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ORIGINAL ARTICLE

The Evolution of Tuberculosis Virulence

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Abstract The evolution of *Mycobacterium tuberculosis* presents several challenges for public health. HIV and resistance to antimycobacterial medications have evolutionary implications for how *Mycobacterium tuberculosis* will evolve, as these factors influence the host environment and transmission dynamics of tuberculosis strains. We present an evolutionary invasion analysis of tuberculosis that characterizes the direction of tuberculosis evolution in the context of different natural and human-driven selective pressures, including changes in tuberculosis treatment and HIV prevalence. We find that the evolution of tuberculosis virulence can be affected by treatment success rates, the relative transmissibility of emerging strains, the rate of reactivation from latency among hosts, and the life expectancy of hosts. We find that the virulence of tuberculosis strains may also increase as a consequence of rising HIV prevalence, requiring faster case detection strategies in areas where the epidemics of HIV and tuberculosis collide.

Keywords Tuberculosis · Virulence · Evolution · Drug resistance · HIV

1. Introduction

Though an ancient and treatable disease, tuberculosis (TB) has continued to cause morbidity and mortality on all continents (World Health Organization, 2007b). Because of the long time-course of TB epidemics (Blower et al., 1995), and given the continued emergence and transmission of drug-resistant strains (Gandhi et al., 2006), the process of bacterial evolution is fundamental to the development of control policies. While the conditions under which drug-resistant TB strains propagate have been previously analyzed (Blower and Chou, 2004; Blower and Gerberding, 1998; Cohen and Murray, 2004; Dye et al., 2002), the manner in which changing evolutionary pressures may affect the pathogenic properties of emergent strains has not been evaluated. Yet *M. tuberculosis* genotype changes are associated with phenotypic changes in human disease pathogenesis (Ernst et al., 2007). Of principal interest is how disease virulence (the rate of pathogen-induced mortality) changes with selective pressures (Levin, 1996).

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The trajectory of tuberculosis epidemics has also been further complicated by HIV. HIV does not necessarily alter the biological susceptibility of hosts to actually acquiring *M. tuberculosis* bacteria, but does appear to accelerate the rate at which persons advance to active TB disease following bacterial acquisition, increase the death rate among human hosts with and without TB, and alter the likelihood of active TB disease following reinfection with TB bacilli (Edlin et al., 1992; Daley et al., 1992; Small et al., 1993; Lawn et al., 2007; Di Perri et al., 1989). In parts of sub-Saharan Africa, over 80% of newly-diagnosed TB cases are coinfected with HIV (Corbett et al., 2006). Between 1990 and 2005, TB incidence increased by 7% annually on average in countries with high (>5%) HIV prevalence, but only by 1.3% annually among countries with low HIV prevalence (Friedland et al., 2007). HIV has also profoundly affected the demography of several countries, changing the average life expectancies among human hosts of TB (World Health Organization, 2005). We examine the impact of these changes in demography and HIV prevalence on selective pressures operating on TB virulence. The advancement of tuberculosis treatment programs may impose additional selection on the virulence of TB.

Traditional epidemiological theory would suggest that pathogens would evolve in ways that increased their transmissibility and decreased their virulence, since these properties would increase their reproductive ratio (the number of secondary infections caused by an average infectious host in a susceptible population) (Mann et al., 2003). But this "runaway evolution" toward highly transmissible and minimally pathogenic bacteria does not seem to occur (Boots and Sasaki, 1999; Bremerman and Thieme, 1989). Instead, it appears that a transmissibility-virulence trade-off may manifest, such that higher pathogen loads offer increased transmissibility at the cost of more rapid host death (shorter duration to transmit) (Anderson and May, 1991).

This trade-off is consistent with tuberculosis pathogenesis, in which bacillary load increases both infectiousness and disease severity (Castro and Dooley, 1993; Crofton et al., 1999). Emerging drug-resistant tuberculosis strains are also typically less transmissible and sometimes more virulent than drug-susceptible strains (Lan et al., 2003; Githui et al., 2004; ECA, 2006; Dye and Espinal, 2001). The transmissibility of drug-resistant strains may also be affected by compensatory mutations (Sherman et al., 1996), and the infectiousness and severity of tuberculosis disease among humans is varied, particularly in the context of HIV (Escombe et al., 2007; Friedland, 2007). Therefore it is difficult, given available data, to determine the functional form of the relationship between transmissibility and virulence (Ebert and Bull, 2003).

Despite the empirical uncertainties, we can specify the direction of bacterial evolution in the context of different selective pressures, without explicit knowledge of the functions that define complex relationships between pathogen virulence and transmissibility. An evolutionary invasion analysis describes what types of mutant strains may successfully propagate under changing evolutionary pressures (Otto and Day, 2007). The invasion analysis specifies the conditions under which an evolutionary stable strategy (ESS) exists; an ESS describes optimal pathogen traits under given pressures (conditions sufficient to prevent invasion from a mutant strain) (Smith and Price, 1973). We further examine how the optima are affected by changes in treatment systems and HIV.

In this analysis, we find that the level of virulence toward which strains evolve depends upon the local treatment success rate, the relative transmissibility of emerging strains, the rate of reactivation from latency among hosts, and the life expectancy of hosts. We also find that the virulence of tuberculosis strains is expected to increase in the context of rising

HIV prevalence, which would require faster case detection strategies in areas where the epidemics of HIV and tuberculosis collide.

2. Invasion dynamics

The direction in which pathogen virulence is likely to evolve can be examined by evaluating the conditions under which a mutant strain can invade a resident strain of tuberculosis. The dynamics of a mutant strain with virulence μ_t^m invading a population with a resident strain of virulence μ_t can be described in terms of the growth rate of the mutant strain, $r(\mu_t, \mu_t^m)$. This quantity must be positive for the mutant to invade (Otto and Day, 2007). To describe the condition $r(\mu_t, \mu_t^m)$, an epidemiological model is required.

