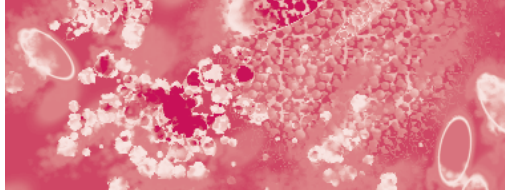

The effect of drug resistance on the fitness of *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis remains a leading infectious cause of morbidity and mortality. While antibiotic resistance is cited as a potential threat to efforts aimed at controlling the spread of this pathogen, it is not clear how drug resistance affects disease dynamics. The effect of mutational events that lead to antibiotic-resistant phenotypes may or may not have a predictable effect on the fitness of drug-resistant tuberculosis strains. Here, we review the literature on laboratory studies of the fitness of drug-resistant tuberculosis, we examine the evidence from cluster studies, and we consider the effect of drug resistance on disease dynamics in mathematical models. On the basis of these diverse lines of evidence, we conclude that the fitness estimates of drug-resistant *M tuberculosis* are quite heterogeneous and that this variation may preclude our ability to predict future trends of this pathogen.

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Much recent attention has focused on assessing the global burden of multidrug-resistant tuberculosis (MDRTB) and predicting the future threat of this pathogen. Despite this attention, there is little consensus on either the magnitude of the problem or its future trend. Some investigators predict a global pandemic of MDRTB whereas others see it as a local problem that can be managed by the proper implementation of currently recommended strategies. If the mutations that lead to multidrug resistance exert a cost on the reproductive effectiveness of the organism, we may expect these strains to be less widely transmitted than drug-sensitive strains. In that case, the main burden of drug resistance will be in people who are infected by sensitive strains and acquire resistance during treatment; therefore, measures that prevent acquisition of resistance mutations during treatment should sharply reduce the incidence of multidrug resistance. However, such strategies will have little effect on those who are initially infected with a drug-resistant strain of tuberculosis. Much of this debate centres on the relative "fitness" of drug-resistant strains. The evidence for and against the fitness cost of drug resistance in tuberculosis comes from several scientific specialties that often use different language to convey similar ideas. Here, we describe current approaches to estimating the fitness of MDRTB, review the available data, and consider potential methodological problems that may contribute to conflicting results.

The concept of "fitness" is derived from the disciplines of ecology and evolutionary biology and implies the existence of heritable variation among individual members of a species. For infectious pathogens, fitness is a composite measure of an organism's ability to survive, reproduce, and be transmitted. It may indicate an individual's growth characteristics within its host, ability to withstand within-host and between-host environmental stresses, and capacity to disseminate and set up residence in a new host. Some of these characteristics can be quantified in the laboratory, although their precise contribution to the empiric success of an individual in the "real world" may not be clear. The laboratory approaches to estimating the fitness of drug-resistant tuberculosis include measurement of growth rates, infectivity in animal models, and ability to withstand specific challenges. Another way to assess the fitness of a transmissible organism is to consider its effectiveness in terms of its epidemic potential. Epidemic potential may be quantified by estimating either the average number of secondary infections or secondary cases of disease caused by a specific genotype after its introduction to an entirely susceptible population. These estimates rely on epidemiological evidence—including cluster studies, traditional epidemiological investigations, and model-based estimates of average fitness—in human populations rather than studies of microbial behaviour in the laboratory.

Laboratory studies

There are over 15 different antituberculosis agents used in clinical practice. We have focused our review on those drugs that have been most extensively studied and are the most important agents in recommended treatment strategies. Postulated mechanisms of resistance and potential effects on fitness are considered first for isoniazid, the antibiotic that has undergone the most scrutiny, and then for other important first-line agents. Table 1 provides a summary of these mechanisms.

Isoniazid resistance and effects on fitness

Isoniazid was first introduced for the treatment of *Mycobacterium tuberculosis* in 1952 and because of its relative efficacy and low toxicity it has become one of the mainstays of first-line therapy for tuberculosis. Although it is also the most intensively studied of antimycobacterial

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agents, the molecular mechanisms of its bactericidal activity are still not well understood.[1] Nonetheless, it is clear that isoniazid is a prodrug that is metabolised to its active form by the bacterial enzyme, catalase-peroxidase (KatG), and that the activated prodrug then exerts its effect by interfering with mycolic acid synthesis.

