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Infectiousness, reproductive fitness and evolution of drug-resistant *Mycobacterium tuberculosis*

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SUMMARY

Mathematical models predict that the future of the multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis (TB) epidemic will depend to a large extent on the transmission efficiency or relative fitness of drug-resistant *Mycobacterium tuberculosis* compared to drug-susceptible strains. Molecular epidemiological studies comparing the spread of drug-resistant to that of drug-susceptible strains have yielded conflicting results: MDR strains can be up to 10 times more or 10 times less transmissible than pan-susceptible strains. Experimental work performed with model organisms has highlighted a level of complexity in the biology of bacterial drug resistance that is generally not considered during standard epidemiological studies of TB transmission. Recent experimental studies in *M. tuberculosis* indicate that drug resistance in this organism could be equally complex. For example, the relative fitness of drug-resistant strains of *M. tuberculosis* can be influenced by the specific drug resistance-conferring mutation and strain genetic background. Furthermore, compensatory evolution, which has been shown to mitigate the fitness defects associated with drug resistance in other bacteria, could be an important factor in the emergence and spread of drug-resistant *M. tuberculosis*. However, much more work is needed to understand the detailed molecular mechanisms and evolutionary forces that drive drug resistance in this pathogen. Such increased knowledge will allow for better epidemiological predictions and assist in the development of new tools and strategies to fight drug-resistant TB.

KEY WORDS: evolution; genotyping; antibiotic; virulence; transmissibility

MULTIDRUG-RESISTANT (MDR) and extensively drug-resistant (XDR) tuberculosis (TB) are urgent public health problems in many parts of the world. Recent surveys indicate that XDR-TB exists in at least 50 countries.[1] Estimates for 2006 indicate that almost 500 000 incident MDR-TB cases occurred during that year.[2] Although 500 000 cases is a large number, it is relatively small compared to the total of the estimated 9.2 million new TB cases that occurred in 2006.[3] However, do MDR and XDR-TB have the potential to increase more dramatically in the future to cause a global pandemic? The answer to this important question will depend on a better understanding of the various factors that determine the emergence and spread of drug-resistant TB. The factors related to the quality of the TB control programmes and socioeconomic aspects have been shown to be important predictors of drug resistance.[4] By contrast, the intrinsic biological factors influencing the emergence and spread of drug resistance in TB remain to a large extent unknown.[5] One longstanding and highly debated question is whether the current problem of drug-resistant TB is primarily attributable to de novo acquisition of resistance during individual patient treatment (secondary resistance) or to direct transmission of drug-resistant strains (primary resistance). A related question is whether drug-resistant strains of *Mycobacterium tuberculosis* are as transmissible as their drug-susceptible counterparts. According to widespread views, drug-resistant bacteria suffer a 'fitness cost' in terms of reduced virulence and transmissibility, following the acquisition of drug resistance (further discussed below). However, this notion appears to be too simplistic.

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The present study is a review of the findings from studies that have approached the topic from an experimental, theoretical and epidemiological perspective. We focus particularly on MDR- and XDR-TB, which have the most profound effects on patient treatment outcomes. We conclude that the available evidence with respect to the 'infectiousness' of drug-resistant *M. tuberculosis* is ambiguous at best, and that more studies are needed.

RELATIVE FITNESS AND DRUG RESISTANCE

'Infectiousness', 'transmissibility' and 'virulence' are terms that have been used interchangeably to refer to the spread of infectious agents. As such, these concepts relate to the Darwinian fitness of pathogenic organisms. Darwinian fitness is often defined as 'the likelihood to survive and reproduce'. In infectious disease epidemiology, the relevant measure that reflects the reproductive fitness of a pathogen is the number of secondary cases generated; this measure is also known as the basic reproductive rate, R_0.[4] In addition to the absolute number of secondary cases (i.e., absolute fitness), an often more useful measure is that of 'relative fitness', where the success of a particular pathogen variant is compared to the success of another. For example, the fitness of a drug-resistant bacterial strain can be expressed relative to the fitness of a drug-susceptible strain. In addition to epidemiological measures of relative fitness, differences in relative fitness can be measured experimentally. Much work has been done on this subject in model organisms and, increasingly, also in pathogenic bacteria (for a comprehensive review, see Andersson and Hughes[6]). One experimental tool widely used to measure the relative fitness of bacteria is competitive fitness assays, where two strains of interest are mixed together in equal proportions and left to compete head-to-head for limited resources in a common environment. This experimental environment can be a simple culture flask or a more complex one, such as a co-infected mouse. At the end of the competition experiment, the fitness of the drug-resistant strain is expressed as the number of generations through which the drug-resistant variant has been relative to the drug-susceptible strain.