We derive $r(\mu_t, \mu_t^m)$ from an updated version of the simple tuberculosis model described by Blower (1995), from which many current tuberculosis models are derived. The model tracks the population of individuals who are susceptible to tuberculosis (S), have latent tuberculosis (L), and have active tuberculosis disease (T), as depicted in the flow diagram in Fig. 1. The model equations are

$$\frac{dS}{dt} = \Pi - \beta T S - \mu S,$$
$$\frac{dL}{dt} = (1-p)\beta T S + dT - (v+\mu)L - px\beta T L,$$
$$\frac{dT}{dt} = p\beta T(S + xL) + vL - (\mu + \mu_t + d)T,$$

where Π is the recruitment rate to the population, β is the transmissibility coefficient, μ is the nontuberculosis (background) mortality rate, p is the proportion of newly-infected persons developing primary progressive disease (rapid progression to active disease) within 1 year, d is the regression rate (natural and chemotherapeutic), v is the rate of latent reactivation, and x is the proportion of exogenously re-infected latent patients who are susceptible to developing primary progressive disease. Table 1 lists typical values of these parameters for both HIV-negative and HIV-positive individuals.

The simplicity of this model permits analytical insights to be derived. However, the equations listed above have also been updated from their original formulation to reflect recent insights into TB pathogenesis. Specifically, reinfection among latently-infected persons is included (generating the term $px\beta TL$), given the likely importance of reinfection to tuberculosis transmission dynamics in high prevalence communities (Cohen et al., 2006). We also follow the standard World Health Organization description of tuberculosis pathogenesis by including regression from active disease (at rate d) to latency upon chemotherapeutic cure (Williams et al., 2005; Dye et al., 1998); natural "self-cure" is added to this regression process, as in prior models (Cohen and Murray, 2004; Basu et al., 2007). The proportion of active tuberculosis patients who are infectious is incorporated into the parameter β (see Table 1). This relatively simple model structure we employ is in accordance with the principal of parsimony, and permits analytical solutions to be derived for our evolutionary invasion analysis.

Replacement of a resident strain with a mutant strain in this system can occur when the reproductive ratio of the mutant exceeds that of the resident (Keeling and Rohani,

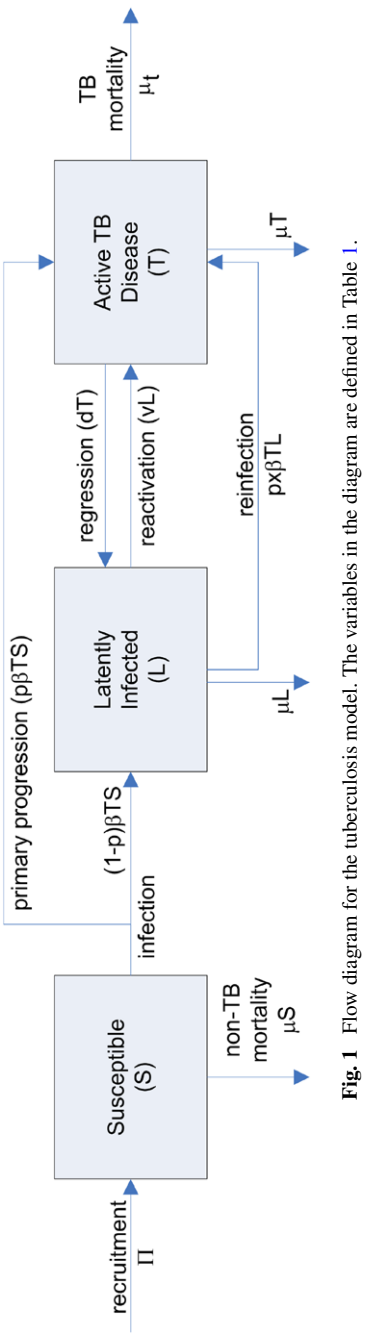

Fig. 1 Flow diagram for the tuberculosis model. The variables in the diagram are defined in Table 1.

Table 1 Typical parameter values and ranges used to describe tuberculosis pathogenesis. Units for the listed rates are 1/year. These parameters produce an average R_0 of ~2 for a population of 400,000 people (Dye et al., 2002)

Parameter	Definition	HIV-negative value (range)	HIV-positive value (range)	Sources
Π	Recruitment rate	8,800[a]		(Blower et al., 1995)
β	Per capita transmissibility coefficient (transmission rate per infectious case × proportion of active cases that are infectious)	3×10^{-5} (2×10^{-5}–5×10^{-5})	2×10^{-5} (6×10^{-6}–3×10^{-5})	(Blower et al., 1995; Dye et al., 1998; Dye and Williams, 2000)
μ	Background (non-TB) mortality rate	0.02 (0.01–0.04)	0.25 (0.1–0.33)	(Blower et al., 1995; Dye et al., 1998)
p	Proportion of infected experiencing primary progressive disease	0.14 (0.08–0.25)[a]	0.67 (0.36–0.8)[a]	(Dye et al., 1998)
d	Regression rate (natural and chemotherapeutic)	0.55 (0.15–0.73)		(Dye and Espinal, 2001; Williams et al., 2005)
v	Reactivation rate from latency	1.13×10^{-4} (10^{-4}–3×10^{-4})	0.17 (0.04–0.2)	(Dye et al., 1998)
x	Proportion of reinfected latents susceptible to primary progressive disease	0.35 (0.1–0.6)[a]	0.75 (0.5–1.0)[a]	(Dye et al., 1998)
μ_t	Virulence (mortality rate due to TB)	0.3 (0.2–0.4)	1.0 (0.75–1.0)	(Dye et al., 1998)

[a]These parameters cancel from the key expressions formulated in the text and, therefore, do not enter into numerical simulations discussed in the Results section