Isoniazid resistance due to katG *mutations*

Most isoniazid resistance in clinical isolates results from blocking prodrug activation. Mutations in the gene *katG* that alter or eliminate mycobacterial catalase-peroxidase activity can prevent activation of the prodrug and induce isoniazid resistance. Although *katG* insertions, deletions, and frameshifts do occasionally happen and induce complete loss of the functional gene product and correspondingly high rates of isoniazid resistance, most mutations identified in clinical isolates are single-point mutations that result in intermediate resistance.[1] The isoniazid-resistance mutation most commonly seen in clinical isolates is a Ser315Thr point mutation in *katG*.[2,3] This variant manifests reduced capacity for prodrug activation while retaining about half the catalase-peroxidase activity of the wild-type enzyme.[4]

Pathogenicity of isoniazid-resistant katG *mutant strains*

Soon after isoniazid came into use, Middlebrook and Cohn[5] reported that isoniazid-resistant strains of tubercle bacilli were less pathogenic or avirulent in guineapigs in comparison with parent isoniazid-sensitive strains. This was true both for isoniazid-resistant variants developed in the laboratory through exposure to isoniazid-imbued media and for some, but not all, isoniazid-resistant strains isolated from treated patients. In the first case, isoniazid-resistant *Mycobacterium bovis* strains killed guineapigs at 33 and 43 days after infection, whereas sensitive strains killed the comparison animals at 12 and 19 days. Animals infected with isoniazid-resistant H37Rv strains remained alive at 60 days, whereas comparison guineapigs infected with isoniazid sensitive strains died at 19 and 25 days. Both of the attenuated resistant strains were reported to be "highly" resistant and to lack any detectable catalase activity. By contrast, the resistant clinical strains showed varying rates of resistance and pathogenicity. Three of 11 strains were "highly" resistant to isoniazid; all of the guineapigs infected with these strains survived, whereas all of those infected with less resistant strains died within 60 days of infection. None of the clinical isolates were tested for catalase activity. Although this work has been cited as evidence that the acquisition of isoniazid resistance attenuates *M tuberculosis*, these data are also consistent with the conclusion that isoniazid-resistant strains are heterogeneous in terms of resistance and pathogenicity.

That conclusion is also supported by subsequent work by Ordway et al,[6] who examined the growth of 15 clinical *M tuberculosis* isolates in the lungs of mice after aerosol exposure. The isolates were selected at random and included isoniazid-resistant strains with and without catalase activity, as well as susceptible and multidrug-resistant strains. Growth patterns fell into three distinct categories which were classified as "avirulent" (defined as growth rate slower than Erdman laboratory strain), "virulent" (defined as growth rate equivalent to Erdman laboratory strain), and "fast-growing" (defined as growth rate faster than Erdman laboratory strain). No correlation between drug-resistance profile (or catalase activity) and growth rate in infected mice was reported. In fact, some catalase-negative isoniazid-resistant strains fell into the fast-growing category and some susceptible strains were apparently avirulent. The investigators reported that in-vitro growth rates did not correspond to in-vivo rates and that several of the fast-

Table 1. Summary of molecular mechanisms of resistance to first-line anti-tuberculosis drugs, postulated fitness costs, and compensatory mutations.

Drug (drug action) and resistance genes (function)	Putative mechanism of resistance	Fitness costs of mutation	Compensation for mutation
Isoniazid (inhibit mycolic acid synthesis)			
katG (catalase-peroxidase)	↓ prodrug activation	↓ protection from oxidative damage	↑ *aphC* may restore detoxification capacity
aphC (alkyl hydroperoxide reductase)	1) promoter mutations give ↑ *aphC*; compensates for *katG* mutations 2) ? ↑ *aphC* causes ↓ prodrug activation	?	?
inhA (enoyl-acp reductase) and *kasA* (ß-ketoacyl-ACP synthase)	Over-production of drug targets	?	?
nadh (NADH dehydrogenase)	? effect of ↑ NADH/NAD ratio	?	?
Rifampicin (inhibit transcription)			
rpoB (RNA polymerase-subunit B)	↓ binding of drug to polymerase	?↓ transcription efficiency	?
Streptomycin (inhibit translation)			
rpsL (ribosomal protein unit 12) and *rrs* (16S rRNA)	↓ binding of drug to ribosomal target	?↓ translation efficiency	Secondary *rspD* and *rpsE* mutations may restore translation efficiency
Pyrazinamide (unknown drug action)			
pncA	↓ prodrug activation	?	?

growing strains grew more slowly in vitro than the Erdman laboratory strain to which they were compared. Although this study did not directly compare isogenic strains that differed only in terms of specific drug-resistance mutations, it did show that the growth rates reported in clinical isolates may vary independently of their drug-resistance profile.