A general picture emerging from these experimental studies is that the ecological and evolutionary consequences of drug resistance are complex.[6] Figure 1 illustrates some of the key features in the evolution of drug resistance and how these can impact the relative fitness of drug-resistant bacteria. The acquisition of drug resistance in bacteria often carries a cost, in terms of reduced bacterial growth in the absence of the drug. This is because antibiotics generally target essential, highly conserved genes. One mechanism by which organisms can become resistant to a given drug is through mutation of these targets, which leads to interference with drug activity or prevents drug activation.[7] However, these mutations will also often (but not always) impact the normal function of these genes, resulting in reduced growth of resistant strains. This reduction in bacterial growth is generally referred to as 'fitness cost'.[6,8] Part of the complexity in the evolution of drug resistance has to do with the fact that drug resistance-conferring mutations can have a variable impact on strain fitness. Although the acquisition of drug resistance determinants is often associated with a loss in fitness, so-called 'low- or no-cost' mutations have been reported in various biological systems (Figure 1).[6,8] Furthermore, although drug-resistant strains often suffer an initial reduction in fitness, they continue to evolve by acquiring one or more secondary-site mutations that can improve or even restore the fitness of these strains over time; this process is known as compensatory evolution.[9] Importantly, this process occurs even in the absence of drug pressure. Thus, one of the consequences of compensatory evolution is that it can lead to a stabilisation of drug resistance in the population, even if antibiotics are withdrawn.[6,8]

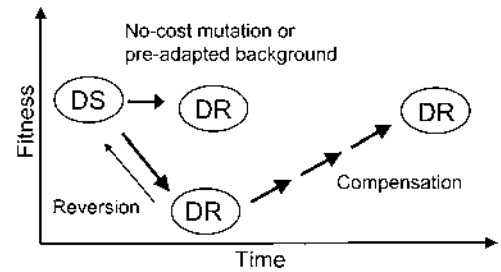

Figure 1 Evolution of drug resistance and its effects on bacterial fitness. A drug-susceptible (DS) bacterium acquires a drug-resistant (DR) determinant, which often, but not always, leads to a reduction in fitness. Reversion to the drug-susceptible state can occur when drug pressure is removed. However, compensatory evolution is more likely, even in the absence of drug pressure, as more evolutionary targets exist in the bacterial genome; true reversion can only occur through a back-mutation at the exact position of the original drug resistance-conferring mutation.

EXPERIMENTAL STUDIES IN *M. TUBERCULOSIS*

Several studies have used experimental approaches to study the effects of drug resistance on the relative fitness or virulence of *M. tuberculosis*. In the 1950s, Middlebrook and Mitchison compared the virulence of different isoniazid (INH) resistant clinical isolates in guinea pigs, and noted that many (but not all) showed reduced virulence compared to drug-susceptible strains.[10,11] Subsequent work in mice confirmed that drug-resistant strains of *M. tuberculosis* exhibit a range of virulence.[12] The most common mechanism by which *M. tuberculosis* acquires resistance to INH is through mutations in the catalase-peroxidase gene *kat*G.[7] A functional *kat*G is required to transform INH into its bioactive form. Mutations that eliminate

*kat*G activity therefore lead to high levels of INH resistance. On the other hand, *kat*G protects *M. tuberculosis* against the oxidative stress encountered during infection, and loss of *kat*G activity usually leads to reduced virulence of INH-resistant strains. However, one particular *kat*G mutation, *kat*G S315T, causes a reduction in the activation of INH while maintaining *kat*G activity and virulence in mice.[13] In other words, the *kat*G S315T mutation causes no significant fitness defect in INH-resistant *M. tuberculosis*, and can thus be considered a 'no-cost' mutation (Figure 1). This notion is further supported by the fact that *kat*G S315T is the most common INH resistance-conferring mutation in clinical settings.[7,14]

Similarly, different mutations conferring resistance to other anti-tuberculosis drugs have also been found to be associated with variable effects on strain fitness. Experimental studies in *M. smegmatis* have shown that the streptomycin (SM) resistance-conferring mutations associated with the least fitness cost were the most frequent in clinical isolates of *M. tuberculosis*.[15,16] Several studies have used competitive fitness assays to measure the fitness impact of rifampicin (RMP) resistance-conferring mutations in *M. tuberculosis*. Again, it was seen that different mutations in the RNA polymerase gene *rpo*B, which confers resistance to RMP, varied in their effects on bacterial fitness.[17–19] In the study by Gagneux et al., the authors found that the strain genetic background could also influence the fitness effects of particular *rpo*B mutations (Figure 2A).[18] Importantly, although all the laboratory-derived RMP-resistant strains were universally associated with a fitness cost (Figure 2A), some clinical strains suffered no defect in fitness compared to their RMP-susceptible ancestor strain (Figure 2B). These findings suggest that initial fitness defects were reduced in the clinical strains, perhaps as a consequence of compensatory evolution during prolonged patient treatment. The *rpo*B S531L mutation, which was associated with the lowest fitness cost in laboratory strains and no fitness defect in clinical strains, is the most frequent RMP resistance-conferring mutation in clinical strains worldwide.[18] By contrast, the *rpo*B mutant exhibiting the highest fitness cost (Figure 2A) has never been observed in any clinical strain.

In sum, the results of experimental studies performed with strains resistant to INH, SM or RMP suggest that, in clinical settings, there is a strong selection pressure for drug resistance-conferring mutations that cause minimal fitness defects.[20] Although these findings support the notion that virulence and competitive fitness assays can be predictive of the epidemiology of drug-resistant TB, they do not capture the overall complexity of the life cycle of *M. tuberculosis*. For example, aspects related to between-host transmission, as opposed to mere growth in culture or virulence in animal models, are difficult to measure experimentally. There is thus a strong need for population-based studies of drug resistance transmission in clinical settings.

