Treating symptomatic infections and the co-evolution of virulence and drug resistance

Samuel Alizon

Laboratoire MIVEGEC (UMR CNRS 5290, IRD 224, UM), Montpellier, France
samuel.alizon@cnrs.fr

Abstract

Antimicrobial therapeutic treatments are by definition applied after the onset of symptoms, which tend to correlate with infection severity. I use mathematical epidemiology models to explore how this link affects the coevolutionary dynamics between the virulence of an infection, measured via host mortality rate, and its susceptibility to chemotherapy. I show that unless resistance pre-exists in the population, drug resistant infections are initially more virulent than drug sensitive ones. As the epidemics unfolds, virulence is more counter-selected in drug-sensitive than in drug-resistant infections. This difference decreases over time and, eventually, the exact shape of genetic trade-offs govern long-term evolutionary dynamics. These results highlight the importance of the feedbacks between epidemiology, public health policies and parasite evolution, and have implications for the monitoring of virulence evolution.

Keywords: epidemiology, evolution, antimicrobial resistance, trade-offs, public health, virulence

1 Introduction

Chemotherapies represent a huge selective force for microbes. It is now commonly accepted that the administration of these treatments always eventually selects for resistant strains (Read et al., 2011). However, drug resistance is not the only infection life-history trait that may evolve, and mathematical (Gandon and Michalakis, 2000, Gandon et al., 2001) and some animal (Schneider et al., 2012, Read et al., 2015) models support the idea that treatments can affect the evolution of virulence, defined here as the increase in host mortality due to the infection.

In epidemiology, the coevolution of drug resistance and virulence is rarely studied. The problem may appear simplistic at first because drug-resistant infections being more difficult to treat, their virulence is likely is higher than drug-sensitive ones in treated hosts. But coevolution can be less straightforward. For instance, as documented for several bacterial species, there can be direct genetic associations between the two traits due to genes with pleiotropic action (for reviews, see Beceiro et al., 2013, Guillard et al., 2016). Note that in these studies, as well as in the existing literature on antibiotic resitance and virulence (e.g. Van Tyne and Gilmore, 2014, Alcalde-Rico et al., 2016, Giraud et al., 2017), the latter trait is usually binary rather than continuous. A final link these two traits, which is the focus of this study, is created by public health policies since less virulent strains are less likely to be treated than more virulent ones. In fact, the delay between the onset of the symptoms and the incubation period is known to largely determine our ability to control an outbreak (Fraser et al., 2004).

Classical theory predicts that if virulence is adaptive for the parasite, defined here to encompass both micro- and macro-parasites, host qualitative resistance selects for increased levels of virulence (Gandon and Michalakis, 2000). If host resistance is quantitative, which would better correspond to efficient drug treatments,

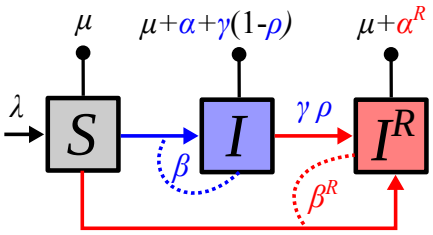

Figure 1 – **Epidemiological model flow diagram.** Drug-sensitive infections are in blue and drug-resistant infections are in red. The latter can be generated through treatment failure or direct transmission. Dashed lines show infection events, arrows transition between states and lines with a circle death events. Parameter notations are detailed in the main text.

virulence is not affected in simple models (Alizon and van Baalen, 2005). However, if multiple infections are allowed in the system, their prevalence decreases, potentially affecting virulence evolution (Gandon et al., 2001). Although the long term effect of host resistance (or prophylactic treatments) on virulence evolution may be equivalent to therapeutic treatments in simple settings, on the short term however, the fact that only a fraction of hosts are treated based on infection life history traits may have affect evolutionary dynamics.

Here, I adopt an evolutionary epidemiology standpoint and introduce strain variation in virulence as well variation in infection drug resistance. A key assumption is that the rate at which an infected host receives treatment correlates with infection virulence. The hypothesis tested here is that this correlation can generate coevolutionary dynamics between virulence and drug resistance on the short and on the long term.

2 Model and Methods

2.1 The SI model

I assume a classical Susceptible-Infected model (Keeling and Rohani, 2008) in which infections caused by a strain i are either drug sensitive or drug resistant (Figure 1). Let us denote by S the density of susceptible hosts, by I_i that of drug-sensitive infections by strain i and by I_i^R that of drug-resistant infections by strain i. Infection traits are assumed to be 'heritable' such that the infection of a susceptible host by a drug-sensitive infection by strain i (at a rate β_i) yields a drug-sensitive infection by strain i. New drug resistant infections appear in two ways: either through the infection of a susceptible host by a drug resistant host (at a rate β_i^R), or following the treatment failure of a drug sensitive host. The latter event occurs at a rate $\rho_i\gamma_i$, where γ_i is the treatment rate and ρ_i is the proportion of drug failure. For simplicity, hosts are assumed not to recover from drug resistant infections. Finally, new hosts enter the system at a density-independent rate λ and die with a natural mortality rate μ. Infected hosts experience an additional mortality, or virulence, α for drug-sensitive infections and α_R for drug-resistant infections. Note that in the model, virulence and transmission rate can differ in drug-sensitive and drug-resistant infections. Biologically, these trait differences could originate from fitness-costs associated to drug resistance (Andersson and Hughes, 2010).

The dynamics of the system can be captured using the following set of Ordinary Differential Equations (ODEs):

$$\frac{\mathrm{d}S}{\mathrm{d}t} = \lambda - \mu\, S - \left(\beta_i\, I_i + \beta_i^R\, I_i^R\right)\, S \tag{1a}$$

$$\frac{\mathrm{d}I_i}{\mathrm{d}t} = \beta_i\, S\, I_i - \left(\mu + \alpha_i + \gamma_i\right)\, I_i \tag{1b}$$

$$\frac{\mathrm{d}I_i^R}{\mathrm{d}t} = \rho_i\, \gamma_i\, I_i + \beta_i^R\, S\, I_i^R - \left(\mu + \alpha_i^R\right) I_i^R \tag{1c}$$

Variations in infection life history traits are captured in different ways based on the biology of the trait. At one extreme, drug resistance is known to evolve rapidly because one or a few mutations can have large phenotypic effects; as illustrated for instance in the case of *Mycobacterium tuberculosis* (Telenti, 1997), *Plasmodium falciparum* (White, 2004) or influenza virus (Foll et al., 2014). Conversely, quantitative traits such as virulence (α_i) or transmission rate (β_i) tend to evolve on a rougher fitness landscape, as estimated for instance in the case of Human Immunodeficiency Virus (HIV) infections through the virus load proxy (Hinkley et al., 2011). Equation system 1 captures this difference between traits. For α, β, γ and ρ, variations between infections are assumed to depend on genetic differences between strains and to vary slowly. Conversely, drug resistance is assumed to be a more labile trait. A possible biological interpretation is that it reflects the state of the majority of the parasites in an infected host at a given time point. We therefore allow this trait to vary, while the others remain constant; whence the existence of the two classes I_i and I_i^R and the possibility to 'mutate' from the former to the latter (reversions are neglected in this model). Genetic mutations from a strain i to a strain j are neglected because if the mutation kernel is symmetric these terms cancel out (Day and Proulx, 2004).