Li et al[7] tested the hypothesis that KatG functions as a virulence factor for *M tuberculosis* by comparing *katG*-deleted H37Rv to recombinants in which *katG* had been restored. As expected, KatG null, isoniazid-resistant *M tuberculosis* lost growth capacity that was subsequently restored by transformation with wild-type tuberculosis *katG* genes. When the organisms were transformed with *katG* genes that contained single point mutations, they either regained their original growth rates or did not, depending on the location of the mutation. These data again suggest that *katG* deletions may result in a different phenotype in terms of growth and virulence than *katG* point mutations that nonetheless confer some degree of isoniazid resistance.

Compensatory mutation in isoniazid-resistant katG *mutants*

Several groups have suggested that bacterial antibiotic-resistance mutations that incur an initial fitness cost may be compensated by later mutations that restore an organism's reproductive potential. Based on their studies of resistance in *Staphylococcus aureus*, Nagaev et al[8] suggested "that fitness-compensatory mutations may be an important aspect of the evolution of antibiotic resistance in the clinical environment, and may contribute to a stabilisation of the resistant bacteria present in a bacterial population". Sherman et al[9] suggested that such a mechanism may pertain to KatG-null isoniazid-resistant mutants. This group showed that when isoniazid-resistant *M bovis* (BCG) strains were selected on solid media, they were highly sensitive to oxidative stress. By contrast with these strains, clinical KatG-null isoniazid-resistant *M tuberculosis* isolates were shown to have mutations in the promoter of the gene *ahpC*. This gene codes for an alkyl hydroperoxide reductase that protects bacilli from the toxic effects of organic peroxides. Mutations in the *ahpC* promoter lead to overexpression of AhpC. These and other studies have led investigators to propose a model of isoniazid resistance in which overexpression of AhpC compensates for the loss of catalase-peroxidase activity by maintaining the ability to withstand oxidative stress mediated through organic peroxides.

Subsequent studies of the role of *ahpC* in isoniazid resistance have yielded inconsistent results. For example, Heym et al[10] again showed that isoniazid-susceptible mycobacteria grew better in immunocompetent mice than did in-vitro-selected isoniazid-resistant strains with very low rates of catalase activity. In this study, impaired growth was noted even in those *katG* mutants that overexpressed AhpC. By contrast, Wilson et al[11] used anti-sense RNA constructs to reduce levels of AhpC in wild type and clinically derived isoniazid-resistant catalase-negative strains. Virulence was significantly reduced in both susceptible and resistant strains with the reduction of AhpC levels. Wilson et al suggest that the discrepancy between their results and those of Heym et al may derive from differences in isoniazid-resistant strains obtained in vitro and those obtained from clinical sources. They argue that clinical strains may have acquired other, not yet identified, mutations that compensate for a residual deficit that the overexpression of AhpC does not completely replace.

Others have suggested that *ahpC* may have a more direct role in isoniazid resistance. Telenti et al[12] proposed that AhpC may be a primary contributor to resistance, noting that a significant percentage of isoniazid-resistant clinical isolates had *ahpC* promoter mutations even in the absence of *katG* mutations. Zhang et al[13,14] hypothesised that excess AhpC may act independently from KatG to block activation of isoniazid.

Isoniazid resistance resulting from other mutations

Other mechanisms of isoniazid resistance seen in clinical *M tuberculosis* isolates involve mutations in genes encoding potential targets of the activated prodrug. Activitated isoniazid interferes with the fatty acid synthetase system (FAS) and leads to cell-wall injury through the disruption of mycolic acid synthesis. Two of the enzymes involved in the FAS system have been implicated in isoniazid resistance. These are encoded by the genes *inhA* and *kasA*; mutations within both of these genes have been found in resistant patient isolates, but are rarely reported in isoniazid-sensitive strains. Some investigators contend that *inhA* inactivation alone accounts for the antibacterial effect of isoniazid,[15] whereas other groups have proposed that mutations in *kasA* have an important but not yet established role in isoniazid resistance. In addition, mutations in the gene *ndh* have been shown to retard NADH oxidation and to result in high NADH/NAD ratios. *Ndh* mutants have also been linked to combined resistance to isoniazid and ethionamide in *Mycobacterium smegmatis*[16] and to be present in isoniazid-resistant isolates of *M tuberculosis* without other known resistance mutations[7]. In each of these cases, it is unclear whether the reported mutations lead directly to isoniazid resistance or whether they are secondary responses to an as yet unidentified mutation that inactivates the actual target of isoniazid action.

Although in general resistance to antibiotics is widely believed to reduce the fitness of bacteria in the absence of antibiotics, there are no current data available to suggest that isoniazid resistance-causing mutations in *inhA*, *kasA*, or *ndh* impose a fitness cost on *M tuberculosis*.