2007). Consistent with prior studies (Blower and Chou, 2004; Cohen and Murray, 2004; Dye and Williams, 2000), we assume that host-related parameters (p, x, v, μ, Π) will not differ between the mutant and resident tuberculosis pathogenesis (but will differ between HIV-negative and HIV-positive hosts). Solving for the reproductive ratio of the above model and rearranging the threshold condition for mutant invasion (see Appendix), we obtain an expression for $r(\mu_t, \mu_t^m)$:

$$r\left(\mu_t, \mu_t^m\right) = -d_m\mu - \left(\mu + \mu_t^m\right)(\mu + v) + \frac{\beta(\mu_t^m)(d\mu + (\mu + \mu_t^m)(\mu + v))}{\beta(\mu_t)},$$

where d_m is the rate of regression from active tuberculosis with the mutant strain, $\beta(\mu_t)$ is the transmissibility of the resident strain in terms of its virulence, and $\beta(\mu_t^m)$ is the equivalent parameter for the mutant strain. We see that the recruitment rate to the population and the proportion of patients who undergo primary progressive disease upon infection or reinfection does not enter into the expression for the invasion potential of the mutant strain. Rather, in this model, the invasion potential has been described only in terms of the regression rate of both resident and mutant strains, the ratio of their transmission coefficients, the rate of reactivation from latency, and the life expectancy of the host.

3. Evolutionary stable strategies

In the absence of explicit data from which to derive the transmissibility-virulence relationship, we can nonetheless derive analytical expressions for the ESS, and characterize how changes in selective pressure could alter that evolutionary optimum.

Differentiating the expression for $r(\mu_t, \mu_t^m)$, we can achieve an expression for the "fitness gradient" (Otto and Day, 2007), which describes the change in a mutant's potential for invasion with respect to its virulence. The gradient is

$$\frac{\partial r(\mu_t, \mu_t^m)}{\partial \mu_t^m} = -\mu - v + \frac{(d\mu + (\mu + \mu_t^m)(\mu + v))}{\beta(\mu_t)} \frac{d\beta}{d\mu_t^m}.$$

At the ESS virulence level, μ_t^*, the growth rate will have maximized, such that the fitness gradient will equal zero, which results in

$$\left.\frac{d\beta}{d\mu_t^m}\right|_{\mu_t^m = \mu_t^*} = \frac{(\mu + v)\beta(\mu_t^*)}{d\mu + (\mu + \mu_t^*)(\mu + v)},$$

and the second derivative must also be less than or equal to zero, which is achieved when

$$\left.\frac{\partial^2 r}{\partial \mu_t^{m2}}\right|_{\substack{\mu_t^m = \mu_t^* \\ \mu_t = \mu_t^*}} = \frac{(d\mu + (\mu + \mu_t^*)(\mu + v))}{\beta(\mu_t^*)} \left.\frac{d^2\beta}{d\mu_t^{m2}}\right|_{\mu_t^m = \mu_t^*} \leq 0.$$

In some cases, strains can evolve away from an ESS (Eshel, 1983), but we are interested in the "convergently stable" ESSs toward which strains will evolve. Convergent stability occurs when the fitness gradient changes in sign from positive to negative as the virulence increases through the value at the ESS. This is equivalent to

$$\frac{d}{d\mu_t}\left\{\left.\frac{\partial r(\mu_t, \mu_t^m)}{\partial \mu_t^m}\right|_{\mu_t^m = \mu_t}\right\}_{\mu_t = \mu_t^*} < 0.$$

Solving this equation results in

$$\frac{1}{\beta^2}\left(\begin{array}{l}-\big(d\mu+\big(\mu+\mu_t^*\big)(\mu+v)\big)\left(\dfrac{d\beta}{d\mu_t}\Big|_{\mu_t=\mu_t^*}\right)^2\\ \quad+\beta\left((\mu+v)\dfrac{d\beta}{d\mu_t}\Big|_{\mu_t=\mu_t^*}+\big(d\mu+\big(\mu+\mu_t^*\big)(\mu+v)\big)\dfrac{d^2\beta}{d\mu_t^2}\Big|_{\mu_t=\mu_t^*}\right)\end{array}\right)<0.$$

But because we known that at the ESS,

$$\frac{d\beta}{d\mu_t^m}\Big|_{\mu_t^m=\mu_t^*}=\frac{(\mu+v)\beta(\mu_t^*)}{d\mu+(\mu+\mu_t^*)(\mu+v)},$$

the condition for convergent stability reduces to

$$\frac{(d\mu+(\mu+\mu_t^*)(\mu+v))}{\beta(\mu_t^*)}\frac{d^2\beta}{d\mu_t^{m^2}}\Big|_{\mu_t^m=\mu_t^*}<0,$$

which repeats the second derivative condition with a strict inequality.

The above conditions tell us that the transmissibility of tuberculosis strains are expected to decrease with increasing virulence around an ESS, consistent with data from drug-resistant TB strains (Lan et al., 2003; Githui et al., 2004; ECA, 2006; Dye and Espinal, 2001). However, the derived expressions also suggest that the rate of decline in the transmissibility would slow as virulence increases.

4. Changing selective pressures

Of principle interest is the question of how the virulence of tuberculosis strains may change as selective pressures are altered, either by different treatment practices (affecting parameter d) or by changes to the human host (as a result of increasing HIV prevalence). We derive general analytical results related to the evolution of tuberculosis virulence, and also explore through simulation what the behavior of invasion may be, given reasonable parameter estimates.

