The evolution of multi-drug resistance in mycobacterium tuberculosis

Tuberculosis is an airborne infectious disease that is caused by *Mycobacterium tuberculosis* . According to the World Health Organization, this disease affects more than 10. 4 million individuals worldwide and is one of humankind’s deadliest disease. While most patients can be cured by existing antibiotics, multidrug-resistant tuberculosis (MDR-TB) – caused by strains of *M. tuberculosis* that are resistant to antituberculotic agents isoniazid and rifampin – kills an estimated 500, 000 individuals annually (Gandhi *et al.* , 2010).

The genus *Mycobacterium* is hypothesized to have originated more than 150 million years ago, before the break up of the Gondwanaland continental landmass (Hayman, 1984). More recently, molecular genetics and genomic sequencing showed that an early progenitor of *M. tuberculosis* was present in East Africa as early as 3 million years ago (Gutierrez *et al.* , 2005). However, it is likely that *M. tuberculosis* and its African variants *Mycobacterium africanum* and others, had a common ancestor about 35, 000 – 15, 000 years ago (Brosch *et al.* , 2002).

Currently circulating *M. tuberculosis* strains comprise of seven phylogenetic lineages that can live in humans, and two that are adapted to various animal species (Brites & Gagneux, 2015). Analysis based on known mutation rate shows that present diversity in the strains originated between 250 and 1000 years ago (Hirsh *et al.* , 2004). Different lineages of *M. tuberculosis* are associated with distinct parts of the world. For example, while lineage 2 is mostly found in East Asia, lineage 5, 6 and 7 are mostly restricted to Africa, and lineage 4 is widely distributed around the world (Stucki *et al.* , 2016). Within these different lineages of *M. tuberculosis* , lineage 2, or the Beijing

lineage, has been most strongly associated with MDR (Borrell & Gagneux, 2009). This higher MDR in lineage 2 can be attributed to the higher mutation rate of this lineage (Ford *et al.* , 2013). The epidemiological data that *M. tuberculosis* is phylogenetically structured has led to the hypothesis that different lineages might have adapted to specific human populations (Sebastien Gagneux, 2012).

There are no fossil records of *M. tuberculosis* , though M. tuberculosis DNA has been recovered from mummied tissues in Egypt and Peru, suggesting that it has existed in East Africa more than 5000 years ago (Nerlich *et al.* , 1997). There are no conflicts regarding the phylogeny of *M. tuberculosis* , since various independent research groups were able to place the common ancestor of modern *M. tuberculosis* to a similar time period (Daniel, 2006).

The cure for tuberculosis came in 1944, where a patient was treated and cured with streptomycin (Keshavjee & Farmer, 2012). However, resistance for this drug was observed not long after. The development of high efficacy drugs like isoniazid and rifampin soon led to the selection of mutations that confer resistance to these drugs as well. With the introduction of every new antituberculotic agent, there is a strong positive selection pressure to acquire drug resistance in order to survive in the patient.

The current global genetic diversity in MDR-TB indicates that MDR-TB evolved independently in many geographical hotspots. This was often due to large outbreaks of tuberculosis and the subsequent ill-management of treatment. Phylogenetic analysis of MDR-TB strains showed that many of the

resistance-associated polymorphisms have multibranch distributions, which suggests multiple ancestry and convergent evolution (Hazbón *et al.* , 2008).

The *de novo* emergence of drug resistance can be attributed to low adherence to treatment, incorrect dosage, or pharmacokinetic-pharmacodynamic (PK/PD) factors (Pasipanodya & Gumbo, 2011). More recently, the molecular mechanism of resistance has also been elucidated. Resistance often result from spontaneous mutations that obstruct drug-target binding (e. g. for rifampin in the *rpo* B gene), interfere with pro-drug activation (e. g. for isoniazid in the *kat* G gene), or cause overexpression of the target (e. g. for isoniazid in the promoter region of *inh* A) (Fonseca *et al.* , 2015). However, not all observed resistance phenotypes can be explained by these mutations. For example, up to 30% of isolates that are resistant to isoniazid have no mutations in these known resistance genes. More work needs to be done to elucidate other mechanism that led to the emergence and fixation of drug resistance in MDR-TB.

The fitness effects of resistance determinants have also not been well characterized. Even as drug resistance mutations can impair bacterial fitness in the absence of drugs, clinical isolates of MDR-TB strains can be more fit than isogenic strains constructed in the lab (Sebastian Gagneux *et al.* , 2006). This suggests other determinants of fitness – either secondary compensatory mutations or different genetic background – that can alleviate the deficiencies of acquiring resistance mutations.

Even as drug resistance in *M. tuberculosis* is often thought to be a single-step process, there is increasing evidence to show that this may occur in a

stepwise process instead that result in gradual resistance (Safi *et al.* , 2013). The first step may be a mutation that does not lead to a clinically significant increase in minimum inhibitory concentration (MIC) of the drug. However, these low-level resistances can persist and be selected for in suboptimal antibiotic concentrations, a channel through which resistance is amplified. These low-level mutants cannot be identified through currently available methods of susceptibility testing, and thus prove a threat to high-level resistance mutations.

Current phylogenetic analysis of MDR-TB also may suffer from sampling and processing biases, where strains from closely related clades are selected for sequencing. Current resistance diagnostics examines a single isolate from a single sample of a single disease episode, which disregards the heterogeneity of MDR-TB populations within a patient. To better infer the evolution of drug resistance in M. tuberculosis, several independent isolates from a single sample should be analysed and genotyped.

The evolution of drug resistance in MDR-TB is complex. By studying the evolution of multi-drug resistance in *M. tuberculosis* , we can better understand the evolutionary forces that drive the emergence and spread of TB around the world. This information can help in the design of more potent antituberculotic drugs, policies to curb its spread, and enhance diagnostic tests to identify drug resistant strains.

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