Resistance to other antituberculosis agents and effects on fitness

Rifampicin resistance

Rifampicin is another widely used first-line antimycobacterial agent to which resistance has become increasingly common in the past decade. Rifampicin acts by binding to bacterial RNA polymerase and disrupting mRNA synthesis. Clinical resistance to this agent results almost exclusively from mutations in the *rpoB* gene that encodes the beta subunit of the bacterial RNA polymerase. Billington et al[17] investigated the in-vitro fitness cost of rifampicin resistance by inducing resistance in *M tuberculosis* H37Rv and measuring fitness with a competitive assay that

estimated the number of generations of comparative strains growing on a drug-free medium. The relative fitness of resistant and sensitive strains was calculated from a simple ratio of the recorded number of generations of rifampicin-resistant to rifampicin-sensitive isolates. Although several mutants were much less fit than their parent strains, isolates of one pattern had comparative fitness values ranging from 0·5–1·2 on different runs of the assay, with a mean of 0·85. This isolate had a Ser531Leu pattern that was identical to the mutation most commonly seen in clinical rifampicin-resistant isolates. These authors conclude that the relative fitness of rifampicin-resistant strains are heterogeneous even when fitness is measured on newly isolated organisms that had no opportunity to develop compensatory mutations. They further suggest that many organisms, including *M tuberculosis,* may have the capacity to adapt to this initial loss of fitness over time and that compensatory mutations may restore or even improve baseline fitness.

Reynolds[18] showed a similar trend of initial fitness cost associated with *rpoB*-based rifampicin resistance in *Escherichia coli,* with a small proportion of resistant isolates showing increased growth. In that study, no association between the rate of rifampicin resistance and the fitness cost of the mutation that produced the corresponding phenotype was seen. Additionally, when low-fitness drug-resistant mutants were "evolved" over 200 generations in vitro each showed increased fitness when compared with its progenitor. Fitness recovery was never associated with reversion to rifampicin sensitivity.

Streptomycin resistance

Like other aminoglycosides, streptomycin acts at the ribosome to interrupt bacterial protein synthesis. Resistance to streptomycin is largely encoded by mutations of ribosomal genes *rpsL* and *rrs,* which block drug binding to the ribosomal target.[19,20] Studies of resistant *E coli* with ribosomal mutations of *rpsL* show significant initial decreases in translation efficiency and consequent fitness costs.[21,22]

More recently, however, several investigators have independently noted evolutionary patterns among resistant strains that improve the fitness costs of antibiotic resistance without compromising the drug-resistant phenotype.[23,24] Bjorkman et al[25] saw that certain mutations of *rpsL* in *Salmonella typhimurium* that cause resistance to (and in some cases dependence on) streptomycin also lead to an initial decrease in the translation efficiency and lower growth rate in a mouse model (restrictive mutations). However, other *rpsL* mutations did not impose any fitness cost (non-restrictive mutations). In-vitro studies of the restrictive strains have shown that compensatory mutations within two gene regions, *rpsD* and *rpsE,* seem to mitigate the translation hyperaccuracy that is responsible for the low growth rates in these *rpsL* mutants. These secondary mutations were capable of restoring the growth rate to near wild-type levels when the bacteria were reintroduced into animals.[26] When they were introduced into a mouse model, restrictive strains developed secondary compensatory mutations within the *rpsL* region but did not show the compensatory mutations in *rpsD* and *rpsE* that were seen in vitro. Furthermore, Bottger et al[27] report that high-level streptomycin resistance acquired in vivo is almost exclusively associated with non-restrictive *rpsL* mutations, and therefore is not likely to confer any fitness disadvantage.

Pyrazinamide resistance

Pyrazinamide is a prodrug that must be metabolised by mycobacterial pyrazinamidase to pyrazinoic acid, which interferes with fatty acid synthesis.[28] Most mutations that provide pyrazinamide resistance in clinical tuberculosis isolates are seen in *pncA* gene region and impair pyrazinamidase-mediated activation of the prodrug[29] although other mutations may interfere with drug uptake or efflux.[30] There are few experimental data that compare the fitness of pyrazinamide-resistant with pyrazinamide-sensitive *M tuberculosis* strains. Nonetheless, it is interesting to note that the virulence of constitutively pyrazinamide-resistant *M bovis* is not attenuated. Although transmission of this mycobacteria happens through exposure to infected animals and only rarely through human-to-human transmission, *M bovis* is a virulent organism that produces a clinical syndrome indistinguishable from tuberculosis in humans.