Even if we do not known the functional form of $\beta(\mu_t)$ to fully characterize the function $r(\mu_t,\mu_t^m)$, we can describe the direction of evolution of tuberculosis by differentiating the fitness gradient with respect to the parameter that is changing (Otto and Day, 2007). Specifically, we obtained an ESS condition such that with regard to any parameter n in the fitness gradient expression,

$$\frac{\partial r(\mu_t,\mu_t^m,n)}{\partial\mu_t^m}\Big|_{\substack{\mu_m^t=\mu_t^*\\ \mu_t=\mu_t^*}}=0.$$

If we differentiate this expression with respect to the parameter n, then we arrive at

$$\frac{\partial}{\partial\mu_t}\left[\frac{\partial r(\mu_t,\mu_t^m,n)}{\partial\mu_t^m}\Big|_{\mu_t^m=\mu_t}\right]_{\mu_t=\mu_t^*}\frac{\partial\mu_t^*}{\partial n}+\frac{\partial}{\partial n}\left[\frac{\partial r(\mu_t,\mu_t^m,n)}{\partial\mu_t^m}\Big|_{\mu_t^m=\mu_t}\right]_{\mu_t=\mu_t^*}=0,$$

which can be rearranged as

$$\frac{\partial \mu_t^*}{\partial n} = -\frac{\frac{\partial}{\partial n}\left[\frac{\partial r(\mu_t, \mu_t^m, n)}{\partial \mu_t^m}\Big|_{\mu_t^m=\mu_t}\right]_{\mu_t=\mu_t^*}}{\frac{\partial}{\partial \mu_t}\left[\frac{\partial r(\mu_t, \mu_t^m, n)}{\partial \mu_t^m}\Big|_{\mu_t^m=\mu_t}\right]_{\mu_t=\mu_t^*}}.$$

The denominator in this expression was found smaller than zero in our convergent stability condition. Therefore,

$$\frac{\partial \mu_t^*}{\partial n} \propto \frac{\partial}{\partial n}\left[\frac{\partial r}{\partial \mu_t^m}\bigg|_{\mu_t^m=\mu_t}\right]_{\mu_t=\mu_t^*}.$$

In other words, the direction of virulence evolution around an ESS as selective pressures change will be determined by the sign of the derivative of the fitness gradient with respect to the parameter that is changing. We differentiate the fitness gradient with respect to the key tuberculosis parameters that change with tuberculosis treatment expansion and increasing HIV prevalence. We also produce simulations of the fate of mutant tuberculosis strains under changing host and environmental selective pressures to describe the evolution of TB virulence.

5. Mutant fixation

The evolutionary invasion analysis we perform infers the direction of evolution under different selective pressures. The speed of such evolution is subject to the stochastic generation and extinction or fixation of mutant strains, and to the random propagation of resident and mutant strains in the context of genetic drift. The time to fixation or extinction, and the nature of random drift, will be affected by aspects of the environment that affect the transmission dynamics and pathogenesis of disease. A new mutant strain that has different transmissibility and virulence than the resident strain may therefore have a different time until establishment or extinction in populations with different HIV prevalence.

The rate of fixation or extinction in a population can be simulated with a classical Wright–Fisher model (Wright, 1931), in which offspring are sampled from the parental generation of cases, and the relative proportion of resident or mutant strains in a population can be followed over subsequent generations. Deterministically, we can simulate the proportion of tuberculosis cases caused by the mutant strain, m, with the expression:

$$m(t+1) = \frac{R_0^m m(t)}{R_0^m m(t) + R_0^r (1 - m(t))},$$

where R_0^m and R_0^r refer to the reproductive ratios of the mutant and resident strains, respectively (see Appendix for the derivation of R_0). In the context of random genetic drift, the stochastic formulation of this model can be simulated by using a binomial distribution to sample the offspring that will reproduce, where the probability of being sampled is given by the prevalence of the strain in each generation.

This formulation allows us to compare the emergence and potential fixation of a tuberculosis strain mutant in the context of a host environment without and with HIV. For example, a hypothetical mutant with altered virulence and transmissibility could be have

a higher reproductive ratio such that $R_0^m - R_0^r = 0.1$. In this case, the mutant is expected to become fixed, and the Wright–Fisher model can be used to simulate how many generations are required to achieve fixation and out-compete the resident strain. Now we can simulate the identical process, with same virulence and transmissibility among resident and mutant strains, and recalculate R_0^m and R_0^r in the context of a population with 20% HIV prevalence (as in some South African communities (Health Systems Trust, 2007), using the typical parameter values listed in Table 1). We can thus compare the rate of emergence of the mutant in the context of selection and drift in the highly HIV prevalent environment against its emergence and fixation in the absence of HIV.

6. Results

By differentiating the fitness gradient with respect to its component parameters, we analytically evaluated the how the ESS virulence would be affected by changes in selective pressures. We found that only a few parameters affect the fitness gradient in our model: the host mortality rate (which increases with HIV prevalence), rate of reactivation from latency (which increases with HIV prevalence), regression rate (which increases with successful treatment), and the transmission coefficient of the strain (which typically decreases for drug-resistant strains) (Dye and Espinal, 2001).

We found that increases in host mortality rate would be expected to select for elevated tuberculosis virulence at a convergently stable ESS, if

$$\left.\frac{d\beta}{d\mu_t^m}\right|_{\mu_t^m=\mu_t^*} > \frac{\beta(\mu_t^*)}{d+2\mu+\mu_t^*+v},$$

and the virulence would decrease or remain unchanged with rising host mortality otherwise.

We previously observed that, at the ESS,

$$\left.\frac{d\beta}{d\mu_t^m}\right|_{\mu_t^m=\mu_t^*} = \frac{(\mu+v)\beta(\mu_t^*)}{d\mu+(\mu+\mu_t^*)(\mu+v)}.$$

When evaluating this expression numerically, given the parameter values in Table 1, we found that increasing host mortality rates resulting from HIV would lead to increased virulence. Figure 2 displays the result of 10,000 Monte Carlo simulations of the emergence potential of new mutant strains using the parameter ranges displayed in Table 1.