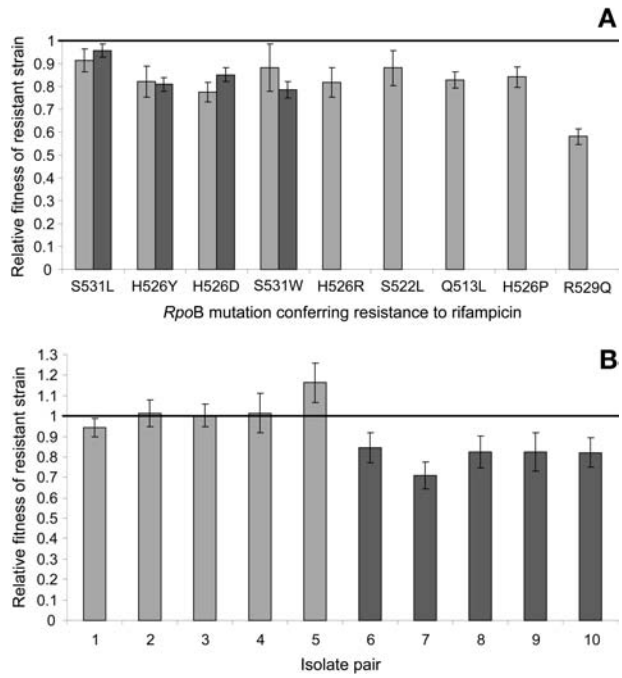

Figure 2 Competitive fitness cost of RMP-resistant *M. tuberculosis* in vitro. (Adapted from Gagneux et al.[18]) **A.** Laboratory-derived strains. All laboratory-derived RMP-resistant strains had a statistically significant reduction in competitive fitness compared to the RMP-susceptible ancestor strain, which by definition has a relative fitness of 1 (black line; error bars indicate 95% confidence intervals). However, differences exist between strains harbouring different RMP resistance-conferring mutations or different genetic backgrounds (light grey bars indicate strains derived from clinical strain CDC1551 and dark grey bars strains derived from clinical strain T85, which belongs to the Beijing lineage). **B.** Clinical isolate pairs. Four of five clinical RMP-resistant strains with the *rpo*B S531L mutation (light grey bars) had no fitness defect compared to the paired RMP-susceptible isolate recovered from the same patient. By contrast, all clinical strains with other *rpo*B mutations (dark grey bars) had a statistically significant fitness defect compared to their RMP-susceptible counterpart. RMP = rifampicin.

Although several mechanisms of compensatory evolution have been described in other bacteria,[9] little work has been done on this topic in *M. tuberculosis*. One compensatory mechanism has been proposed for INH-resistant strains that lack a functional *kat*G enzyme. Clinical strains lacking *kat*G activity have been associated with promoter mutations in *ahp*C, which encodes an alkyl hydroperoxide reductase. It has been shown that upregulation of *ahp*C expression can partially compensate for the lack of *kat*G activity.[21] However, *ahp*C promoter mutations are rare in clinical settings, and strains harbouring *ahpC* promoter mutations but without *kat*G mutations have been reported.[22] Hence, the role and relevance of *ahp*C mutations for INH resistance remain controversial.[7] A recent study used next-generation high-throughput DNA sequencing to compare the genomes of one drug-

susceptible, one MDR and one XDR *M. tuberculosis* isolate that belonged to the strain family that caused the recent outbreak of XDR-TB in KwaZulu-Natal, South Africa (reviewed in Jassal and Bishai[1]). The hope was that, by analysing closely related isolates, some of the key genomic differences between drug-susceptible and drug-resistant strains, including putative compensatory mutations, would become evident. This analysis revealed that only few mutations separated drug-resistant from drug-susceptible isolates. Although some of the changes specific to the two drug-resistant isolates could represent putative compensatory mutations, more work is needed to confirm this possibility.

MATHEMATICAL MODELS

In addition to experimental studies, theoretical approaches have been used extensively to study the emergence and spread of drug-resistant bacteria. In TB, mathematical models have been developed to study various aspects of the natural history of the disease (for a detailed review, see Cohen et al.[23]). For example, mathematical modelling has been used to explore how MDR *M. tuberculosis* is selected within individuals undergoing TB treatment, or what type of interventions can limit the spread of drug-resistant TB in the community. Much theoretical emphasis has been put on predicting the future spread of drug-resistant TB.

As discussed above, the concept of relative fitness can be applied to both experimental and epidemiological settings. In the epidemiological context, the reduced reproductive fitness of a drug-resistant pathogen is reflected in fewer secondary cases generated when compared to susceptible strains, corresponding to a reduction in the basic reproductive rate R_0.[23] Early mathematical models aiming at predicting the future spread of drug-resistant TB assumed that drug resistance was universally associated with a reduction in bacterial fitness.[4,24,25] The resulting predictions were that MDR-TB would remain a local problem.[4] More recent models have allowed for variation in the relative fitness of drug-resistant strains, along the lines discussed in the previous section, and have come to very different conclusions.[26,27] For example, the model by Cohen and Murray found that even when the average relative fitness of MDR strains is low and a well-functioning TB control programme is in place, in the long term, a small subpopulation of relatively fit MDR strains may outcompete both the drug-susceptible strains and the less fit MDR strains.[27]

Taken together, available experimental and theoretical evidence suggests that the relative fitness of drug-resistant strains is one of the key parameters dictating the future of the MDR and XDR epidemics. How does this evidence compare with the current epidemiological evidence for transmission of drug-resistant *M. tuberculosis*?

