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# Strain diversity, epistasis and the evolution of drug resistance in *Mycobacterium tuberculosis*

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## Abstract

*Mycobacterium tuberculosis* harbours little DNA sequence diversity compared with other bacteria. However, there is mounting evidence that strain-to-strain variation in this organism has been underestimated. We review our current understanding of the genetic diversity among *M. tuberculosis* clinical strains and discuss the relevance of this diversity for the ongoing global epidemics of drug-resistant tuberculosis. Based on findings in other bacteria, we propose that epistatic interactions between pre-existing differences in strain genetic background, acquired drug-resistance-conferring mutations and compensatory changes could play a role in the emergence and spread of drug-resistant *M. tuberculosis*.

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## Introduction

Tuberculosis (TB) remains an important global health problem, with close to 10 million new cases per year and a pool of 2 billion latently infected individuals worldwide [1]. Of particular concern are the ongoing epidemics of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB), which threaten to make TB incurable [2]. Tuberculosis is a complex phenomenon driven by multiple biological, socioeconomic and environmental factors [3]. In this review, we discuss some of the bacteriological factors involved in TB with a particular focus on drug resistance. We start by reviewing our current knowledge of the genetic diversity in the *Mycobacterium tuberculosis* complex (MTBC), the causative agent of human TB, and how this diversity influences the emergence of drug-resistant strains. We then summarize some recent findings from other bacteria on the role of epistasis in the evolution of drug resistance, and end by discussing possible implications for our understanding of MDR-TB and XDR-TB.

## Strain Diversity in MTBC

Despite exhibiting limited DNA sequence diversity compared with other bacteria [4], the extent of strain diversity in MTBC is more pronounced than previously believed [5]. Recent comparative genomic studies have shown that genomic deletions and duplications represent an important source of genomic plasticity in MTBC [6–13]. Furthermore, investigation of global collections of clinical strains revealed that human MTBC exhibits a phylogeographic population structure with different strain lineages associated with particular geographic regions and human populations [6,14–18]. In a study by Hershberg *et al.* [19], 89 genes were sequenced in each of 108 strains representative of the global diversity of MTBC. Based on these gene sequences, the authors con-

structed the most comprehensive DNA sequence-based phylogeny of MTBC to date [20]. The authors also showed that the genetic distances between the most distant human MTBC strains were equivalent to the genetic distance between an average human strain and animal-adapted MTBC [19]. Because animal-adapted MTBC such as *Mycobacterium bovis* and *Mycobacterium microti* are believed to form distinct ecotypes within MTBC [21], the findings by Hershberg *et al.* [19] support the notion that the different human-adapted lineages of MTBC might represent different ecotypes adapted to different human populations [6,18]. In 2010, Comas *et al.* [22] used 21 whole genome sequences to generate the first whole genome-based phylogeny of human-adapted MTBC. This phylogeny confirmed that human-adapted MTBC consists of six main phylogenetic lineages, two of which are also known as *M. africanum* [23].

DNA sequencing studies in MTBC clinical isolates found consistently that two-thirds of the single nucleotide polymorphisms in MTBC were non-synonymous [19,22,24]. Furthermore, the study by Hershberg *et al.* [19] showed that about 40% of these non-synonymous single nucleotide polymorphisms occurred at positions that were highly conserved in other mycobacteria, and therefore were likely to affect gene function. The authors concluded that purifying selection against non-synonymous single nucleotide polymorphisms was reduced in MTBC. Despite this high proportion of non-synonymous single nucleotide polymorphisms in MTBC, the study by Comas *et al.* [22] found that essential genes were more evolutionarily conserved than non-essential genes. Hence, even though purifying selection in MTBC might be reduced compared with other bacteria [19], natural selection is still acting on MTBC and differentiating between various gene classes [22].

The studies reviewed here show that strain genetic diversity in MTBC includes genomic insertions/deletions and duplications, as well as non-synonymous single nucleotide polymorphisms, many of which are predicted to have functional effects. An important question is how this genetic diversity translates into phenotypic diversity. A recent review compiled the results of 100 published studies that evaluated the effect of MTBC strain diversity on experimental and clinical phenotypes [25]. Based on this review, one concludes that clinical strains of MTBC differ in immunogenicity and virulence in infection models. However, the role of strain diversity in clinical settings is less clear. One of the difficulties when studying the impact of strain diversity in patient populations is that many additional variables need to be taken into account [3]. Furthermore, strain classification for MTBC remains an issue as no universally accepted standard has been defined. Such a new standard is urgently needed and should be based on whole genome sequencing [26].

In addition to the possible impact of strain variation on the outcome of TB infection and disease, this diversity is relevant for our understanding of drug resistance. For example, the so-called 'Beijing' family of strains has been associated with drug resistance in multiple reports (reviewed in [27]). However, the underlying cause of this association remains unknown, even though multiple hypotheses have been put forward. One thought is that the genetic background of Beijing strains might predispose to the acquisition of mutations that confer drug resistance [28]. However, evidence for an increased mutation rate in Beijing strains is still lacking [29]. Before discussing other possible effects of MTBC diversity on drug resistance, let us briefly review some of the most important features of the current epidemic of drug-resistant TB.

## Factors Driving MDR/XDR-TB

The past 20 years have seen the worldwide appearance of MDR-TB, followed by XDR-TB [2], and, most recently, strains that are resistant to all antituberculosis drugs, the so-called 'totally drug-resistant' or TDR strains [30]. MDR-TB is caused by MTBC that is resistant to at least isoniazid and rifampicin, the most important first-line drugs against TB [2]. XDR-TB is caused by MDR strains with additional resistance to any fluoroquinolone and one of the three injectable drugs, capreomycin, kanamycin and amikacin. MTBC acquires MDR and XDR through a stepwise accumulation of chromosomal mutations. In most cases, each of these mutations confers resistance to an individual drug [31].

Drug resistance in MTBC can be acquired *de novo* in individual patients undergoing TB treatment, either because of lack of patient adherence or through an interrupted drug supply. Alternatively, drug-resistant strains can spread through direct transmission of MTBC strains that are already drug-resistant. In addition, treatment for other diseases can also contribute to acquired resistance in TB. For instance, widespread use of fluoroquinolones for respiratory tract and other infections might drive resistance to fluoroquinolones in TB [32]. Given the financial burden and logistical difficulties associated with treating MDR-TB and XDR-TB [2], it is important to understand the evolutionary mechanisms that promote the emergence of highly drug-resistant MTBC.

Mathematical models predict that one of the most important factors influencing the future of MDR/XDR-TB is the relative fitness of drug-resistant strains compared with drug-susceptible strains [33–35]. As in other bacteria [36], resis-