Similarly, we found that tuberculosis virulence would rise with an increasing latent reactivation rate if

$$\left.\frac{d\beta}{d\mu_t^m}\right|_{\mu_t^m=\mu_t^*} > \frac{\beta(\mu_t^*)}{\mu+\mu_t^*},$$

and would decrease or remain unchanged otherwise. Using the parameter ranges from Table 1, we found that the virulence would again increase given the impact of HIV on the latent reactivation rate. The parameter describing the rate of latent reactivation among HIV-infected patients has been imprecisely estimated, however (Dye et al., 1998); thus, an

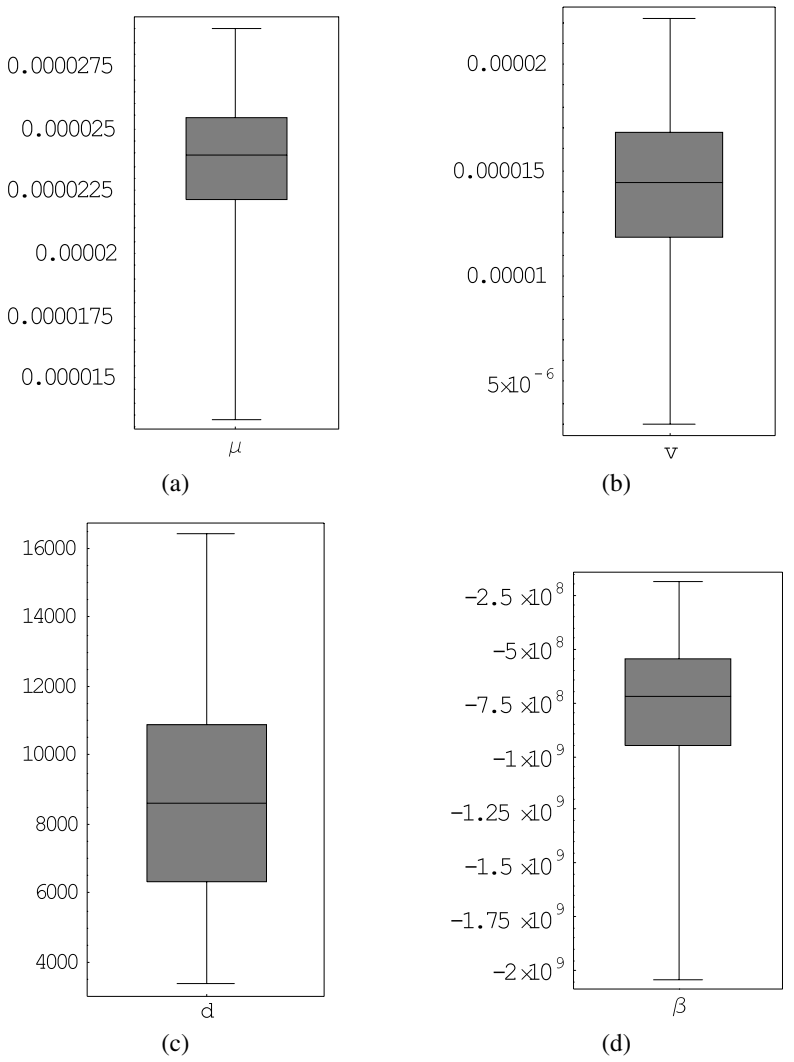

Fig. 2 The evolution of tuberculosis virulence. The boxplots indicate the direction of virulence evolution under changing selective pressures. Positive values indicate that higher virulence would evolve, and negative values indicate that lower virulence would evolve, as pressures change from the HIV-negative to the HIV-positive values (in the case of μ and v, plots **a** and **b**, respectively), as treatment improves (in the case of d, plot **c**), or as transmissibility increases (in the case of β, plot **d**). The boxplots display the median and interquartile range of 10,000 Monte Carlo simulations from the parameter ranges in Table 1. Whiskers extend to the extreme values obtained from each simulation. Note that the values on the y-axis are simply the numerical result of evaluating the expressions for the fitness gradient derivatives shown in the results section; they do not indicate the relative magnitude of the virulence change or selective pressure.

exact threshold of HIV prevalence above which this condition is fulfilled was not possible to determine.

The relationship of virulence evolution to the other two parameters in the fitness gradient expression was also examined. When the fitness gradient was differentiated with respect to the regression rate parameter (d), we found that virulence would increase with improved treatment rates whenever

$$\frac{\mu}{\beta(\mu_t^*)}$$

is positive. Since the parameters in this expression are always positive, the condition will always be fulfilled.

In contrast, the virulence of tuberculosis strains is expected to decrease with increasing transmissibility, since the differentiation of the fitness gradient with respect to β indicated that virulence would decrease with rising transmissibility whenever

$$-\frac{(d\mu + (\mu + \mu_t^*)(\mu + v))}{\beta(\mu_t^*)^2}$$

is negative. The component parameters are always positive, thus the expression is always negative. Consistent with this expression, newly emerging drug-resistant tuberculosis strains are typically less transmissible than resident drug-susceptible counterparts (Dye and Espinal, 2001). However, it is unclear whether the transmission coefficient will change in the context of HIV (Cruciani et al., 2001); the proportion of patients who become infectious has been thought to be reduced by HIV in some prior analyses (resulting in the parameter ranges shown in Table 1) (Dye et al., 1998). If true, this would further increase tuberculosis virulence, according to this expression.

Using the Wright–Fisher formulation to simulate the time to fixation of a new mutant where $R_0^m - R_0^r = 0.1$, we find that the new mutant will typically dominate within 200 generations of tuberculosis cases in an HIV-negative population (Fig. 3a). If we take the identical mutant and resident strains, having same virulence and transmissibility, and simulated their propagation in a population with 20% HIV prevalence, the rate of fixation of the new mutant nearly doubled (Fig. 3b).