2.2 Short-term evolutionary dynamics

The dynamics of the total densities of infected hosts (I_T and I_T^R) are governed by the following ODEs:

$$\frac{\mathrm{d}I_T}{\mathrm{d}t} = \overline{\beta}^I\, S\, I_T - \left(\mu + \overline{\alpha}^I + \overline{\gamma}^I\right)\, I_T \tag{2a}$$

$$\frac{\mathrm{d}I_T^R}{\mathrm{d}t} = \overline{\rho\,\gamma}^I\, I_T + \overline{\beta^R}^R\, S\, I_T^R - \left(\mu + \overline{\alpha^R}^R\right)\, I_T^R \tag{2b}$$

where $\overline{x}^I$ (resp. $\overline{x}^R$) is the average trait value of x in the drug sensitive (resp. drug resistant) compartment. Mathematically, $\overline{x}^I = \sum_i p_i x_i$, where $p_i = I_i/I_T$ is the fraction of strain i among all the drug-sensitive infections. Similarly, $\overline{x}^R = \sum_i q_i x_i$, where $q_i = I_i^R/I_T^R$ is the fraction of strain i among all the drug-resistant infections.

Even for this simple SI model, each strain i is characterised by 6 infection traits (α_i, α_i^R,β_i, β_i^R, γ_i and ρ_i). Furthermore, at the population level, the average values of each of these trait can differ in the drug-sensitive and drug-resistant compartments (I and I^R). Finally, note that there is an identifiability issue between the treatment failure ratio (ρ) and the treatment rate (γ) since both are averaged simultaneously in equation 2b.

After some calculations following the Price equation formalism introduced by Day and Proulx (2004) and described in Appendix A, we obtain the following ODEs to capture the dynamics of the average trait value of a trait x in the drug sensitive compartment and a trait y in the drug-resistant compartment:

$$\frac{\mathrm{d}\overline{x}^I}{\mathrm{d}t} = \mathrm{Cov}^I(\beta, x)\; S - \mathrm{Cov}^I(\alpha, x) - \mathrm{Cov}^I(\gamma, x) \tag{3a}$$

$$\frac{\mathrm{d}\overline{y}^R}{\mathrm{d}t} \approx \frac{I_T}{I^R}\; \left(\overline{y}^I - \overline{y}^R\right)\overline{\rho\,\gamma}^I + S\,\mathrm{Cov}^R\left(\beta^R, y\right) - \mathrm{Cov}^R\left(\alpha^R, y\right) \tag{3b}$$

where $\mathrm{Cov}^A(x, y)$ is the genetic covariance between traits x and y measured in compartment A.

2.3 Long-term evolution

System 1 accepts three equilibria for $(\tilde{S}\,,\,\tilde{I}\,,\,\tilde{I}^R)$, which are detailed in Appendix A. The first equilibrium corresponds to a trivial state, where the parasite goes extinct $(\lambda/\mu\;,0,0)$. In the second, resistant infections are only maintained via treatment failure ($\gamma_i\;\rho_i$). Without it, the equilibrium simplifies into $\left(\frac{\mu+\alpha_i+\gamma_i}{\beta_i}, \frac{\lambda}{\mu+\alpha_i+\gamma_i} - \frac{\mu}{\beta_i}, 0\right)$. In the third equilibrium, all infections are drug resistant $\left(\frac{\mu+\alpha_i^R}{\beta_i^R}\;,\;0\;,\;\frac{\lambda}{\mu+\alpha_i^R} - \frac{\mu}{\beta_i^R}\right)$.

By adopting an adaptive dynamics approach, we can investigate the convergence and evolutionary stability

of fixed points (Dieckmann, 2002). As described in Appendix A, the next generation theorem (Diekmann et al., 1990, Driessche and Watmough, 2002, Hurford et al., 2010) tells us that the fate of a rare mutant in a resident population is governed by the largest of the two following eigenvalues

$$\lambda_1 = \frac{\beta^R}{\mu + \alpha_m^R}\,\tilde{S} \quad \text{and} \quad \lambda_2 = \frac{\beta_m}{\mu + \alpha_m + \gamma_m}\,\tilde{S} \tag{4}$$

where $\tilde{S}$ is the equilibrium density of susceptible hosts in the resident population before the apparition of the mutant. This dichotomy originates from the assumption that the mutant is initially rare, making the generation of drug-resistant infections via mutation a second order term that vanishes when evaluating the eigenvalues of the Jacobian matrix. One possible biological interpretation of these two eigenvalues is either the parasite spreads via drug-resistant infections or via drug-sensitive infections.

2.4 Numerical simulations

To validate the analytical predictions, I perform numerical simulations in `R` with $n = 20$ strains the traits of which are drawn at random (the `R` script used with the default parameter values is in Appendix B). These simulations assume a positive covariance between virulence (α_i) and treatment rates (γ_i), therefore considering that more virulent infections are treated more. Following empirical and experimental data (Anderson and May, 1982, Dwyer et al., 1990, Fraser et al., 2007, de Roode et al., 2008, Råberg, 2012, Doumayrou et al., 2012, Williams et al., 2014), transmission rate (β) is assumed to be traded-off against infection duration due to the cost virulence (α) such that

$$\beta_i = \beta_0\ \alpha_i^p \tag{5}$$

where β_0 is a scaling constant. If $0 < p < 1$, theory predicts there is an intermediate evolutionary stable level of virulence (van Baalen and Sabelis, 1995, Alizon and van Baalen, 2005).

This relationship between transmission and virulence can also be imperfect. Practically, this can be captured by assuming the realised transmission rate is assumed to be normally distributed around the value predicted from the trade-off (see Figure S3).

Finally, for simplicity, I assume that $\alpha_i^R = a\alpha_i$ and $\beta_i^R = b\beta_i$, meaning that all strains undergo similar variations in infection traits with the acquisition of drug-resistance. Note that this assumption implies that $\beta_i^R = b\ \beta_0\ (a\ \alpha_i)^p$. Typically, if drug resistance is costly for the parasite, we expect to have $a > 1$ or $0 \leq b < 1$, where a and $1/b$ can be interpreted as fitness costs.

3 Results

3.1 Virulence rapid evolution

We first use the Price equation formalism to get a qualitative understanding of the selective forces acting on infection traits. The short term dynamics of our main trait of interest, virulence in drug-sensitive infections, are

obtained from equation system 3:

$$\frac{\mathrm{d}\overline{\alpha}^I}{\mathrm{d}t} = \overbrace{\mathrm{Cov}^I(\beta,\alpha)\ S}^{\text{trade-off}} - \overbrace{\mathrm{Var}^I(\alpha)}^{\text{virulence cost}} - \overbrace{\mathrm{Cov}^I(\gamma,\alpha)}^{\text{treating symptoms}} \tag{6a}$$

$$\frac{\mathrm{d}\overline{\alpha}^R}{\mathrm{d}t} \approx \underbrace{\frac{I_T}{I^R}\left(\overline{\alpha}^I - \overline{\alpha}^R\right)\overline{\rho\,\gamma}^I}_{\text{treatment failure}} + \underbrace{b\ S\ \mathrm{Cov}^R(\beta,\alpha)}_{\text{trade-off}} - \underbrace{a\ \mathrm{Var}^R(\alpha)}_{\text{virulence cost}} \tag{6b}$$

In drug sensitive infections, virulence evolutionary dynamics are similar to that described for classical SIR systems (Day and Proulx, 2004). The first term in the right hand side of equation 6a indicates that the average virulence increases (i.e. more virulent strains spread more) if there is a positive covariance between transmission rate and virulence, which is also known as the transmission-virulence trade-off hypothesis (Alizon and Michalakis, 2015). This effect is amplified by the availability of susceptible hosts (S), as demonstrated experimentally in a bacteria-phage system (Berngruber et al., 2013). According to the second term, virulence is costly and if there is genetic variation for this trait, more virulent strains are counter-selected; a phenomenon introduced more than a century ago by Theobald Smith as the 'law of declining virulence' (Méthot, 2012).

The third term in equation 6a deserves some explanations because it involves the rate at which infections are treated (γ_i). In most epidemiological models, this rate is assumed to be the same for all the strains. Here, this term allows us to assume that treatments are triggered by infection symptoms, which are themselves correlated to virulence. If the covariance is positive, the term acts as a selective force against virulence in the drug sensitive compartment.