EPIDEMIOLOGICAL STUDIES

Various molecular tools have been developed to genotype *M. tuberculosis* strains.[28] These tools have been applied to molecular epidemiological investigation of TB transmission for many years. According to the standard concept, patient isolates sharing a particular genotype or DNA 'fingerprint' can be considered epidemiologically linked and represent cases of active TB transmission (i.e., they are clustered TB cases), whereas strains with distinct or 'unique' DNA patterns are thought to reflect reactivation of latent infections. The proportion of genotypic clustering can be used as an approximate measure of ongoing TB transmission. Following this approach, studies have been conducted to compare the relative proportion of genotypic clustering in drug-resistant and drug-susceptible strains. This proportion can then be transformed into a measure equivalent to the relative fitness used in the experimental studies discussed above.

In Figure 3, we compiled molecular epidemiological fitness estimates from two previous reviews and more recent studies.[4,5] Overall, the relative fitness estimates for MDR-TB vary dramatically, ranging from an almost 10-fold increased fitness compared to fully drug-susceptible strains found in a study from Russia,[29] to about 10-fold lower fitness in Mexico;[30] other studies have reported that MDR strains do not cause any secondary cases at all.[31] The reasons for this high variability in relative fitness of MDR strains have likely to do with the differences in study design and setting, differences in sample size and different

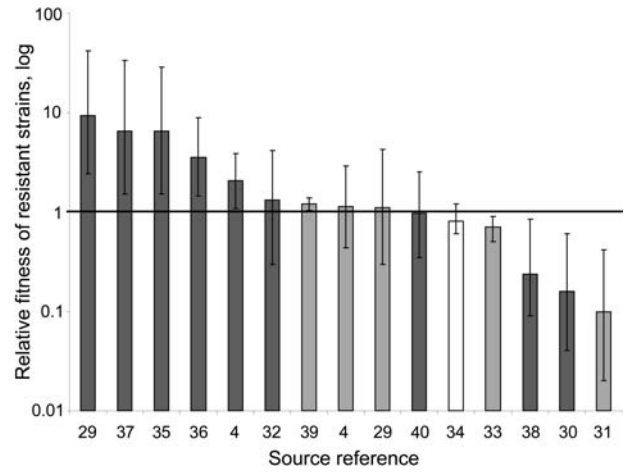

Figure 3 Relative fitness of MDR (dark grey bars) and INH-monoresistant (light grey bars) strains as measured in molecular epidemiological studies. (Data compiled from references 4 and 29–40.) The white bar indicates the relative fitness of INH-monoresistant strains with the S315T mutation in *kat*G. Relative fitness corresponds to the difference in genotypic clustering between drug-resistant and drug-susceptible strains calculated according to Dye et al.[4] The black line indicates equal fitness and error bars 95% confidence intervals. MDR = multidrug-resistant; INH = isoniazid.

methodologies. Variation in the quality of the TB control programme could also play a role. For example, standard DOTS is likely to be more efficient in reducing the duration of infectiousness in patients carrying drug-susceptible strains compared with patients infected with drug-resistant strains, which likely complicates the measurement of relative fitness in clinical settings.[4] Interestingly, a well-established DOTS programme has been shown to reduce the incidence of both drug-susceptible and drug-resistant TB in Mexico.[32] In Peru, on the other hand, DOTS has been well-established for many years, but rates of MDR-TB are still increasing.[2] These contrasting observations highlight complexities that we will only be able to decipher by acquiring more comprehensive data on the global trends of drug-resistant TB.

In addition to methodological, socio-economic and environmental factors, the variation in MDR fitness illustrated in Figure 3 might also reflect biological heterogeneity. To date, few molecular epidemiological studies have taken into account the possible variable effects of drug resistance-conferring mutations on TB transmission. Studies in the Netherlands have shown that even though INH-resistant strains overall exhibited a significantly lower level of epidemiological clustering, strains harbouring the no-cost mutation *kat*G S315T suffered no significant reduction in transmission (Figure 3).[33,34,41] A study in San Francisco also found statistically significant differences in transmission of INH-resistant strains depending on the specific resistance-conferring mutation. Although some strains with the *kat*G S315T mutation resulted in successful transmission, none of the strains harbouring any other *kat*G mutation (i.e., mutations likely to abrogate enzyme activity) generated any secondary case.[22] All of these studies were undertaken in areas with a well-functioning TB control programme. Nevertheless, a subset of drug-resistant strains managed to transmit and cause secondary cases. These findings illustrate the variable effects of drug resistance-conferring mutations on the transmissibility of drug-resistant TB.