7. Discussion

Our results provide theoretical insights into how the evolutionary pressures on *M. tuberculosis* can direct the evolution of tuberculosis virulence, using a simple model of tuberculosis pathogenesis. We find that the level of virulence toward which strains evolve can vary with the local treatment success rate, the relative transmissibility of emerging and resident strains, the rate of reactivation from latency among hosts, and the life expectancy of hosts. We find that TB virulence may increase in the context of rising HIV prevalence. The shortened life expectancy resulting from HIV, and the increasing tuberculosis reactivation rate among HIV-infected persons, leads to selective pressures that lead to increased TB virulence in our model. HIV is now a leading coinfection among new tuberculosis diagnoses (Friedland, 2007). Additionally, the speed of fixation of new robust mutants increases in the context of HIV, such that the evolution of tuberculosis strains may require

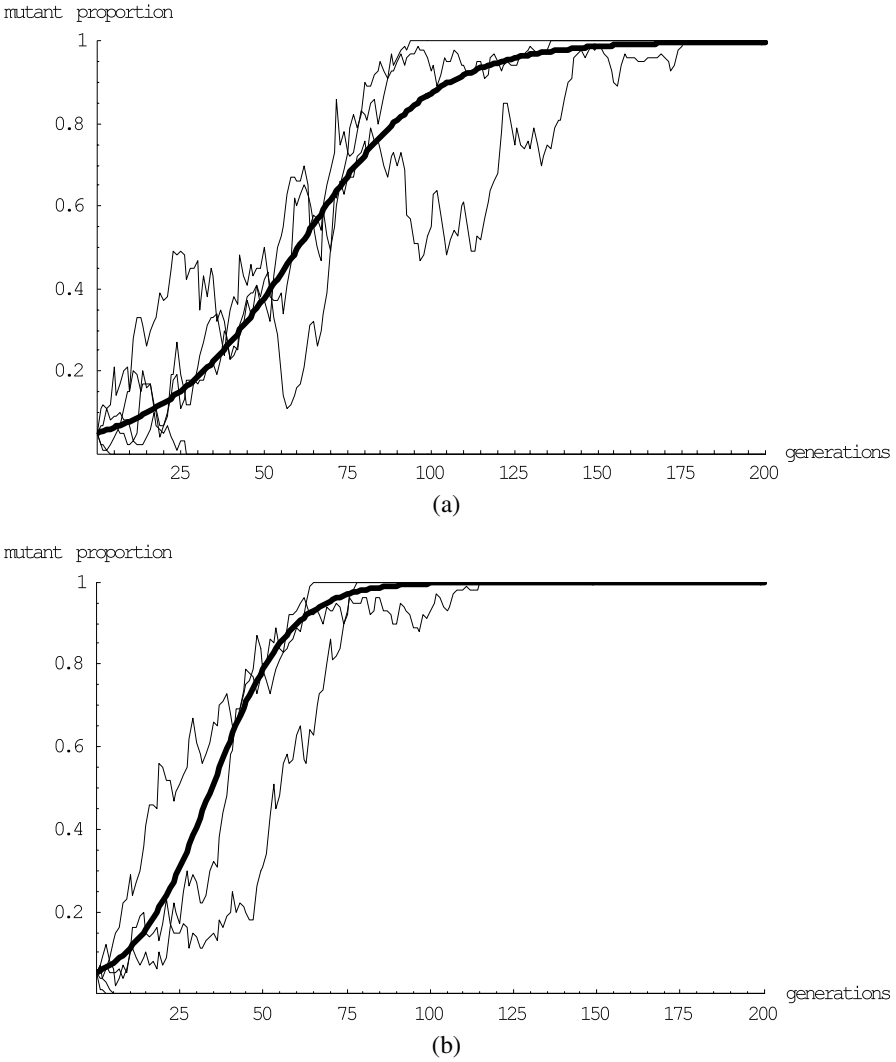

Fig. 3 Rate of tuberculosis evolution. The proportion of TB cases that are attributable to a mutant strain is plotted over time, in the context of selection and genetic drift. A population of 100 tuberculosis cases is simulated, where 5% are mutant at the start of the simulation. (**a**) The graphs illustrate the deterministic (*bold lines*) and five representative stochastic (*thin lines*) trajectories of fixation of a robust mutant where $R_0^m - R_0^r = 0.1$ and $R_0^r = 2.0$ in the HIV-negative environment (Dye et al., 2002). The mutant was created by increasing the rate of virulence by 0.01 from the typical HIV-negative value in Table 1, then calculating the transmission coefficient necessary to increase the reproductive ratio by 0.1 for such a mutant. (**b**) Trajectories in a population with 20% HIV prevalence. We recalculated R_0^m and R_0^r when the other parameters in the reproductive ratio formula (Appendix) were adjusted to simulate a population with 20% HIV prevalence (parameters given in Table 1), maintaining the transmissibility and virulence of the two strains the same.

less time to cause pathogenic changes in human disease. As the epidemics of tuberculosis and HIV collide, the increasing virulence of tuberculosis strains may require faster case detection to prevent increased mortality among tuberculosis patients. Currently, case detection can take an extended period of time (Special Programme for Tropical Diseases Research, 2006), though new diagnostic approaches have been developed that may allow detection to occur with greater speed (Moore et al., 2006).

Many patients in resource-poor areas with large HIV/TB coepidemics do not yet have access to antiretroviral therapy (World Health Organization, 2006). Antiretroviral therapy slows the rate of latent reactivation and increases the lifetime of hosts with HIV (Williams and Dye, 2003), which would lessen the impact of HIV on tuberculosis virulence according to the equations derived in this analysis. Therefore, expanding effective antiretroviral therapy programs may be a mechanism to avert the increase in tuberculosis virulence that we describe.

Our analysis suggests that increasing tuberculosis virulence may also be an inadvertent side-effect of expanding tuberculosis therapy. As tuberculosis treatment efforts are advanced in several areas that had previously low rates of treatment, rapid detection may be necessary to avert the mortality increase from the evolution of virulent strains.