Virulence evolutionary dynamics in the drug resistant infections compartment are partly governed by similar forces than in the drug sensitive compartment. The second term in the right hand side of equation 6b is the transmission-virulence trade-off, while the third is the law of declining virulence. The proportionality constants a and b originate from our simplifying assumption regarding the link between traits expressed both in drug-sensitive and drug-resistant infections (see the Model section).

The first term in equation 6b corresponds to treatment failure events and homogenises trait values between drug-sensitive and drug-resistant compartments. If virulence is the same in the two compartments, this term is zero. As mentioned above, variations in the fraction of treatments that fail (ρ_i) cannot be separated from variations in the treatment rate (γ_i). Finally, this term is weighted by the density of drug-sensitive and drug-resistant infections. Initially, infections are drug-sensitive and this treatment failure term governs most of the dynamics. It is only in a second phase that transmitted drug-resistance can matter and that the second and third terms weight in. This term is not present in 6a because reversions from drug-resistant to drug-sensitive infections are neglected in this model.

Overall, virulence in the drug-resistant compartment should be less selected against than in the drug sensitive compartment because of the absence of covariance term between α and γ in equation 6b.

3.2 Numerical simulations of short-term dynamics

To validate these analytical predictions, I perform numerical simulations with $n = 20$ strains with sets of traits drawn at random and assuming a transmission-virulence trade-off. Figure 2 shows the short term evolutionary dynamics predicted by the Price equation formalism (dashed lines) and those obtained via simulations (plain lines).

Figure 2A illustrates the diversity of the epidemiological dynamics among all of the 20 strains. We see that not all the strains spread during the epidemics. We also see that initially, all the infections are drug-sensitive (dotted lines start from 0). Figure 2B shows the total densities of each type of infection (drug-sensitive or drug-

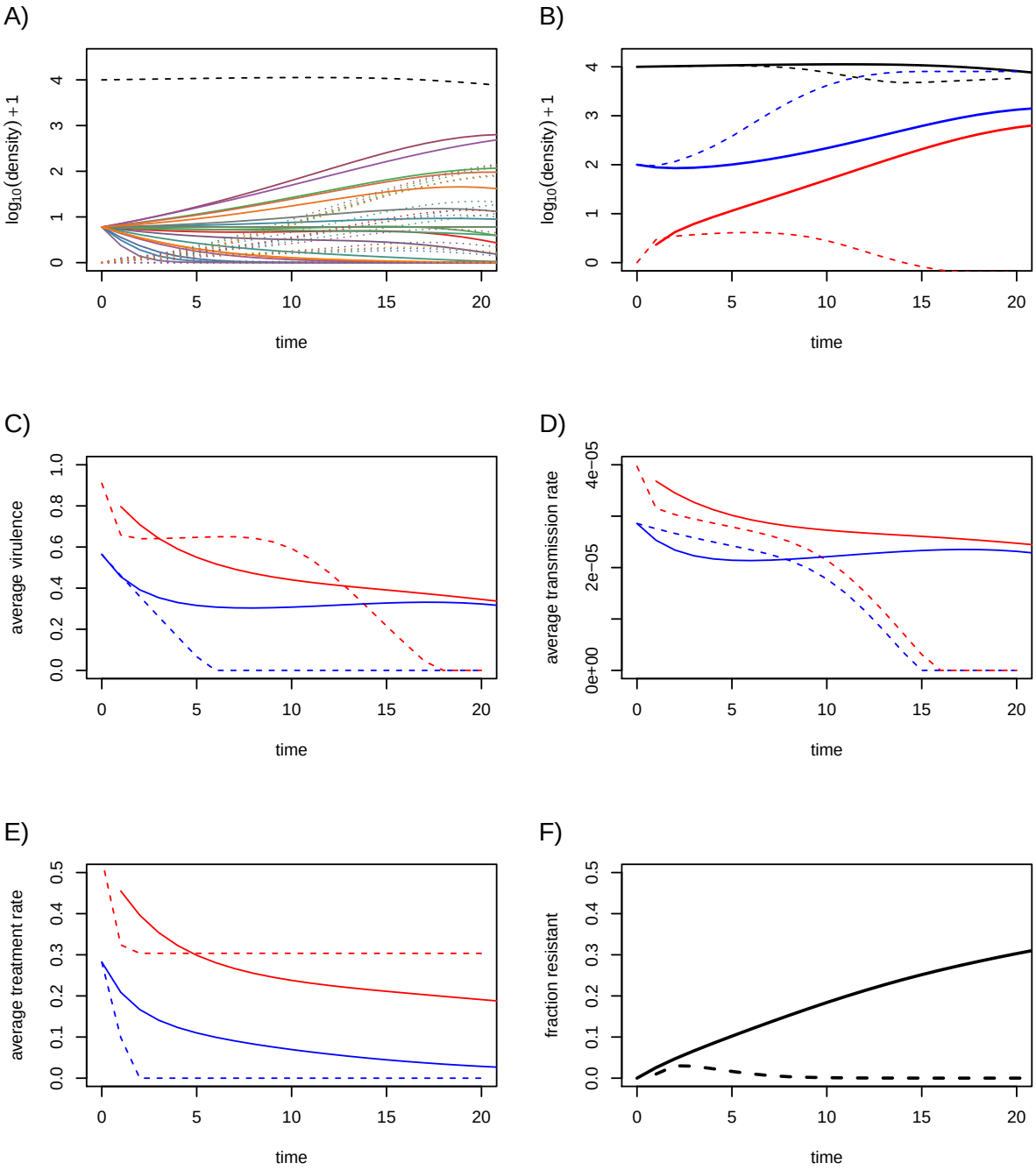

Figure 2 – **Short term evolutionary dynamics.** A) Dynamics of the densities of susceptible hosts (dashed black line), of drug-susceptible infections (plain coloured lines) and drug-resistant infections (dotted coloured line). Each color corresponds to one of the $n = 20$ strains. B) Same as A but the blue line shows the total density of drug-susceptible infections and the red line the total density of drug-resistant infections. The dashed lines are the predictions from the Price equation system. C) Average virulence in the drug-sensitive (blue) and drug-resistant infections (red) for numerical multi-strain simulations (plain line) and the Price equation system (dashed line). D) Same as panel C for transmission rate. E) Same as panel C for treatment rate. F) Fraction of the infections that are drug-resistant in the multi-strain simulation (plain line) or using the Price equation (dashed line). We assume not fitness cost ($a = b = 1$) and a transmission-virulence trade-off ($\beta(\alpha) = 10^{-4}\sqrt{\alpha}$). Other parameter values are $\lambda = 0.02$, $\mu = 4.5 \times 10^{-5}$, $a = b = 1$, $\rho = 0.1$, $\mathrm{Cov}(\alpha, \gamma) = 0.1$, $\mathrm{Var}(\gamma) = 1/12$, $\mathrm{Var}(\alpha) = 1/6$, $\overline{\alpha}(0) = 1/2$, $\overline{\gamma}(0) = 1/4$, $S(0) = 10^4$, $I_i = 5$ and $I_i^R = 0$.

resistant) and the predictions from the Price equation formalism (dashed lines). These predictions are correct at

first but they rapidly overestimate the spread of drug-sensitive infections (in blue) and underestimate the spread of drug-resistant infections (in red).

Focusing on average infection trait values, Figure 2C shows that, as predicted, virulence decreases faster in the drug sensitive infections than in the drug resistant infections. However, it also shows the limits of the Price equation because parasites eventually evolve to complete avirulence. Note that in the Price equation approach, I assumed different initial trait values for the drug-sensitive and drug-resistant compartments. Indeed, drug-resistance is initially absent in the simulations (the plain red line starts with a lag) and avergae infection virulence is much higher in this compartment. This is because resistant infections first emerge from treatment failure and not all strains have the same exposition to treatments. Therefore, instead of using the average virulence of all the strains in the population as our initial value, we weight these virulence by the recovery rates. This approximation is consistent with the simulated dynamics (Figure 2C).