Multiple-drug and multidrug resistance

Simultaneous resistance to more than one antituberculosis drug results from the independent accumulation of individual resistance-generating mutations. These mutations are identical to those that are seen in isolates that are singly resistant.[31] Only one laboratory study has examined the relative fitness of multidrug-resistant strains compared with drug-sensitive strains.[32] This report described two clinical cases of tuberculosis in adult siblings; one of these patients was non-compliant with medication and developed resistance to six drugs while the other had an isolate that remained fully sensitive to standard therapy. A common source of these infections was suspected; this hypothesis was supported by the fact that molecular fingerprints of the patients' isolates were found to be indistinguishable. The isolates were assessed using an in-vitro fitness assay that compared the number of generations over time of the resistant strain and the sensitive strain. This comparison showed the multidrug-resistant strain to be significantly less "fit" than the matched sensitive strain with a relative fitness of 0·73. Despite this reduced in-vitro fitness, the multidrug-resistant strain produced cavitary disease and resulted in the patient's death. Thus, the authors conclude that such a measure of fitness may have little bearing on the virulence of the organism in its natural host.

Lessons from laboratory studies

While it is difficult to reach any substantive conclusions from these sometimes conflicting laboratory data about the fitness cost of drug resistance in *M tuberculosis*, the evidence highlights several points. First, there is significant heterogeneity in the fitness phenotypes of drug resistant organisms which may or may not correlate with the degree of resistance manifested or with the specific mutation(s) that

lead to resistance. Second, in-vitro assays of fitness may not correspond to in-vivo assays of virulence in animal models or to the capacity of an organism to cause disease in human beings. Third, even in the event that a fitness cost is incurred by a specific drug resistance mutation, compensatory mutations may restore or increase the fitness of the organism.

Population-based studies

Epidemiologists have used several methods to estimate the relative fitness of drug-resistant compared with drug-sensitive *M tuberculosis*. The purpose of these studies is to compare the basic reproductive number (R_0) of resistant and sensitive organisms and thus establish whether a person who harboured a resistant strain would cause the same number of secondary cases as a person with a sensitive strain. In principle, since R_0 represents the cases caused by a single infectious host in an entirely susceptible population, it is a hypothetical construct; epidemiologists rarely, if ever, have the opportunity to count the number of secondary cases produced by the first infectious person in a population without any acquired immunity. Instead, researchers can compare the numbers of people who were infected or who developed disease with drug-sensitive and drug-resistant strains, assuming that the people infected with and exposed to these different strains are equal in all other ways. This approach generates studies that compare the frequency and size of "clusters" of cases of drug-resistant and drug-sensitive *M tuberculosis*. A cluster is defined as a group of cases in a community whose isolates share similar or identical DNA fingerprints and are therefore presumably "epidemiologically" related—ie, a cluster includes members of a transmission chain or network.

There are, of course, many ways in which people infected with or exposed to multidrug-resistant and drug-sensitive strains may not be equivalent and a systematic difference between them could lead to a bias in the estimate of fitness. For example, people infected with or exposed to multidrug-resistant strains in the USA might be more likely to be homeless or in prison than those infected with or exposed to drug-sensitive strains. Since incarceration[33] and homelessness[34] are independent risk factors for having a positive tuberculin skin test (TST), this group may be partly immune to subsequent infection and thus less likely to be infected with a drug-resistant strain. Conversely, in some populations, people exposed to multidrug-resistant strains may be especially likely to be infected with HIV, as was the case during the early 1990s outbreak of MDRTB in New York City.[35] Although HIV infection is not known to modify the risk of infection with *M tuberculosis*, it does greatly increase the risk of disease progression after infection and, therefore, may increase the likelihood of disease given exposure.[36] A high prevalence of HIV among exposed people may thus bias an estimate of the fitness of MDRTB, making fitness seem higher than it would be if it had been measured in a non-HIV-infected population. To address this bias some cluster studies have done analysis stratified on potential confounders such as HIV infection, while other studies have been too small to permit such an approach.

A second way to estimate relative transmissibility of drug-resistant and drug-sensitive strains is to compare the secondary attack rates of resistant and sensitive strains. This method requires the researcher to compare the number of secondary infections resulting from a single drug-resistant case with those resulting from a single drug-sensitive case.

Cluster studies

There have been studies of the molecular epidemiology of tuberculosis in many different populations throughout the world. In some, the frequency and sizes of clusters involving drug-resistant and drug-sensitive strains have been explicitly compared and an odds ratio for the effect of drug resistance on clustering has been reported. In others, data has been presented that makes this comparison possible in retrospect. Table 2 summarises the results of the reports in which odds ratios were either reported or could be calculated. The studies incorporate various settings, different rates of HIV prevalence in study participants, and use a range of statistical approaches to these data. This table illustrates the heterogeneity of population-based estimates of the comparative fitness of sensitive and multidrug-resistant strains.