EVIDENCE FOR TRANSMISSION OF PRIMARY MDR- AND XDR-TB

Irrespective of the *relative* transmission of drug-resistant *M. tuberculosis* compared to drug-susceptible strains, another key question is how much of the current drug resistance problem in TB is attributable to primary (i.e., transmitted) vs. secondary (i.e., acquired) resistance.[2] Human immunodeficiency virus (HIV) co-infection has long been recognised as an important risk factor for MDR-TB. Many nosocomial outbreaks of MDR- and XDR-TB have been reported in HIV-positive individuals, and these include cases of heterogeneous re-infection with MDR or XDR strains during standard treatment for drug-susceptible TB.[42] Several possible explanations have been proposed for the association between HIV and drug-resistant TB (for discussion, see Dye et al.[4] and Cohen et al.[23]). In the context of this review, an interesting possibility is that, because of their putative reduction in fitness, MDR strains might be less likely to thrive outside of immune-compromised patients. Here we decided to specifically review the current published evidence for transmission of MDR-TB in HIV-negative individuals. We only considered studies where transmission of MDR-TB was confirmed by appropriate molecular epidemiological techniques[28] and where the HIV-negative status of patients was laboratory confirmed. Of 442 studies identified initially, only 12 fulfilled all of these inclusion criteria (Table 1). It is surprising that despite a wealth of molecular epidemiological studies on TB transmission,[28] a total of only ~300 laboratory-confirmed instances of transmission of MDR strains in HIV-negative individuals have been described during the

Table 1 Evidence for transmission of multidrug-resistant *M. tuberculosis* in HIV-negative individuals

Year	Reference*	City, country	Study setting	HIV-negative %	Cases involved in transmission *n*
1995	Shafer et al.[44]	New York, USA	Hospital	33	3
1999	Van Rie et al.[45]	Cape Town, South Africa	Community	100	16
2000	Fandinho et al.[35]	Rio de Janeiro, Brasil	Hospital	100	23
2001	Sofia et al.[46]	France	Family	100	3
2002	Quitugua et al.[47]	Texas and Mexico, USA	Community	100	85
2003	Palmero et al.[48]	Buenos Aires, Argentina	Family, community and hospital	100	36
2005	Samper et al.[49]	Spain	Community	100	38
2005	Mardassi et al.[50]	Tunisia	Community	100	18
2005	Oeltmann et al.[51]	Thailand	Refugees	100	20
2006	Kodmon et al.[52]	Hungary	Community	100	21
2007	Umubyeyi et al.[53]	Rwanda	Hospital	100	3
2007	Vazquez-Gallardo et al.[54]	Galicia, Spain	Hospital	100	30

*Relevant studies were identified (n = 142) by searching PubMed (accessed March 2009) using the search terms 'tuberculosis', 'drug resistance' and 'transmission'. Only publications in English were considered. Articles were included if they contained molecular epidemiology and laboratory-confirmed HIV data. Articles were excluded if the transmission occurred just among HIV-positive patients or if the molecular epidemiology data were based solely on low resolution techniques (e.g., spoligotyping).
HIV = human immunodeficiency virus.

last 20 years. By contrast, individual population-based molecular epidemiological studies of TB transmission have reported thousands of clustered drug-susceptible TB cases in a single area.[33,41] We realise that our inclusion criteria were quite strict and that, as a consequence, we might have missed epidemiologically well-documented cases of primary resistance or heterogeneous re-infection. The point we would like to stress here is that, from a scientific point of view, the actual evidence for primary transmission of MDR-TB in HIV-negative individuals that has been confirmed by molecular methods is very limited, and that more studies including molecular data are needed to know the true extent of primary MDR-TB in the general population.

Another important point is whether drug resistance-conferring mutations can exhibit a cumulative effect on strain fitness. It is possible that as bacteria acquire mutations conferring resistance to multiple drugs (i.e., they are experiencing amplification of resistance), they will suffer increasing fitness defects. XDR strains of *M. tuberculosis* are currently defined as resistance to the two first-line drugs, INH and RMP, with additional resistance to quinolones and at least one of the injectable second-line drugs.[1] At a minimum, XDR strains thus have mutations in at least four key enzymes, which collectively could impact the growth physiology of XDR strains. In reality, many of these XDR strains are resistant to many more drugs, with each additional drug resistance-conferring mutation potentially adding to the fitness burden. It is interesting to speculate that although compensatory evolution might be able to mitigate the deleterious effects of a few drug-resistant mutations, there might be a limit to the degree to which the fitness defects of highly resistant XDR strains might be compensated. No study has yet investigated this possibility. However, several recent reports have highlighted the high rate of mortality among XDR-TB patients who are HIV-co-infected, indicating that, at least in the context of reduced immune competence, XDR-TB can thrive.[1] Outbreaks of XDR-TB have been associated with nosocomial transmission, but it remains unclear how transmissible XDR strains are in the general population. To review the current evidence for XDR transmission, we analysed all the studies that have reported molecular epidemiologically confirmed cases of XDR transmission, following inclusion criteria similar to those outlined earlier. As summarised in Table 2, only four studies have documented laboratory-confirmed transmission of XDR strains. Three of the four involved a hospital outbreak, and a large proportion of the affected individuals were HIV-co-infected. The term 'XDR' was coined relatively recently,[1] which will have limited the number of search hits during our review process. Again, the point here is that the current published scientific evidence for transmission of XDR-TB is very limited and more studies are needed.