Importantly, the mismatch in virulence we observe in drug-resistant infections is not solely due to this initial effect of more virulent infections being more treated. Indeed, as shown in Supplementary Figure S1, even if initial densities of drug-sensitive and drug-resistant infections are assumed to be equal, therefore implying a pre-existence of drug resistance, the initial virulence in the two compartments are identical but there is still a more rapid decrease in virulence in the drug-sensitive compartment, as predicted by the Price equation model. This cannot be explained by a fitness difference between the two strains because we do not include a fitness cost in these simulations.

For the transmission rate (Figure 2D), the Price equation does not predict any difference. In the simulations, the transmission rate in the drug-resistant compartment remains higher than that in the drug-sensitive compartment but this is due to the initial increase in virulence in this compartment (explained above). For the rate at which infections are treated (Figure 2E), we see a similar pattern than for virulence, which is consistent with the fact that the two are strongly correlated. Finally, Figure 2F shows the fraction of infections that are drug-resistant. Again, the Price equation is initially accurate but it rapidly underestimates the spread of drug resistance.

3.3 Long-term evolutionary dynamics

Using the adaptive dynamics approach, we can determine which of the strains present in the system will initially dominate over the long run. Since there is no density-dependent feedback (eigenvalues only depend on $\tilde{S}$ and not on $\tilde{I}_i$ or $\tilde{I}_i^R$), the fittest strain, denoted with a star ($*$), is the one with the highest value of

$$\max\left(\frac{\beta_*^R}{\mu+\alpha_*^R}\,,\,\frac{\beta_*}{\mu+\alpha_*+\gamma_*}\right) \tag{7}$$

This fittest strain is either found only in drug-resistant infections or in a majority of drug-sensitive infections depending on the existence of fitness cost associated with drug-resistance. Without such a cost and with sufficient genetic variation, the system will always tend towards drug-resistant infections.

Assuming a fitness cost such that $\alpha_i = a\alpha_i^R$ and $\beta_i = b\beta_i^R$ (with $a \geq 1$ and $0 \leq b \leq 1$) and a transmission-virulence trade-off (equation 5), we know from equation 7 that, on the long run, the system will be invaded by drug-resistant infections if

$$\mu(b\,a^p - 1) + \alpha_*\,(b\,a^p - a) + b\,a^p\,\gamma_* > 0 \tag{8}$$

If natural mortality is assumed to be negligible compared to virulence ($\mu \ll \alpha_*$), this equation simplifies into $\gamma_*/\alpha_* > a^{1-p}/b - 1$. This suggests that strains with high treatment rate and low virulence tend to be more prone to the evolution of drug-resistance. Also, lower values of b and higher values of a obviously make the

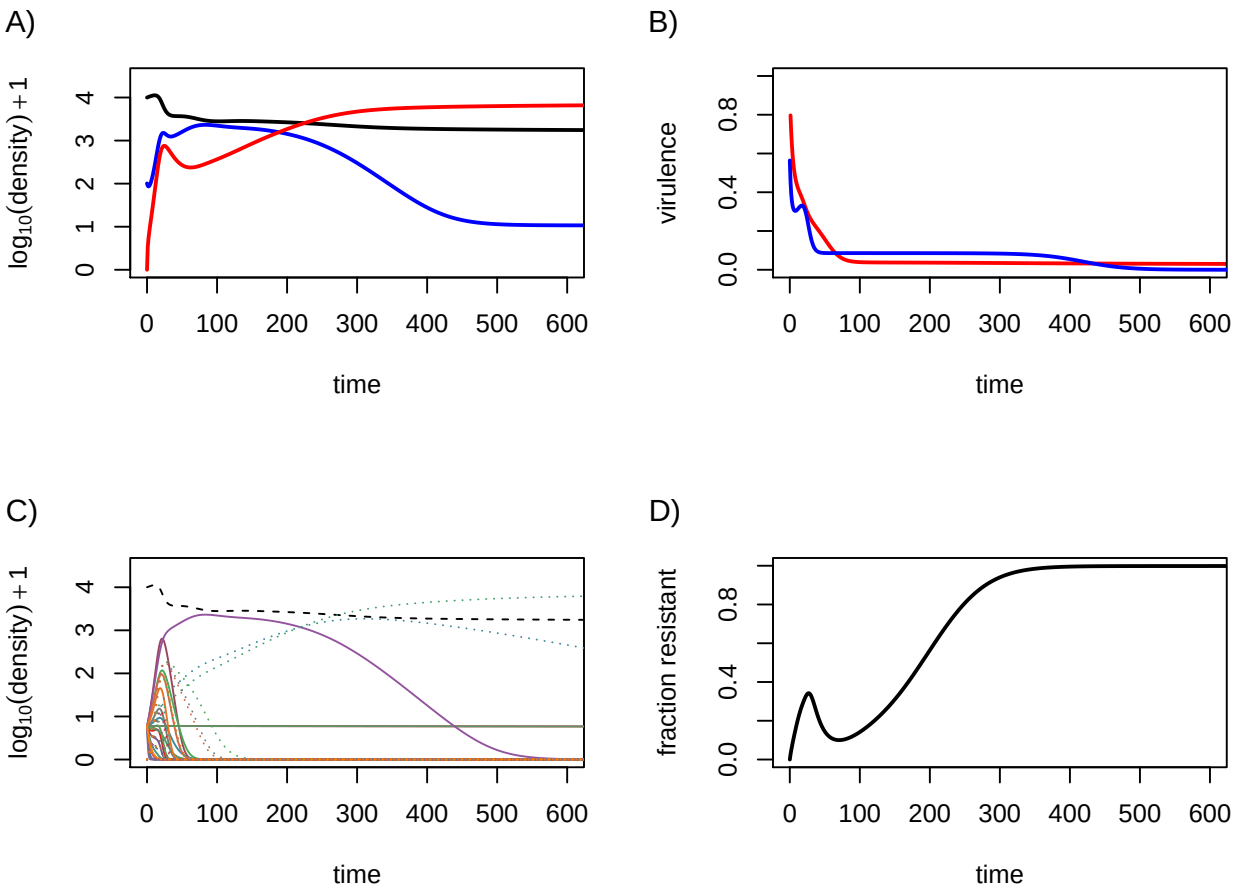

Figure 3 – **Long term evolutionary dynamics.** A) Dynamics of the densities of susceptible hosts (black), drug-susceptible infections (blue) and drug-resistant infections (red). B) Average virulence in the drug-sensitive (blue) and drug-resistant infections (red) for numerical multi-strain simulations. C) Dynamics of the densities of susceptible hosts (dashed black line), of drug-susceptible infections (plain coloured lines) and drug-resistant infections (dotted coloured line). Each color corresponds to one of the $n = 20$ strains. D) Fraction of the infections that are drug-resistant. Parameter values are the same as in Figure 2 for the multi-strain simulation.

evolution of drug resistance more difficult.

3.4 Numerical simulations of long-term dynamics

Long-term simulations show that the parasite can persist (Figure 3A). We also see that the average virulence in the drug-sensitive compartment (in blue) increases before reaching avirulence (Figure 3B). After a long transient phase, it even drops below the average virulence in the drug resistant compartment (in red). Figure 3C shows that strains can be maintained by different types of infections. For instance, for times between 100 and 300 the purple strain is present mostly in drug-sensitive infections, whereas the green strain is present in drug-resistant infections. We also see that only three of the strains persist after time 200 and that one of the strains seems to eventually take over the population. The nature of these strains can be predicted before running the simulation by calculating the two eigenvalues from equation 4. Based on equation 7, we also know whether this fittest strain is found mostly in drug-resistant or drug-sensitive infections.