The results vary widely even among cluster studies that describe very similar populations. For example, in a study of tuberculosis dynamics in New York City in the early 1990s, Alland et al[47] saw that resistant organisms comprised nearly half the isolates and resistance to any drug was significantly associated with clustering (adjusted OR 4·52, 95% CI 1·75–13·01). However, a study of patients in San Francisco during the same period did not find any association between drug resistance and clustering.[48]

There is some evidence that these disparate results may indicate heterogeneity in the specific drug-resistance profiles of the isolates studied. A large study in the Netherlands found that isoniazid-resistant strains were less likely to be clustered than sensitive strains (OR 0·7, 95% CI 0·5–0·9), whereas streptomycin-resistant strains were more often clustered than their sensitive counterparts.[43] When this group further stratified their results on the specific mutations causing isoniazid resistance, they saw that mutations in *katG* resulting in aminoacid substitutions at the 315 position were more likely to be clustered than other isoniazid-resistance-conferring mutations, and equally likely to be clustered as the susceptible strains.[44] However, in a much smaller study, investigators reported no clustering among strains with a Ser315Thr mutation despite the fact that this mutation occurred in 22 of 24 isoniazid-resistant isolates from a group of Russian patients.[49]

Molecular epidemiologists have also used these data to estimate the proportion of drug-resistant tuberculosis cases that has arisen through the transmission of drug-resistant strains and the proportion of cases that has been acquired through inadequate therapy. Like the cluster analyses cited above, efforts to quantify the contribution of primary and acquired resistance among drug-resistant isolates in populations have used different study designs and arrived at different conclusions. In Scotland, a country with low incidence of both drug-susceptible and drug-resistant tuberculosis, an analysis of a very small sample (n=10) of

MDRTB isolates showed substantial IS6110 heterogeneity. This diversity led the authors to conclude that transmission of resistant bacilli was not a major problem in that region.[50] An analysis of 167 drug-resistant strains from patients in Germany, another low prevalence country, showed a higher proportion of clustered cases implying that the transmission of drug-resistant strains accounts for a larger proportion of the drug-resistant tuberculosis in that country.[51] A study in a much higher-incidence setting in Cape Town, South Africa, saw the proportion of clustered cases among resistant isolates to be 60%, again leading researchers to infer that much resistant tuberculosis is acquired through the transmission of resistant strains.[52] A recent molecular epidemiological study of patients with persistent disease showed that the clinical definition of acquired drug resistance (due to treatment failure) may be unreliable.[53] In this retrospective study in a high-incidence setting, 11 patients with initially drug-susceptible isolates were treated and had multiple smear-positive follow-up sputum specimens. Five of these 11 patients were subsequently found have MDRTB. In each of these cases, IS6110 typing showed that the multidrug-resistant isolate had a different molecular fingerprint than the original infecting strain. This study shows the inadequacy of the clinical definition of treatment failure as well as the ability of multidrug-resistant isolates to infect those with partial immunity.

Since cluster studies only include people who have tuberculosis and not the cohort of people who are at risk for infection, they are relatively easy to conduct. Nonetheless, this case-only approach can lead to bias if the people exposed to drug-resistant tuberculosis differ systematically from those exposed to sensitive strains. Another difficulty with inferring the relative transmissibility of strains from the cluster size of those strains involves the stochastic nature of infectious disease transmission. The number of secondary cases caused by a single infectious case (R_0) is an average; in practice, we expect that the numbers of people infected by an

Table 2. Odds ratios of clustering of drug resistant versus drug susceptible isolates and odds ratios of clustering among HIV positive versus HIV negative individuals in recent studies