RELEVANCE OF STRAIN LINEAGE

Based on the evidence discussed in the previous sections, it is clear that part of the heterogeneity in fitness and transmissibility of drug-resistant strains can be linked to the variable impact of drug resistance-conferring mutation. In addition to these direct effects, studies in other bacteria have shown that the strain's genetic background can significantly influence the fitness effects of particular drug resistance-conferring mutations (Figure 1). For example, in vivo experiments conducted with *Campylobacter jejuni* showed that a specific quinolone resistance-conferring mutation in the DNA gyrase gene *gyr*A reduced the relative fitness of some quinolone-resistant strains, but increased strain fitness when transferred into another strain background.[57]

In *M. tuberculosis*, different strains have been shown to differ in immunogenicity and virulence in animal models (reviewed in Gagneux and Small[58]). There is also increasing evidence that strain diversity can influence the outcome of infection and disease in humans.[59] Genomic analyses of strain collections from global sources have revealed that *M. tuberculosis* has a phylogeographic population structure, in which different strain lineages are associated with particular geographic regions.[58,60] A recent theoretical study found that simulated populations with immunologically distinct strain groups had a higher risk of drug resistance than populations without strain

Table 2 Evidence for transmission of XDR *M. tuberculosis*

Year	Reference*	Country	Study setting	HIV-positive %	Total XDR cases in the study *n*	Clustered XDR strains *n/N* (%)
2006	Gandhi et al.[43]	South Africa	Hospital	100	53	39/46 (85)
2006	Masjedi et al.[55]	Iran	Family/community	25	12	12/12 (100)
2008	Mlambo et al.[56]	South Africa	Hospital/community	ND	41	15/41 (37)†
2008	Cox et al.[42]	Uzbekistan	Hospital	ND	10	7/10 (70)

*Relevant studies were identified (n = 20) by searching PubMed (accessed March 2009) using the terms 'tuberculosis', 'XDR' and 'transmission'. Only studies where transmission of XDR strains was confirmed by molecular epidemiology were retained.
†Molecular epidemiology data obtained by spoligotyping.
XDR = extensively drug-resistant; HIV = human immunodeficiency virus; ND = no data provided.

diversity, even if the quality of TB control was the same.[61] In addition to immunological effects, the variable genetic background of strains belonging to different strain lineages could play a role in the evolution of drug resistance. The Beijing lineage of *M. tuberculosis* has repeatedly been associated with drug resistance. Here, we decided to compile all the available evidence about the association between MDR and the Beijing lineage. Table 3 summarises all published studies that have reported a statistically significant association between Beijing strains and MDR-TB when compared to other strains and pan-susceptible TB. Of the 12 studies that fulfilled our inclusion criteria (Table 3), nine were performed in countries of the former Soviet Union and three in South-East Asia. The association between the Beijing lineage and drug resistance appears particularly strong and consistent in these two geographic areas. Although poor TB control strategies (for example in Russian prisons) have likely contributed to the large problem of drug resistance in these regions, it is hard to imagine how differences in TB control would affect the emergence of drug resistance in a lineage-dependent manner. Moreover, the fact that the association between Beijing lineage and drug resistance holds across different countries and continents where TB control measures are likely to differ is indicative of a biological effect.

Several biological factors could contribute to the association between Beijing strains and drug resistance. According to one hypothesis, Beijing strains might exhibit an increased mutation rate, a phenomenon known as mutator phenotype.[72] This notion is based on the fact that several missense mutations have been discovered in DNA repair genes of Beijing strains.[73] These mutations could lead to a higher overall mutation rate and to an accelerated acquisition of drug resistance. However, the only study to date to have measured spontaneous mutation rates across strain lineages has found no difference in mutation rate between Beijing and other strain lineages in vitro.[74] Furthermore, if the rate of spontaneous mutations in Beijing strains was elevated compared to other strains, one would expect Beijing strains to accumulate mutations all over the genome. However, according to a recent study that compared DNA sequences of 89 genes in 108 strains, no evidence for such an accumulation can be found.[75] Alternatively, Beijing strains could better tolerate the fitness effects of drug resistance-conferring mutations. In other words, the Beijing strain background could be 'pre-adapted' to the fitness effects of drug resistance (Figure 1). In support of this view, a study looking at the in vitro growth of clinical strains found that, in contrast to non-Beijing strains, some drug-resistant strains belonging to the Beijing lineage had no growth defect compared to their drug-susceptible counterparts.[76] Furthermore, a study in San Francisco showed that Beijing strains were significantly associated with INH resistance-conferring mutations that were likely to abrogate *kat*G activity. Because *kat*G helps protect the bacteria against oxidative stress during infection, loss of *kat*G usually results in attenuation.[13] The findings from San Francisco suggest that Beijing strains might be less dependent on an intact *kat*G, perhaps because they are generally less susceptible to oxidative stress or better able to compensate for the loss of *kat*G activity.