Since the system only contains 20 strains, the presence of drug resistance at equilibrium does not only depend on the model parameter. In Supplementary Figure S2, I show that the presence of drug resistance can be negligible with the same parameter values but with 20 other strains chosen at random using the same

distribution.

Discussion

As attested by the resolution from the United Nations in 2016, anti-microbial drug resistance is a world issue with strong implications for public health policies (Wernli et al., 2017). However, efforts are still required to popularise it as an evolutionary process (Antonovics et al., 2007). This is not a mere semantic issue because it means envisaging antimicrobial resistance as a dynamical process, which directly impacts the design of optimal therapies (zur Wiesch et al., 2011, Read et al., 2011).

This study analyses the evolution of antimicrobial resistance in parasite strains that can vary in virulence, which is defined here as the decrease in host fitness due to the infection (Read, 1994). This trait was long seen as binary, following Pasteur's view was that 'virulent strains killed whereas attenuated strains did not' (Mendelsohn, 2002), which led to the successful identification of 'virulence factors'. In fact, many recent studies identify links between these factors and drug resistance (Beceiro et al., 2013, Van Tyne and Gilmore, 2014, Guillard et al., 2016, Alcalde-Rico et al., 2016, Giraud et al., 2017, Geisinger et al., 2018, Copin et al., 2019). However, the field is increasingly considering virulence as a quantitative trait under the control of many genes (Casadevall et al., 2011) and, in this context, experimental data is lacking.

Using an epidemiological modelling framework to analyse short-term and long-term evolution, I show that public health policies can generate differences in virulence between drug-resistant and drug-sensitive infections. First, assuming that more virulent infections are treated in priority, the initial emergence of drug-resistance should occur first in more virulent strains. Second, even if drug-resistance pre-exists in the population, virulence can be expected to be less counter-selected in the drug-resistant population, leading to drug-resistant infections being less virulent on average. Third, on the long term, genetic trade-offs determine the nature of the strain that takes over the host population. The infections this strain causes are either mostly drug-sensitive or drug-resistant depending on the nature of the fitness costs associated with drug resistance. These analytical predictions are confirmed using numerical simulations with limited parasite genetic diversity.

One direct consequence of these results has to do with the estimation of infection virulence, especially in an outbreak situation. Indeed, in addition to controlling for host differences that can affect virulence (since it is a shared trait between the host and the parasite), the drug resistant nature of the infection should also be taken into account. This could be combined with other proxies, for instance less severe cases tend to be less reported than severe ones. The advantage of drug-resistance is that it can be assessed *a posteriori* on clinical samples. The downside is that there can also be a direct link between drug resistance and virulence. Ideally, one would want to also assess parasite virulence in vitro, but this is much less straightforward than drug resistance.

There are several ways to test the predictions of this study. One possibility could be to use an experimental system where treatment can be applied to hosts based on the symptoms they exhibit. One difficulty is that it requires to have hosts large enough to detect the symptoms and apply a treatment without affecting the rest of the population. Another difficulty is that it imposes to be able to generate real epidemics. Fungal or bacterial parasites of plants could provide the ideal system. Another possibility would be to analyse existing clinical or agronomical data. A potential parasite could be HIV infections because drug resistance is well characterised (Beerenwinkel et al., 2003) and virulence can be estimated through set-point virus load (Fraser et al., 2014). However, a major difficulty is that in order to test the model, we would either need to know the nature of the fitness cost potentially associated with drug resistant mutations. A possibility could be to use algorithms that can partly predict virus load based on the virus sequence (Hinkley et al., 2011).

A number of simplifying assumptions were made in order to analyse the model. One of them is that drug resistance is a binary trait, when virulence is a continuous one. Modelling drug-resistance as a continuous

trait is possible. However, this would only leave one type of infected hosts. The drug-resistant trait would then be diluted in the equations and the feedback between the ecology (treatment policies) and trait evolution (drug resistance) would disappear. Conversely, it would also be possible to model virulence as a discrete trait. This would have the advantage that by modelling the system with (at least) two loci, one governing the drug resistance phenotype and another governing the virulence phenotype, we could draw parallels with and perhaps use data on virulence factors. In fact, an earlier mathematical model of multi-locus drug resistance has a similar structure (Day and Gandon, 2012). The main issue is that this binary structure is poorly adapted to capture quantitative variations in virulence, which have been shown to lead to trade-off relationships with other traits such as transmission rate (Anderson and May, 1982, Dwyer et al., 1990, Fraser et al., 2007, de Roode et al., 2008, Råberg, 2012, Doumayrou et al., 2012, Williams et al., 2014). In the end, the main results would most likely depend on the genetic linkage between the two loci and on the assumption regarding the fitness effect of the alleles at the virulence locus (Day and Gandon, 2012).

Our formalism has the advantage to allow for a continuum of strains with varying values of virulence and transmission rate. Regarding the latter parameter, one simplifying assumption of the model is that it is defined a function of virulence. In reality, the realised transmission rate will be distributed around this expected value. As illustrated in Supplementary Figure S3, I also allowed some degree of noise on this transmission rate. In general, the results are qualitatively robust to this noise. The main variable that is affected is the fraction of infections that are drug-resistant (Supplementary Figure S4). The model also assumes a saturating transmission-virulence trade-off relationship, which means there exists an intermediate optimal level of virulence (van Baalen and Sabelis, 1995, Alizon and Michalakis, 2015). Without the trade-off, parasites evolve to avirulence but they still do so more rapidly in the drug-sensitive than in the drug-resistant infections.

Overall, the qualitative predictions from the Price equation appear to be correct initially but there is an increasing mismatch for some traits as the epidemic unfolds. This is expected because the variances and covariances are here assumed to be constant. Initially, they are set to be identical to the multi-strain simulations. However, the relative frequency of each of the 20 strains varies over time (as illustrated by Figure 2A) therefore altering the variances and covariance. To take an extreme example, if a single strain dominates, the variances will be 0. Because the formalism used here does not update the covariance matrix, it is bound to eventually be unable to predict the dynamics accurately.

Results on the long run that drug resistant strains may persist may seem surprising given the Price equation predictions but, again, these are only valid on the short term. The ability for drug resistance to invade depends on potential fitness costs acting on virulence and transmission rate. Furthermore, everything else being equal, strains with low virulence that are treated at a high rate tend to lead more often to drug resistance than strains with high virulence that are treated at a low rate. This makes sense because the latter have more to benefit from evolving drug resistance (their treatment rate vanishes in our model).

Further studies are needed to investigate the effect of the nature of the fitness cost and its interaction with transmission-virulence trade-offs. It would also be interesting to allow for compensatory mutations to mitigate the effect of drug resistance costs. Finally, modelling explicitly within-host dynamics, and explicitly tracking the competition between drug-sensitive and drug-resistant variants of a given strain through nested models (Mideo et al., 2008) could be a way to study the interaction between levels of adaptation.