Study (setting)	Total isolates	Comparison group	OR resistant cluster (95% CI)	% isolates from HIV+	OR HIV+ cluster (95% CI)
Toungoussova et al Russia, 2002[37] (regional)	119	MDR Any single drug resistant Streptomycin resistant Rifampicin resistant Isoniazid resistant Ethambutol resistant	9·2* (2·4–47 7) 4·3* (1·8–10·4) 3·2 (0·9–10·7) 3·2 (1·3–44·8) 1·1 (0·3–4·2) 0·4 (0·1–1·7)	0	Not defined
Kruuner et al Estonia, 2001[38] (country-wide)	209	MDR	2·8* (1·0–7·8)	0	Not defined
Hannan et al. Portugal, 2001[39] (HIV hospital unit)	134	MDR	∞	100	Not defined
Fandinho et al. Brazil, 2000[40] (2 inpatient hospitals)	120	MDR Any single-drug resistant	6·5* (1·5–28·5) 2·8* (0·9–9·2)	31†	0·7* (0·3–1·7)
Garcia-Garcia et al. Mexico, 2000[41] (community)	326	MDR	0·31 (0·12–0·81)	3	‡
Godfrey-Faussett et al. South Africa, 2000[42] (occupational cohort; gold miners)	371	MDR Any single-drug resistant	0·27 (0·09–0·83) 1·49 (0·68–3·26)	48	0·8* (0·5–1·2)
van Soolingen et al. Netherlands, 1999 & 2000[43,44] (country-wide)	4266	All isoniazid resistant aa315 isoniazid-resistant (Dutch) aa315 isoniazid-resistant (others) All streptomycin-R	0·7 (0·5–0·9) 1·6 (0·5–6·4) 0·8 (0·5–1·1) 1·2* (0·9–1·5)	4§	1·2* (0·9–1·6)
Diaz et al. Cuba, 1998[45] (country-wide)	160	Any drug resistant	6·0* (1·8–22·5)	NA	‡
Samper et al. Spain, 1998[46] (community)	226	Any drug resistant	0·15* (0·01–1·18)	44‖	0·6* (0·3–1·3)
Alland et al. USA, 1994[47] (community)	104	Any drug resistant MDR	4·52 (1·75–13·01) 6·5* (1·5–33·3)	43	4·0* (1·6–10·4)

*Unadjusted OR. †97/120 (81%) of study population was tested for HIV.‡Data necessary to calculate OR not provided. §181/4266 (4%) were known to be HIV+; since the whole sample was not tested, the actual HIV prevalence is unknown; OR for HIV+ cluster for this study is based on the assumption the HIV status of all unknown subjects was negative. ‖126/226 (56%) of study population was tested for HIV. NA=HIV data was not available

infectious case will vary because of random events. For example, one infectious host may travel on a crowded and poorly ventilated aeroplane infecting many others, whereas another may be sentenced to solitary confinement and have minimum opportunity to expose others to his pathogen. The cluster sizes for the culprit organisms will vary widely for these two scenarios even if the infecting strains do not differ in any way. Sampling introduces another source of bias in cluster studies. The figure gives a simplified illustration of this sampling bias.

Secondary attack rates

A more direct approach to estimating the transmissibility of two strains is to measure and compare the rates of infection in two cohorts of people exposed to different strains. Studies designed to estimate the secondary attack rates of drug-resistant and drug-sensitive *M tuberculosis* compare the number of people with a positive TST (presumably infected with tuberculosis) and/or cases of clinical tuberculosis among the household contacts of source cases. One retrospective analysis reported that paediatric household contacts of patients with drug-resistant tuberculosis were less likely to be infected or have active tuberculosis than a similarly aged cohort of household contacts of drug-sensitive cases.[54] However, two more recent studies of household contacts of tuberculosis patients in both high-prevalence and low-prevalence areas have not found differences in secondary attack rates based on drug susceptibility. Using a random sample of cases from *M tuberculosis* registries in the USA, Snider et al[55] saw that the proportions of TST-positives were similar among the young household contacts of drug-resistant and drug-susceptible cases. A study in Brazil that assessed both infection and progression to disease found that whereas the multidrug-resistant cases were, on average, infectious for a longer period of time, neither the prevalence of TST positivity nor the rate of clinical tuberculosis among household contacts of cases was associated with the drug susceptibility profile of the index case's isolate. Using the molecular typing techniques described previously, these researchers found that every secondary case tested was infected by a strain with an identical IS6110 pattern as the index case in that house.[56]

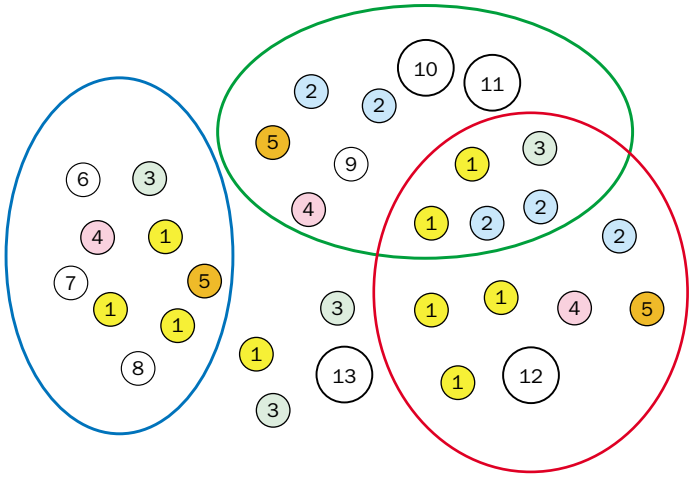
Lessons from population-based studies

First, population-based estimates of fitness are as heterogeneous as laboratory-based estimates. Second, fitness estimates derived from cluster studies are subject to biases that arise from the interpretation of population-based studies in the presence of confounding or stochastic processes. Variance in fitness estimates may reflect true phenotypic differences in the organisms or be an artifact of these methodological problems. Third, efforts to estimate the fitness cost of drug resistance may have less heterogeneous results if there are analyses on the effect at the level of specific mutation rather than at the level of drug-resistance phenotype.