Table 3 Associations between the Beijing lineage of *M. tuberculosis* and multidrug resistance in published studies

Year	Reference*	Country/region	Study setting	Beijing strains/ total strains *n/N* (%)	MDR strains/ total Beijing *n/N* (%)	OR (95%CI)	*P* value†
2001	Pfyffer et al.[62]	Azerbaijan	Prison	46/65 (70.8)	28/46 (60.9)	3.4 (1.0–12.7)	<0.05
2001	Kruuner et al.[36]	Estonia	Community	61/209 (29.2)	34/61 (55.7)	17.0 (5.3–54.9)	ND
2002	Toungoussova et al.[29]	Russia	Community	53/119 (44.5)	23/53 (43.4)	11 (3.4–37.0)	<0.001
2005	Park et al.[63]	Korea	Community	569/743 (76.6)	190/569 (33.4)	1.8 (1.1–2.9)	<0.01
2005	Kovalev et al.[64]	Russia	Community	50/92 (54.3)	19/50 (38.0)	9.1 (2.3–43.1)	<0.001‡
2005	Cox et al.[65]	Uzbekistan	Community	190/382 (49.7)	51/190 (26.8)	4.8 (2.5–9.6)	<0.001
2005	Drobniewski et al.[66]	Russia	Prison/community	586/880 (66.6)	216/586 (36.9)	2.4 (1.9–3.0)	<0.001
2006	European Concerted	West Europe	Community	253/7340 (3.5)	3/253 (1.0)	4.2 (1.2–14.7)§	<0.001
	Action for the Control	Eastern Europe	Community	248/564 (44)	126/248 (51.0)	11.2 (6.9–18.3)	<0.001
	of Tuberculosis[67]	South-East Asia	Community	366/1027 (35.6)	29/366 (8.0)	3.3 (1.74–6.4)	<0.001
2007	Nikolayevskyy et al.[68]	Ukraine	Community	89/231 (38.5)	31/89 (34.8)	1.43 (1.08–1.9)	ND
2007	Sun et al.[69]	Singapore	Community	328/598 (54.9)	31/328 (9.5)	2.66 (1.28–5.5)	<0.01
2009	Mokrousov et al.[70]	Russia	Community	41/90 (45.6)	28/41 (68.3)	5.8 (2.2–16.6)	<0.001
2009	Phyu et al.[71]	Myanmar	Community	99/310 (32)	21/99 (21.2)	3.2 (1.34–7.67)¶	<0.01

*Relevant studies were identified (n = 190) by searching PubMed (accessed March 2009) using the terms 'tuberculosis', 'Beijing' and 'drug resistance'. Articles were included if they provided evidence for a statistically significant association between Beijing and MDR when compared with non-Beijing and pan-susceptible strains.

†χ^2 test for proportions.

‡Two-sided Fisher's exact test.

§Calculated for non-immigrants in Western Europe.

¶Comparison of the Beijing lineage with the other main lineage in the area; adjusted for previous TB treatment.

MDR = multidrug-resistant; OR = odds ratio; CI = confidence interval; ND = no data; odds ratios copied from original paper, but *P* values could not be calculated based on the data provided.

AREAS FOR FUTURE RESEARCH

As is becoming evident from this review, our current understanding of drug resistance in TB appears too limited to be able to predict the future of MDR- and XDR-TB with confidence. Our ignorance is particularly marked with respect to the biological factors involved. There is hence an urgent need to improve our insight into all aspects of TB drug resistance. A particularly important point relates to the development of molecular diagnostics, which have the potential to dramatically improve and accelerate the diagnosis of drug-resistant TB.[1] Although many drug resistance-conferring mutations have been identified in *M. tuberculosis*,[77] for many clinical strains the resistance mechanisms and the associated resistance-conferring mutations remain unknown, particularly in those resistant to second-line agents. In addition to enhancing our understanding of the primary drug resistance determinants, we need to learn more about the putative compensatory mechanisms operating in *M. tuberculosis*. For example, some MDR Beijing strains circulating in countries of the former Soviet Union are arguably among the most successful drug-resistant strains of *M. tuberculosis*.[65] What are the molecular and evolutionary mechanisms that have contributed to this success? Another important issue emerging from this review is that the current epidemiological evidence for transmission of MDR- and XDR-TB, particularly compared to pan-susceptible TB, is very inconclusive. This can be partially explained by the fact that *M. tuberculosis* is more genetically diverse than is often appreciated,[75] and because drug-resistant strains can exhibit heterogeneous fitness compared to drug-susceptible strains. Future epidemiological studies on the transmission of drug-resistant TB should incorporate more comprehensive strain data, including specific drug resistance-conferring mutations and information on the strain genetic background. These variables, as well as their interaction, could play an important role in the transmission success of particular drug-resistant variants.