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Appendix A: Supplementary methods

Price equation calculations

Following the Price equation formalism introduced by Day and Proulx (2004), we take the derivative with respect to time of $p_i = I_i/I_T$, we get

$$\frac{\mathrm{d}p_i}{\mathrm{d}t} = \frac{1}{I_T}\left(\frac{\mathrm{d}I_i}{\mathrm{d}t} - \frac{\mathrm{d}I_T}{\mathrm{d}t}\, p_i\right) \tag{S1}$$

From equations 1b and 2a, we get

$$\frac{\mathrm{d}p_i}{\mathrm{d}t} = \left[\left(\beta_i - \overline{\beta}^I\right) S - \left(\alpha_i - \overline{\alpha}^I\right) - \left(\gamma_i - \overline{\gamma}^I\right)\right] p_i \tag{S2}$$

Let us look at the evolution of the average value of a given trait x. If we assume that the trait of a genotype i does not value with time, we have:

$$\frac{\mathrm{d}\overline{x}}{\mathrm{d}t} = \sum_i x_i \frac{\mathrm{d}p_i}{\mathrm{d}t} \tag{S3}$$

From equation S2, we have:

$$\frac{\mathrm{d}\overline{x}}{\mathrm{d}t} = \sum_i x_i \left[\left(\beta_i - \overline{\beta}^I\right) S - \left(\alpha_i - \overline{\alpha}^I\right) - \left(\gamma_i - \overline{\gamma}^I\right)\right] p_i \tag{S4}$$

This can also be written as

$$\frac{\mathrm{d}\overline{x}}{\mathrm{d}t} = S\,\mathrm{Cov}\left(\beta, x\right) - \mathrm{Cov}\left(\alpha, x\right) - \mathrm{Cov}\left(\gamma, x\right) \tag{S5}$$

Similarly, we can define the proportion of genotype i in the drug-resistant compartment as $q_i = I_i^R/I_T^R$ and derive it with respect to time to get:

$$\frac{\mathrm{d}q_i}{\mathrm{d}t} = \frac{1}{I_T^R}\left(\frac{\mathrm{d}I_i^R}{\mathrm{d}t} - \frac{\mathrm{d}I_T^R}{\mathrm{d}t}\, q_i\right) \tag{S6a}$$

$$= \rho_i\, \gamma_i\, \frac{I_i}{I_T^R} + \beta_i^R\, S\, q_i - \left(\mu + \alpha_i^R\right) q_i - \overline{\rho\,\gamma}^I\, \frac{I_T}{I_T^R}\, q_i - \overline{\beta^R}^R\, S\, q_i + \mu + \overline{\alpha^R}^R\, q_i \tag{S6b}$$

$$= \left(\rho_i\, \gamma_i\, p_i - \overline{\rho\,\gamma}^I\, q_i\right) \frac{I_T}{I_T^R} + \left(\beta_i^R - \overline{\beta^R}^R\right) S\, q_i - \left(\alpha_i^R - \overline{\alpha^R}^R\right) q_i \tag{S6c}$$

For a given trait y in the drug-resistant compartment, we have

$$\frac{\mathrm{d}\overline{y}}{\mathrm{d}t} = \sum_i y_i \left[\left(\rho_i\, \gamma_i\, p_i - \overline{\rho\,\gamma}^I\, q_i\right) \frac{I_T}{I_T^R} + \left(\beta_i^R - \overline{\beta^R}^R\right) S\, q_i - \left(\alpha_i^R - \overline{\alpha^R}^R\right) q_i\right] \tag{S7a}$$

$$= \frac{I_T}{I_T^R} \sum_i y_i\, \rho_i\, \gamma_i\, p_i - \frac{I_T}{I_T^R} \sum_i y_i\, \overline{\rho\,\gamma}^I\, q_i + S\,\mathrm{Cov}\left(\beta^R, y\right) - \mathrm{Cov}\left(\alpha^R, y\right) \tag{S7b}$$

$$\approx \frac{I_T}{I_T^R} \left(\overline{y}^I - \overline{y}^R\right) \overline{\rho\,\gamma}^I + S\,\mathrm{Cov}^R\left(\beta^R, y\right) - \mathrm{Cov}^R\left(\alpha^R, y\right) \tag{S7c}$$

The assumption made to reach the last step is that $y_i\, \gamma_i \approx \overline{\gamma}^I\, p_i$.

As before, for the main trait of interest (virulence) and with the same assumptions as before we get:

$$\frac{\mathrm{d}I_T^R}{\mathrm{d}t} = \overline{\rho\,\gamma}^I\, I_T + \overline{\beta^R}^R\, S\, I_T^R - \left(\mu + \overline{\alpha^R}^R\right)\, I_T^R \tag{S8a}$$

$$\frac{\mathrm{d}\overline{\alpha^R}^R}{\mathrm{d}t} = \frac{I_T}{I_T^R}\left(\overline{\alpha^R}^I - \overline{\alpha^R}^R\right)\overline{\rho\,\gamma}^I + S\,\mathrm{Cov}^R\left(\beta^R, \alpha^R\right) - \mathrm{Var}^R\left(\alpha^R\right) \tag{S8b}$$

$$\frac{\mathrm{d}\overline{\beta^R}^R}{\mathrm{d}t} = \frac{I_T}{I_T^R}\left(\overline{\beta^R}^I - \overline{\beta^R}^R\right)\overline{\rho\,\gamma}^I + S\,\mathrm{Var}^R\left(\beta^R\right) - \mathrm{Cov}^R\left(\alpha^R, \beta^R\right) \tag{S8c}$$

Adaptive dynamics approach

We now adopt an adaptive dynamics approach to study long term evolution based on the functional relationship between treatment rate and virulence ($\gamma(\alpha)$). For generality, we also initially denote mutation rate as a function of virulence ($\rho(\alpha)$). Finally, we assume a transmission virulence trade-off ($\beta(\alpha)$) otherwise virulence will not be adaptive and always be selected against.

Let us rewrite the ODE system:

$$\frac{\mathrm{d}S}{\mathrm{d}t} = \lambda - \mu\, S - \beta(\alpha)\, S\, I - \beta^R(\alpha^R)\, S\, I^R \tag{S9a}$$

$$\frac{\mathrm{d}I}{\mathrm{d}t} = \beta(\alpha)\, S\, I - (\mu + \alpha + \gamma(\alpha))\, I \tag{S9b}$$

$$\frac{\mathrm{d}I^R}{\mathrm{d}t} = \rho(\alpha)\, \gamma(\alpha)\, I + \beta^R(\alpha^R)\, S\, I^R - \left(\mu + \alpha^R\right) I^R \tag{S9c}$$

We can assume that virulence in resistance infections is proportional to that in sensitive infections ($\alpha^R \propto \alpha$) and that the functional relationship between transmission rate and virulence is unaffected by the resistance.

Following the next-generation matrix theorem (Diekmann et al., 1990, Driessche and Watmough, 2002, Hurford et al., 2010), the Jacobian matrix of the system (J) can be decomposed into a 'birth' (F) and a 'death' (V) matrix:

$$F = \begin{bmatrix} \beta(\alpha)\, S & 0 \\ \rho(\alpha)\, \gamma(\alpha) & \beta^R(\alpha^R)\, S \end{bmatrix} \tag{S10a}$$

$$V = \begin{bmatrix} \mu + \alpha + \gamma(\alpha) & 0 \\ 0 & \mu + \alpha^R \end{bmatrix} \tag{S10b}$$

The eigenvalues of $F.V^{-1}$ are

$$\lambda_1 = \frac{\beta^R(\alpha_m)}{\mu + \alpha_m^R}\, S(\alpha) \quad \text{and} \quad \lambda_2 = \frac{\beta(\alpha_m)}{\mu + \alpha_m + \gamma(\alpha_m)}\, S(\alpha)$$

Note that if we allow for 'reversions' of drug-resistant infections, the eigenvalues can still be derived but their expression is less clear. We can also see that any link between treatment failure probability and virulence does not matter on the long run.

System equilibria

The detailed equilibria of the main equation system are the following:

$$
\begin{aligned}
(\tilde{S}\ ,\ \tilde{I}\ ,\ \tilde{I}^R) &= \left(\frac{\lambda}{\mu}\ ,\ 0\ ,\ 0\right) \\
(\tilde{S}\ ,\ \tilde{I}\ ,\ \tilde{I}^R) &= \left(\frac{\mu+\alpha_i+\gamma_i}{\beta_i}\ ,\ \frac{\lambda-\mu\tilde{S}}{\beta_i\ \tilde{S}\left(\frac{\beta_i^R}{\beta_i}\ \frac{\gamma_i\ \rho_i}{\alpha_i^R+\mu-\beta_i^R\tilde{S}}+1\right)}\ ,\ \frac{\gamma_i\ \rho_i\ (\lambda-\mu\tilde{S})}{\tilde{S}\left(\beta_i^R\ \gamma_i\ \rho_i+\beta_i\ (\alpha^R+\mu-\beta_i^R\ \tilde{S})\right)}\right) \\
(\tilde{S}\ ,\ \tilde{I}\ ,\ \tilde{I}^R) &= \left(\frac{\mu+\alpha_i^R}{\beta_i^R}\ ,\ 0\ ,\ \frac{\lambda}{\mu+\alpha_i^R}-\frac{\mu}{\beta_i^R}\right)
\end{aligned}
$$

Appendix B: simulation `R` code

This appendix contains the `R` code used for the simulations and generating the figures.