Effects of fitness estimates in epidemic models

The debate about the fitness cost of drug resistance in tuberculosis is of more than academic interest. Questions concerning the relative fitness of drug-resistant tuberculosis have a central role in the uncertainty surrounding current treatment guidelines and goals, and their ability to prevent the further emergence of drug-resistant disease. Several mathematical models have been developed to predict tuberculosis dynamics and to examine how key parameters may affect the success or failure of current policy decisions. Blower and Geberding[57] developed a transmission model in which they considered the effect of the relative fitness of drug-resistant strains on the success of WHO-recommended treatment policies. Sensitivity analysis of relative fitness allowed for three possibilities: (1) that transmissibility is the same for susceptible and resistant strains; (2) that transmissibility is slower for the resistant strain due to a putative fitness cost; or (3) that transmissibility is in fact greater for the resistant strain. In this model, the authors allowed for the development of "fast" (due to primary disease) and "slow" (due to latency and subsequent reactivation) sub-epidemics of both drug-sensitive and drug-resistant disease. Their results implied that there may be a large number of drug-resistant latent infections that are clinically silent and may only become evident over a very long period of time. The number of hidden resistant infections depends on the parameter reflecting the relative transmissibility of the drug-resistant strain. This finding implies that the ability of drug-resistant organisms to infect susceptible hosts might have a powerful effect on the long-term outcome of an epidemic.

The effect of assigning different values to the relative transmissibility of drug-resistant disease in tuberculosis transmission models is highlighted in two subsequent papers that present similar models but arrive at different conclusions.



	Clustered (c)	Unique (u)	Ratio c/u
"True"	24	8	3
Red sample	8	4	2
Green sample	6	6	1
Blue sample	3	6	0·5

Bias associated with sampling from a hypothetical population of cases. Identical cases are identified by type number and colour (types 1–5); unique cases are left uncoloured (types 6–13). Sampling is represented by the larger coloured ovals.

Using a parameter value for the relative fitness of MDRTB of 0·7–1·0, Dye and Williams[58] predicted that second-line drugs would be needed to raise MDRTB cure rates to a level that would prevent future epidemics of MDRTB. However, in a second study, Dye and Espinal[59] revised their relative fitness estimates for MDRTB to 0·4–0·6 and concluded that the prevalence of MDRTB has been at the saturation point for decades and that the proportion of cases due to multidrug-resistant mycobacteria should not rise significantly above 5%. The authors note that their revised fitness estimate was based on a recently published cluster study in which multidrug resistance had a strong negative association with clustering.[41]

In view of the effect of fitness parameters on predicting the future spread of drug-resistant tuberculosis and the potential policy ramifications of these predictions, accurate estimates of the fitness of MDRTB are essential. The impressive heterogeneity of resistance-conferring and compensatory mutations belies the notion, however, that MDRTB has a "true" average reproductive fitness that researchers should be seeking to discover. For the purposes of policy analysis, what matters is the distribution: even if highly transmissible strains of MDRTB are only two of several thousand less-transmissible extant strains, outbreaks of these potential "superbugs" are nonetheless threatening. Publications on fitness cost suggest that deterministic models of tuberculosis resistance and treatment can be misleading, at the very least.

Future directions

The complex and often contradictory data generated in studies that aim to assess the fitness costs associated with resistance to individual drugs or combinations of drugs may indicate that the fitness question has been too broadly stated. Specific drug-resistance phenotypes may be achieved through a range of mutations, each of which is likely have a different effect on fitness. With the publication of the complete sequence of the *M tuberculosis* genome has come the opportunity to study the phenotypes associated with individual mutations, both in the laboratory through comparison of isogenic strains and through observing the behaviour of different strains in communities. Our ability to predict the future of drug-resistant disease and to design a well-informed global tuberculosis-control policy depends on a more refined understanding of the impact of specific drug-resistance mutations on bacterial survival, reproduction, and transmission.

Search strategy and selection criteria

Data for this review were identified through searches of Medline and references from relevant articles. Search terms were "*Mycobacterium tuberculosis*", "drug therapy", "drug resistance", "mutation", "fitness", "cluster", "DNA fingerprinting", and "mathematical model". English language papers were reviewed.

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Conflict of interest

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