Theoretical studies, too, need to be expanded. Mathematical models are based on a number of specific assumptions and the model parameters are set based on empirical evidence.[23] However, although much about the ecological and evolutionary complexity of drug resistance has been learnt from model organisms, the corresponding data for MDR- and XDR-TB remain scarce. Nevertheless, some of the more recent studies reviewed here suggest that similar phenomena occur in *M. tuberculosis*. Although mathematical modelling of TB transmission dynamics is increasingly incorporating part of this complexity,[61] some important features of the biology of drug resistance have not been addressed. For example, the effects of compensatory evolution and improving fitness of drug-resistant strains over time have not yet been explored. Moreover, bacterial species consist of genetically and phenotypically heterogeneous populations, some of which can exhibit increased mutation rates or phenotypic drug tolerance, all of which can facilitate the development of genetically encoded drug resistance.[72,78]

Finally, current drug resistance surveillance data are very limited with respect to the associated molecular information because routine drug susceptibility testing (DST) relies on phenotypic assays.[2] A wider adoption of molecular-based tests would help generate valuable data on the frequency of various drug resistance alleles in different parts of the world. This information is crucial for the development and evaluation of new tools for molecular-based DST, as well as for a better understanding of the impact of drug resistance on the global spread of *M. tuberculosis*.[77]

CONCLUDING REMARKS

Anti-tuberculosis drugs have been used for many decades, yet only a small proportion of today's TB cases are MDR or XDR.[2,3] Thus, overall, global TB control appears to be quite successful at limiting the emergence and global spread of MDR- and XDR-TB.[79] However, in other places drug resistance is still increasing. The important question is how will these trends look in the future? Drug-resistant TB is a complex phenomenon. Although good TB control measures are able to limit the emergence of new drug-resistant strains, the long-term fate of the existing drug-resistant strains, and with it the future of the MDR- and XDR-TB epidemics, remains uncertain. More studies are needed to address these important questions.

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RÉSUMÉ

Les modèles mathématiques prédisent que l'avenir de l'épidémie de la tuberculose multirésistante (TB-MDR) et de la tuberculose ultrarésistante (TB-XDR) dépendra dans une large mesure de la capacité de transmission et de la vitalité relative de *Mycobacterium tuberculosis* résistant aux médicaments par comparaison aux souches sensibles. Les études d'épidémiologie moléculaire comparant la dispersion des souches résistantes aux médicaments à celle des souches sensibles ont donné des résultats contradictoires. Les souches MDR peuvent être

jusqu'à dix fois plus ou dix fois moins contagieuses que les souches sensibles à tous les médicaments. Des travaux expérimentaux réalisés sur des organismes type ont mis en évidence un niveau de complexité dans la biologie de la résistance bactérienne aux médicaments, complexité qui n'est généralement pas prise en compte au cours des études épidémiologiques standard sur la transmission de la TB. Des études expérimentales récentes portant sur *M. tuberculosis* indiquent que la résistance de cet organisme aux médicaments pourrait être tout aussi complexe. Par exemple, la vitalité relative des souches de *M. tuberculosis* résistantes aux médicaments pourrait être influencée par la mutation spécifique responsable de la résistance aux médicaments et par le contexte génétique de la souche. De plus, l'évolution compensatoire qui s'est avérée amoindrir les déficiences de vitalité associées à la résistance aux médicaments dans d'autres bactéries pourrait être un facteur important dans l'apparition et la dispersion de *M. tuberculosis* résistant aux médicaments. Toutefois, des travaux bien plus nombreux sont nécessaires pour comprendre les mécanismes moléculaires détaillés et les forces évolutives qui stimulent la résistance de cet agent pathogène aux médicaments. Un tel accroissement des connaissances permettra des prédictions épidémiologiques de meilleure qualité et aidera à l'élaboration de nouveaux outils et de nouvelles stratégies pour lutter contre la TB à germes résistants aux médicaments.

RESUMEN

Los modelos matemáticos prevén que el futuro de la epidemia de tuberculosis multidrogorresistente (TB-MDR) y extensivamente drogorresistente (TB-XDR) dependerá en gran medida de la eficiencia de transmisión o de la relativa adaptabilidad de las cepas resistentes de *Mycobacterium tuberculosis*, en comparación con las cepas sensibles. Los estudios epidemiológicos moleculares que comparan la diseminación de cepas drogorresistentes con la diseminación de las normosensibles han dado resultados contradictorios. Las cepas con MDR pueden presentar una transmisibilidad hasta 10 veces superior o 10 veces inferior a las cepas sensibles a todos los medicamentos. Los estudios llevados a cabo con estas cepas en modelos experimentales han puesto en evidencia la complejidad de las características biológicas de la drogoresistencia bacteriana, la cual no suelen evaluar los estudios epidemiológicos corrientes de transmisión de la TB. Las investigaciones recientes con *M. tuberculosis* indican que las resistencias en este microorganismo podría tener una complejidad equivalente. Por ejemplo, la mutación específica que confiere la resistencia y el repertorio genético de las bacterias podrían influir sobre la relativa adaptabilidad de las cepas resistentes de *M. tuberculosis*. Es más, la compensación evolutiva, que al parecer aminora las deficiencias de la adaptabilidad asociadas con la farmacorresistencia en otras bacterias, podría ser un factor importante en la aparición y diseminación de cepas resistentes de *M. tuberculosis*. Sin embargo, se precisa mucha más investigación a fin de comprender los mecanismos moleculares precisos y las fuerzas evolutivas que promueven la resistencia a los medicamentos en este patógeno. Esos nuevos conocimientos favorecerán mejores predicciones epidemiológicas y contribuirán a la elaboración de nuevas herramientas y estrategias de lucha contra la TB drogorresistente.