Supplementary Figures

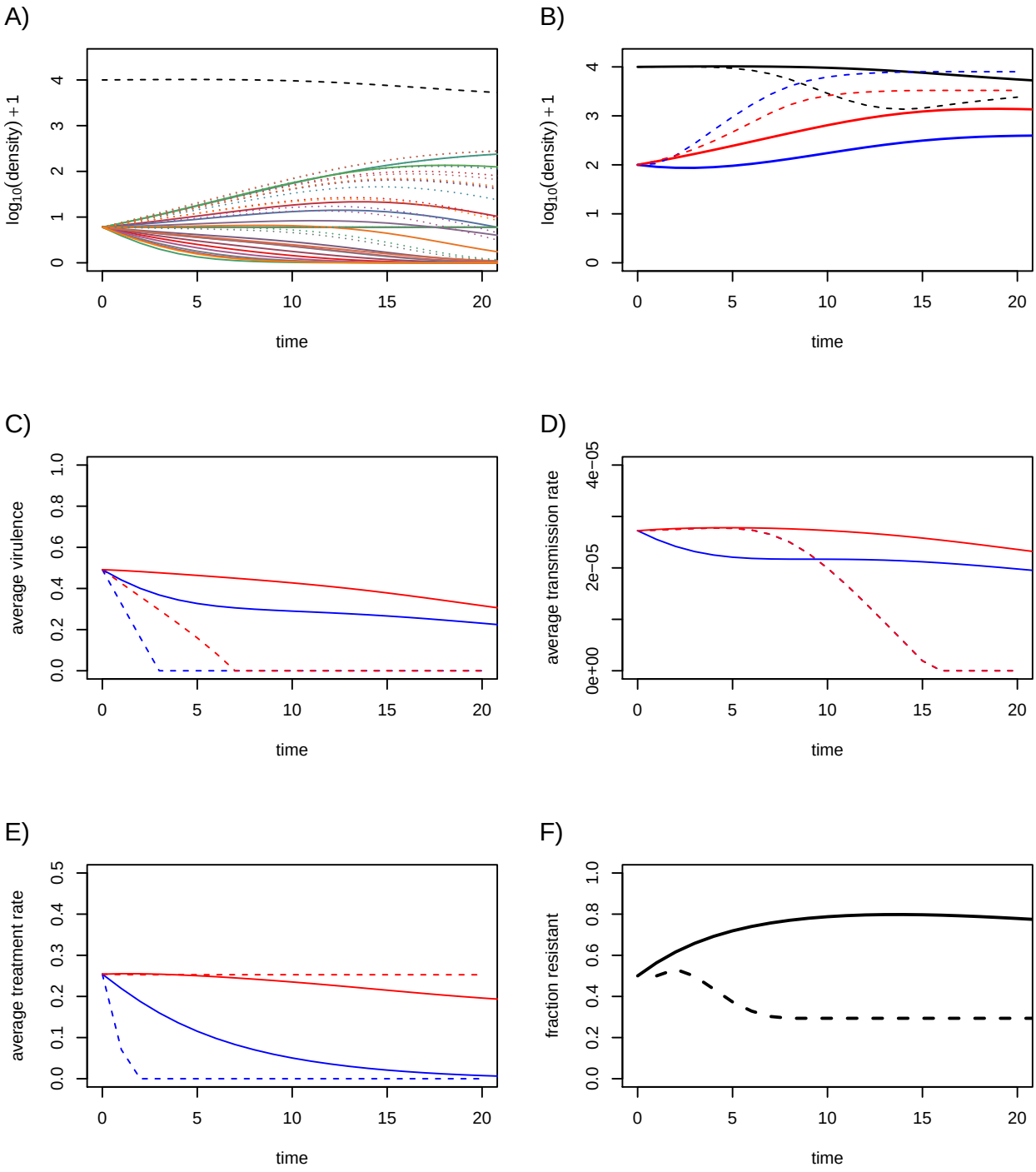

Figure S1 – **Short-term dynamics with pre-existing drug resistance.** Virulence in the drug-resistant compartment still decreases more slowly than in the drug-sensitive compartment if we assume that the initial density of the two types of infections is equal (therefore that drug resistance is not only generated by treatment failure initially). $I_i^R = 5$ and other parameter values are identical to that in the main text.

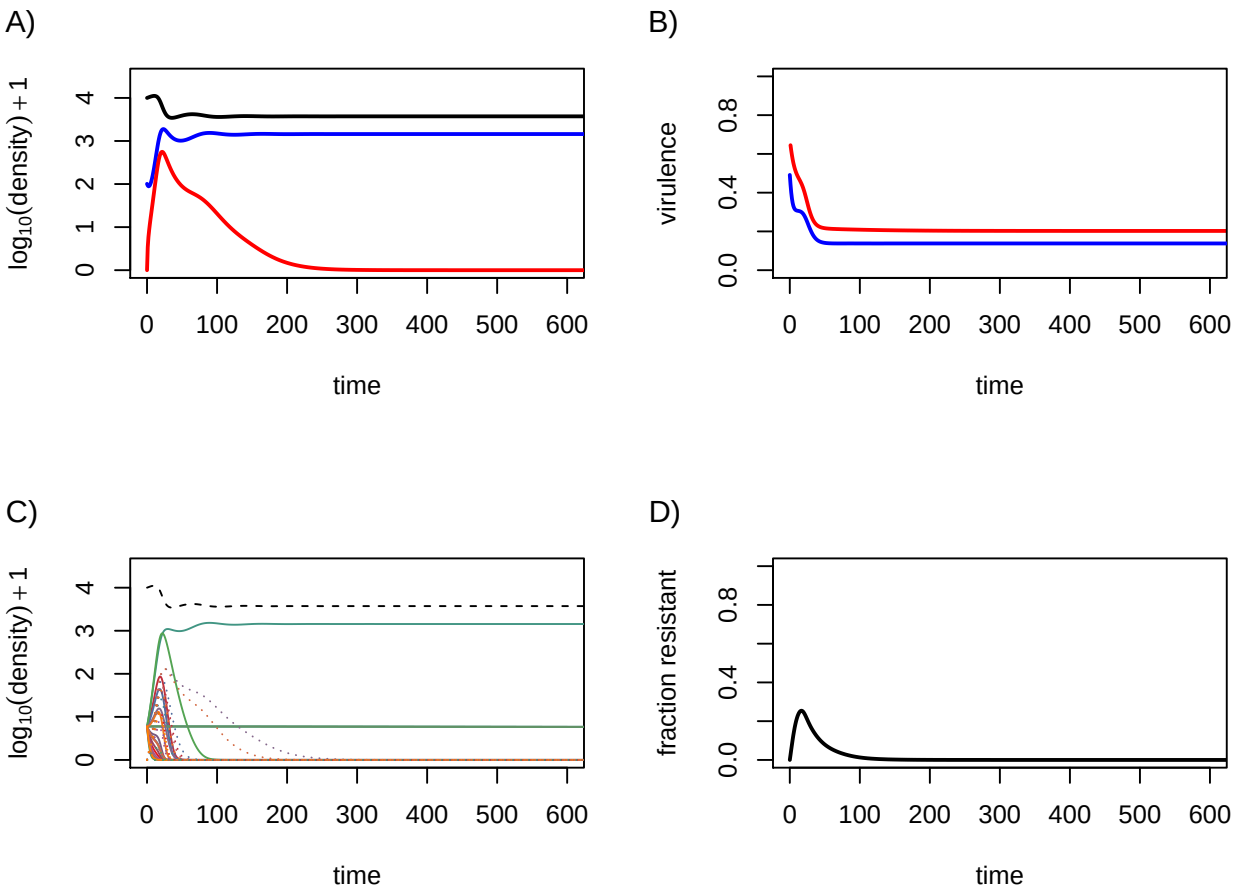

Figure S2 – **Long-term evolutionary dynamics.** Even with the same parameter values as in the main text, drug-sensitive infections can eventually dominate the system. This is because we only have a limited number of strains in the simulations. Parameter values are the same as in the main text but the $n = 20$ strains are different (although drawn using the same covariance matrix as in the main text).