The expansion of treatment efforts is typically accompanied by the emergence of drug-resistant tuberculosis strains. These strains often have lower transmissibility than their drug-susceptible counterparts. Our analysis assumes a classical virulence-transmissibility trade-off. However, this does not incorporate the impact of potential compensatory mutations that can lead to heterogeneities in the transmissibility of drug-resistant strains (Sherman et al., 1996). It is also unclear whether and how transmission coefficients for tuberculosis strains will change in the context of HIV's impact on demography. The degree of tuberculosis infectiousness among HIV-infected patients also appears to be heterogeneous; a recent meta-analysis has been inconclusive in determining whether HIV-infected persons are more or less infectious than HIV-negative patients, given the conflicting results from multiple studies (Cruciani et al., 2001). Recent analytical experiments suggest that the distributions of infectiousness are wide-ranging among HIV-infected patients, overlapping with the distribution of infectiousness among HIV-negatives (Escombe et al., 2007).

Evolutionary invasion analysis describes the common scenario in which resident tuberculosis strains have become endemic in populations, and new mutants appear to potentially challenge the resident strains (World Health Organization, 2007a). This analysis applies to those mutants that are generally small deviations from the resident strain, which is a typical scenario (Pillay and Sturm, 2007). However, rarely, large mutants emerge to which the ESS may not be resilient, and thereby can produce changes that evolutionary invasion analysis cannot anticipate.

Our study revealed that the continued evolution of tuberculosis strains should be considered as human demography changes with HIV, and as treatment efforts expand. In both cases, tuberculosis virulence may increase. Therefore, areas with high-HIV/TB coinfection, or areas in which tuberculosis treatment efforts are expanding, may be key regions for the introduction of new technologies to rapidly detect active tuberculosis (Keeler et al., 2006).

Appendix

To derive an analytical expression for R_0, we computed the Jacobian matrices of new (**F**) and transported (**V**) infections at the disease-free equilibrium:

$$F = \begin{bmatrix} 0 & (1-p)\beta \\ 0 & p\beta \end{bmatrix},$$

$$V = \begin{bmatrix} -(v+\mu) & d \\ v & -(\mu+\mu_t+d) \end{bmatrix}.$$

The dominant eigenvalue of the next generation matrix $\mathbf{F}(-\mathbf{V}^{-1})$ is R_0 (van den Driessche and Watmough, 2002)

$$R_0 = \frac{p\beta(\mu+v)}{d\mu + (\mu+\mu_t)(\mu+v)}.$$

Mutant invasion occurs where

$$\frac{p\beta_m(\mu+v)}{d_m\mu + (\mu+\mu_t^m)(\mu+v)} > \frac{p\beta(\mu+v)}{d\mu + (\mu+\mu_t)(\mu+v)},$$

which can be rearranged to the mutant growth rate expression as detailed elsewhere (Otto and Day, 2007).

As observed in prior analyses, reinfection of latently infected individuals does not affect the expression for the reproductive ratio (Blower and Chou, 2004). Some authors have observed that re-infections may lead to backwards bifurcations when $R_0 < 1$, implying that R_0 may not be a suitable threshold parameter to use when simulating the invasion of tuberculosis strains (Feng et al., 2000). However, we agree with other authors that such backward bifurcations only occur for a model using highly unrealistic assumptions, such that R_0 remains the appropriate critical threshold parameter for invasion analysis (Lipsitch and Murray, 2003).

References

Anderson, R.M., May, R.M., 1991. Infectious Diseases of Humans: Dynamics and Control. Oxford University Press, London.

Basu, S., et al., 2007. Prevention of nosocomial transmission of extensively drug-resistant tuberculosis in rural South African district hospitals: an epidemiological modelling study. Lancet 370(9597), 1500–1507.

Blower, S.M., Chou, T., 2004. Modeling the emergence of the 'hot zones': tuberculosis and the amplification dynamics of drug resistance. Nat. Med. 10(10), 1111–1116.

Blower, S.M., Gerberding, J.L., 1998. Understanding, predicting and controlling the emergence of drug-resistant tuberculosis: a theoretical framework. J. Mol. Med. 76(9), 624–636.

Blower, S.M., et al., 1995. The intrinsic transmission dynamics of tuberculosis epidemics. Nat. Med. 1(8), 815–821.

Boots, M., Sasaki, A., 1999. 'Small worlds' and the evolution of virulence: infection occurs locally and at a distance. Proc. Biol. Sci. 266, 1933–1938.

Bremerman, H.J., Thieme, H.R., 1989. A competitive-exclusion principle for pathogen virulence. J. Math. Biol. 27, 179–190.

