little to no selective pressure on the bacteria but this has not yet been proved *in vivo*. Anti-virulence drugs could target a range of mechanisms, including bacterial adhesion to host cells, toxin delivery or virulence gene regulation, all necessary for successful infection (Clatworthy et al., 2007; Mellbye and Schuster, 2011) and many of these are under investigation. In the vast majority of cases these approaches have proved to attenuate but not clear infection and often only when used in infection-prevention (as opposed to post-infection treatment), see for example Hentzer et al. (2003), Rasmussen et al. (2005), Wright et al. (2005), Jakobsen et al. (2012), Curtis et al. (2014), Hraiech et al. (2014), Sully et al. (2014) (quorum sensing and signalling inhibitors), Felise et al. (2008) (secretion inhibitor), Krachler et al. (2012) (adhesion inhibitor), Liu et al. (2008) (direct modulator of the bacteria's ability to suppress an immune response) and Hung et al. (2005) (colonisation inhibitor).

We adopt a modelling approach to investigate the viability of anti-virulence drugs *in silico*. Our results suggest that, in combination with antibiotics and under specific treatment strategies, anti-virulence drugs can be effective in clearing bacterial infections where antibiotic resistance is a concern. Optimal treatment strategies are likely to be specific to the patient, infection and bacterial strain and (rather than attempt to pin-point exact strategies) we use this theoretical framework as a "proof of concept", exploiting parameter surveys to investigate a range of scenarios, highlighting the potential and the need for targeting infections with tailored treatments in the future. Given that every patient, infection and strain of bacteria are different, it is impossible to obtain a conclusive set of parameters which will suit all situations. We therefore exploit parameter surveys to ascertain under what conditions certain behaviour will occur.

This work lays the foundations for more detailed models incorporating mechanisms of specific types of anti-virulence drugs. This will facilitate testing of the likelihood that microbial resistance to these novel drugs could emerge. We address this briefly in Section 4 but leave this largely to further work due to the vast array of targets and resistance mechanisms that can be associated with anti-virulence drugs. Unless explicitly stated otherwise therefore, the description 'resistant' refers only to antibiotic-resistance in this work.

2. Materials and methods

2.1. Model formulation

There are two main approaches usually taken when modelling the emergence of antibiotic-resistant bacteria: within-host models, or within-hospital compartmental models. Models of hospital resistance usually follow a similar form to the classic "Susceptible–Infectious–Resistant" (SIR) models of epidemiology, looking specifically at how nosocomial infections will spread throughout the hospital, for example Austin and Anderson (1999), Lipsitch et al. (2000), and Webb et al. (2005). While such models are useful to develop strategies to prevent the spread of resistance, our focus is on treatment strategies to eliminate the emergence of an antibiotic-resistant subpopulation that has either arisen through random mutation and clonal expansion or through cross-contamination within a particular infection and under a specific treatment regimen. If the resistant bacteria within a single host can be eliminated then the spread of resistance throughout the hospital is a less pressing concern.

Existing mathematical models that focus on the within-host emergence of antibiotic-resistance examine how treatment strategies can both cause and be adapted to prevent the emergence of antibiotic resistance, for example Lipsitch and Levin (1997) and D'Agata et al. (2008). Such models often neglect the effect of the host immune response, assuming it to be negligible or constant under the effect of the antibiotic. Since the efficacy of anti-virulence drugs will depend at least in part on the host's innate immunity, we include cell-mediated innate immune response in the model.

The system consists of five interacting components: populations of antibiotic-susceptible bacteria (S), antibiotic-resistant bacteria (R) and immune cells e.g. phagocytes (P), and concentrations of antibiotic (A) and anti-virulence drug (A^*). These interact as demonstrated in Fig. 1 and represent a generalised model of a local bacterial infection, such as a urinary tract or wound infection.

The growth of bacteria within a host is likely to saturate over time as a result of space and nutrient limitations. We therefore use a logistic growth term with baseline growth rate η_i, $(i=S,R)$ and a combined carrying capacity K, rather than simple exponential growth, to represent the bacterial dynamics. We include a removal rate, ψ, representing the body's endogenous, physical clearance mechanisms (this can be expected to vary depending upon infection type).

Due to the potential for multidrug resistance (Tenover, 2006), the primary cause for concern in hospitals is resistance due to horizontal evolution: acquiring new genetic material from other resistant organisms. Horizontal evolution involves the transfer of the resistance gene, normally found in sections of DNA known as transposons, from one plasmid to another. This takes place via one of three main mechanisms: conjugation, transformation or transduction (Alanis, 2005; Tenover, 2006). We focus on the most common of the three transmission mechanisms conjugation (Alanis, 2005), whereby one bacterium transfers plasmid containing the genes for resistance to an adjacent bacterium. Research suggests that these plasmid-bearing, and thus antibiotic-resistant, bacteria are subject to a fitness cost, c, lowering their growth rate (Levin et al., 1997; Austin and Anderson, 1999; Bergstrom et al., 2000; Lipsitch et al., 2000; Lipsitch, 2001), hence we choose the growth rate $\eta_R=(1-c)\eta_S$ where $0<c<1$. Since this plasmid transfer occurs between adjacent bacteria, and we assume a well mixed population, we represent this interaction through mass action kinetics with a conjugation rate, λ, proportional to the levels of both antibiotic-susceptible and -resistant bacteria in the population (Bergstrom et al., 2000; Webb et al., 2005; Imran and Smith, 2006; D'Agata et al., 2008). It has also been observed that it is possible to lose the plasmid carrying the resistance genes (Webb et al., 2005; Imran and Smith, 2006) and so this too is incorporated into the model via a constant reversion rate, ρ.