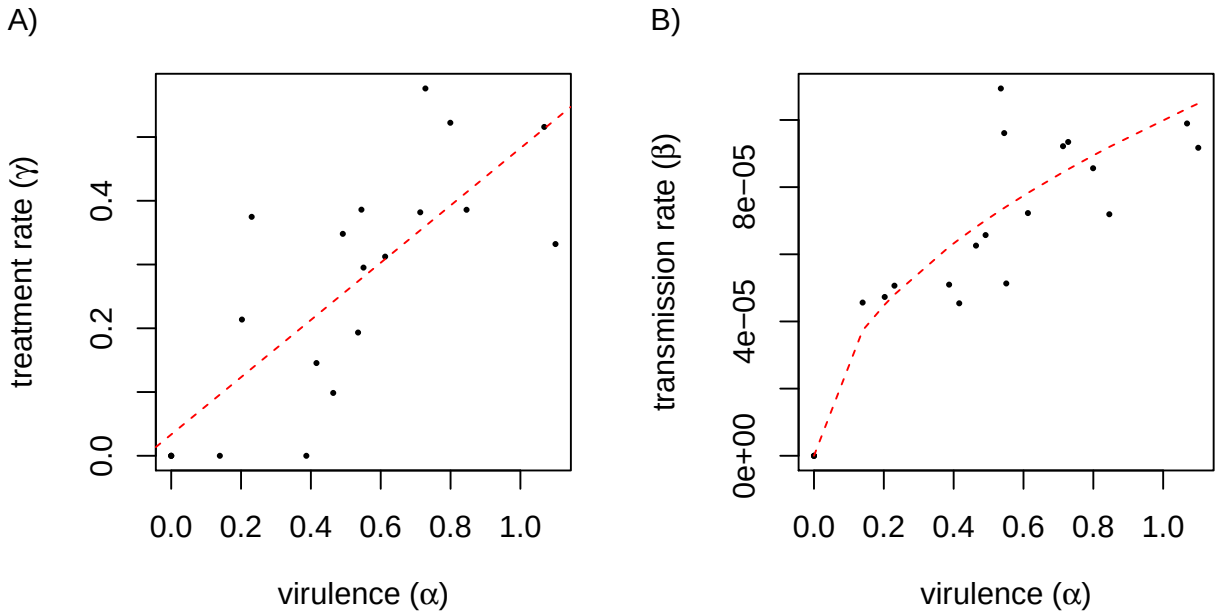

Figure S3 – **Trade-off relationships between model parameters.** A) Positive covariance between virulence and treatment rate. B) Trade-off relationship assumed between transmission rate and virulence (dashed line) and points obtained when adding noise to the transmission rates.

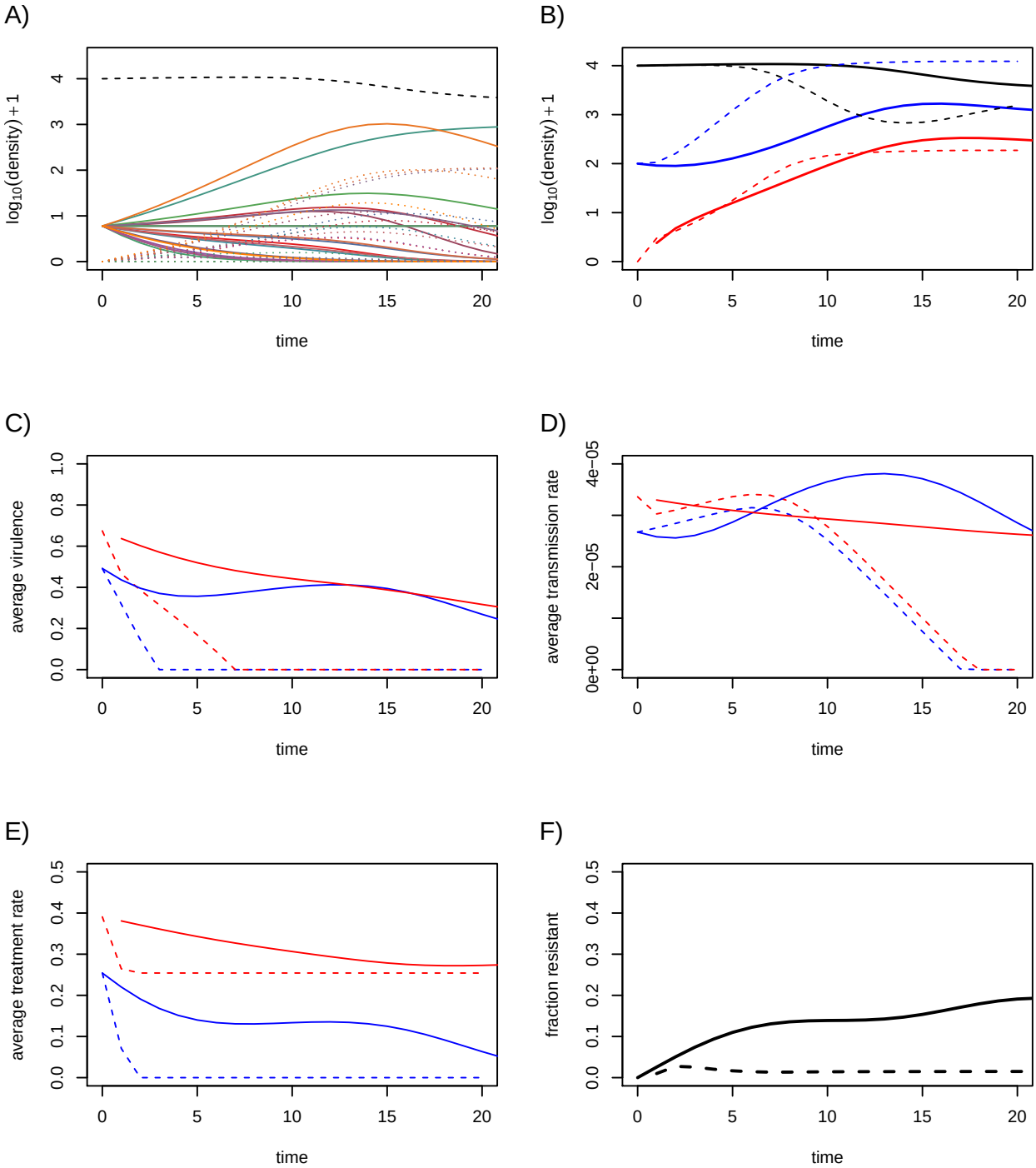

Figure S4 – **Short-term evolutionary dynamics assuming noise in transmission rates.** Figure captions are identical to that in the main text. Parameter values are identical to that in the main text, except for the noise, which is generated by a Gaussian distribution centred around the transmission value predicted by the trade-off shown in Figure S3B and with relative standard deviation 20%.