Castro, K.G., Dooley, S.W., 1993. Mycobacterium tuberculosis transmission in healthcare settings: is it influenced by coinfection with human immunodeficiency virus? Infect. Control. Hosp. Epidemiol. 14(2), 65–66.
Cohen, T., Murray, M., 2004. Modeling epidemics of multidrug-resistant *M*. tuberculosis of heterogeneous fitness. Nat. Med. 10(10), 1117–1121.
Cohen, T. et al., 2006. Exogenous re-infection and the dynamics of tuberculosis epidemics: local effects in a network model of transmission. J. R. Soc. Interface.
Corbett, E.L., et al., 2006. Tuberculosis in sub-Saharan Africa: opportunities, challenges, and change in the era of antiretroviral treatment. Lancet 367(9514), 926–937.
Crofton, J., Horne, N., Miller, F., 1999. Pulmonary tuberculosis in adults. In: Clinical Tuberculosis. MacMillan, London.
Cruciani, M., et al., 2001. The impact of human immunodeficiency virus type 1 on infectiousness of tuberculosis: a meta-analysis. Clin. Infect. Dis. 33(11), 1922–1930.
Daley, C.L., et al., 1992. An outbreak of tuberculosis with accelerated progression among persons infected with the human immunodeficiency virus. An analysis using restriction-fragment-length polymorphisms. N. Engl. J. Med. 326(4), 231–235.
Di Perri, G., et al., 1989. Nosocomial epidemic of active tuberculosis among HIV-infected patients. Lancet 2(8678–8679), 1502–1504.
Dye, C., Espinal, M.A., 2001. Will tuberculosis become resistant to all antibiotics? Proc. Biol. Sci. 268(1462), 45–52.
Dye, C., Williams, B.G., 2000. Criteria for the control of drug-resistant tuberculosis. Proc. Natl. Acad. Sci. USA 97(14), 8180–8185.
Dye, C., et al., 1998. Prospects for worldwide tuberculosis control under the WHO DOTS strategy. Directly observed short-course therapy. Lancet 352(9144), 1886–1891.
Dye, C., et al., 2002. Erasing the world's slow stain: strategies to beat multidrug-resistant tuberculosis. Science 295(5562), 2042–2046.
Ebert, D., Bull, J.J., 2003. Challenging the trade-off model for the evolution of virulence: is virulence management feasible? Trends Microbiol. 11(1), 15–20.
ECA, 2006. Beijing/W genotype mycobacterium tuberculosis and drug resistance. Emerg. Infect. Dis. 12(5), 736–743.
Edlin, B.R., et al., 1992. An outbreak of multidrug-resistant tuberculosis among hospitalized patients with the acquired immunodeficiency syndrome. N. Engl. J. Med. 326(23), 1514–1521.
Ernst, J.D., Trevejo-Nunez, G., Banaiee, N., 2007. Genomics and the evolution, pathogenesis, and diagnosis of tuberculosis. J. Clin. Invest. 117(7), 1738–1745.
Escombe, A.R., et al., 2007. The detection of airborne transmission of tuberculosis from HIV-infected patients, using an in vivo air sampling model. Clin. Infect. Dis. 44(10), 1349–1357.
Eshel, I., 1983. Evolutionary and continuous stability. J. Theor. Biol. 103, 99–111.
Feng, Z., Castillo-Chavez, C., Capurro, A.F., 2000. A model for tuberculosis with exogenous reinfection. Theor. Popul. Biol. 57(3), 235–247.
Friedland, G., 2007. Tuberculosis, drug resistance and HIV/AIDS: a triple threat. Curr. Infect. Dis. Rep. 9(3), 252–261.
Friedland, G., Churchyard, G.J., Nardell, E., 2007. Tuberculosis and HIV coinfection: current state of knowledge and research priorities. J. Infect. Dis. 196(1), S1–S3.
Gandhi, N.R., et al., 2006. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet 368(9547), 1575–1580.
Githui, W.A., et al., 2004. Identification of MDR-TB Beijing/W and other mycobacterium tuberculosis genotypes in Nairobi. Kenya. Int. J. Tuberc. Lung. Dis. 8(3), 352–360.
Health Systems Trust, 2007. HIV Prevalence. HST, Durban.
Keeler, E., et al., 2006. Reducing the global burden of tuberculosis: the contribution of improved diagnostics. Nature 444(1), 49–57.
Keeling, M.J., Rohani, P., 2007. Modeling Infectious Diseases in Humans and Animals. Princeton University Press, Princeton.
Lan, N.T., et al., 2003. Mycobacterium tuberculosis Beijing genotype and risk for treatment failure and relapse. Vietnam. Emerg. Infect. Dis. 9(12), 1633–1635.
Lawn, S.D. et al., 2007. Early mortality among patients with HIV-associated TB in Africa: implications for the time to initiate ART. In: 14th Conference on Retroviruses and Opportunistic Infections, Los Angeles.

Levin, B.R., 1996. The evolution and maintenance of virulence in microparasites. Emerg. Infect. Dis. 2(2), 93–102.
Lipsitch, M., Murray, M.B., 2003. Multiple equilibria: tuberculosis transmission require unrealistic assumptions. Theor. Popul. Biol. 63(2), 169–170.
Mann, N.H., et al., 2003. Marine ecosystems: bacterial photosynthesis genes in a virus. Nature 424(6950), 741.
Moore, D.A., et al., 2006. Microscopic-observation drug-susceptibility assay for the diagnosis of TB. N. Engl. J. Med. 355(15), 1539–1550.
Otto, S.P., Day, T., 2007. A Biologist's Guide to Mathematical Modeling in Ecology and Evolution. Princeton University Press, Princeton.
Pillay, V., Sturm, A.W., 2007. Evolution of the extensively dRUG-resistant F15/LAM4/KZN strain of mycobacterium tuberculosis in KwaZulu-Natal, South Africa. Clin. Infect. Dis. 45(11), 1409–1414.
Sherman, D.R., et al., 1996. Compensatory ahpC gene expression in isoniazid-resistant mycobacterium tuberculosis. Science 272(5268), 1641–1643.
Small, P.M., et al., 1993. Exogenous reinfection with multidrug-resistant Mycobacterium tuberculosis in patients with advanced HIV infection. N. Engl. J. Med. 328(16), 1137–1144.
Smith, J.M., Price, G.R., 1973. The logic of animal conflict. Nature 246, 15–18.
Special Programme for Tropical Diseases Research, 2006. Diagnostics for Tuberculosis: Global Demand and Market Potential. WHO, Paris.
van den Driessche, P., Watmough, J., 2002. Reproduction numbers and sub-threshold endemic equilibria for compartmental models of disease transmission. Math. Biosci. 180, 29–48.
Williams, B.G., Dye, C., 2003. Antiretroviral drugs for tuberculosis control in the era of HIV/AIDS. Science 301(5639), 1535–1537.
Williams, B.G., et al., 2005. The impact of HIV/AIDS on the control of tuberculosis in India. Proc. Natl. Acad. Sci. USA 102(27), 9619–9624.
World Health Organization, 2005. The Impact of HIV on TB in Africa. WHO, Paris.
World Health Organization, 2006. Progress on Global Access to HIV Antiretroviral Therapy: A Report on "3 by 5" and beyond. WHO, Paris.
World Health Organization, 2007a. Global Tuberculosis Control: Surveillance, Planning, Financing. WHO, Paris.
World Health Organization, 2007b. Global Tuberculosis Control—Surveillance, Planning, Financing. WHO, Paris.
Wright, S., 1931. Evolution in Mendelian populations. Genetics 16, 97–159.