Antimicrobial Resistance in *Mycobacterium tuberculosis*: the Odd One Out

Vegard Eldholm[1] and François Balloux[2]

Correspondance: Vegard Eldholm v.eldholm@gmail.com

François Balloux f.balloux@ucl.ac.uk

1. Division of Infectious Disease Control, Norwegian Institute of Public Health, Lovisenberggata 8, Oslo 0456, Norway

2. Department of Genetics, Evolution and Environment, University College London, Darwin Building, Gower Street, London WC1E 6BT, UK

Keywords

Tuberculosis, Antibiotics, Evolution, Mutation, Transmission, Latency, Biofilms

Abstract

Antimicrobial resistance (AMR) threats are typically represented by bacteria capable of extensive horizontal gene transfer (HGT). One clear exception is *Mycobacterium tuberculosis* (*Mtb*). It is an obligate human pathogen with limited genetic diversity and a low mutation rate which further lacks any evidence for HGT. Such features should in principle reduce its ability to rapidly evolve AMR. We identify key features in its biology and epidemiology that allows it to overcome its low adaptive potential. We focus in particular on its innate resistance to drugs, unusual life cycle including an often extensive latent phase and its ability to shelter from exposure to antimicrobial drugs within cavities it induces in the lungs.

So Special

The rapid increase of **antimicrobial resistance (AMR; see Glossary)** in bacteria is driven by the widespread use, abuse and misuse of antibiotics, and constitutes one of the most challenging healthcare problems globally. With the notable exception of *Mycobacterium tuberculosis* (*Mtb*) the agent of **tuberculosis (TB)**, all other bacterial species listed as current AMR threats by the Centers for Disease Control http://www.cdc.gov/drugresistance/biggest_threats.htmltrend frequently exchange genetic material and frequently acquire novel mutations through gain of new genes by horizontal gene transfer (HGT) rather than *de novo* mutations (Figure 1). *Mtb* has a virtually non-existent accessory genome, meaning that almost all genes are common to all strains. Evidence suggests that there is little or no recombination occurring in the species. In addition to its strictly clonal reproduction, *Mtb* is characterized by a low mutation rate and limited genetic diversity, which has led to it being considered as a 'monomorphic bacterium' [1]. *Mtb* also stands out from most other bacteria considered as AMR threats by being an obligate pathogen.

The lack of HGT combined with a low mutation rate makes *Mtb* an *a priori* unlikely foe outwitting considerable efforts aiming at defeating it. Yet, **multi-drug resistant TB** [**MDR-TB** resistant to **isoniazid (INH)** and **rifampicin (RIF)**] and **extensively-drug resistant TB** (**XDR-TB**; MDR-TB with additional resistance to second-line injectable drugs and fluoroquinolones) represent a significant and growing threat to global health accounting for nearly half a million new cases and around 200,000 deaths in 2014 alone http://www.who.int/tb/publications/global_report/en/. Resistance has also been shown to emerge essentially immediately after the introduction of new drugs and highly resistant strains of *Mtb* have been in circulation for at least four decades [2, 3]. In this review we explore why *Mtb* is such a severe public health problem despite being an outlier amongst other AMR threats. To search for clues, we review the key features of its biology, life history and epidemiology in the wider phylogenetic context of the bewildering diversity of mycobacteria.

We conclude that compared to other bacterial resistance threats, the strict reliance of *Mtb* on *de novo* chromosomal mutations has led to a relatively slow rate of emergence of AMR. However, the proportion of resistant strains is increasing and this trend is proving difficult to contain due to various public health failings, the intrinsic resistance of mycobacteria to a range of antibiotics and life-style properties such as the ability of *Mtb* to hide in pulmonary cavities and lesions with limited drug penetrance.

Roots Bloody Roots

The genus *Mycobacterium* contains well over 100 recognized species and probably an equally large number of species yet to be discovered. The genetic diversity within described species varies significantly, and this discrepancy follows a simple rule: the more attention a group of mycobacteria has received, the more likely it has been split into multiple species. The genus mainly comprises environmental bacteria, but a number of these can cause opportunistic infections in humans (Figure 2). The more host-specialized members exhibit a clonal mode of inheritance, but recombination is frequent in the genus as a whole.

A comparative analysis including 13 mycobacterial species found only sporadic evidence of recombination in core genes within Mycobacteria, but genome content analyses suggest that horizontal acquisition of genes is frequent and played an important role in the evolution of this group [4]. The presence of numerous genomic islands dispersed across different mycobacterial species is also suggestive of extensive horizontal gene transfer from outside the genus [5]. A meiosis-like conjugational mechanism termed distributed conjugal transfer (DCT), controlled by a chromosomally encoded mating-identity locus was recently discovered in *Mycobacterium smegmatis* [6]. This intriguing mechanism enables the transfer of large unlinked stretches of DNA across entire chromosomes. Genomic signatures indicative of DCT have subsequently been identified in *Mycobacterium canettii*, an environmental mycobacterium closely related to *Mtb*, suggesting that this mode of horizontal gene transfer may play an important role in shaping the evolution of mycobacteria [7].

Mycobacteria are intrinsically resistant to a number of antimicrobial compounds, an observation that is often explained by the presence of an impermeable mycolic acid-rich cell envelope. In addition, mycobacteria are members of the order Actinomycetales which also includes *Streptomyces* species, well known for their ability to produce a wide range of antibiotics. Antibiotic-producing bacteria must have defence mechanisms in place to guard them against their own toxic compounds. The inducible *whiB7* multidrug-resistance system common to all Actinomycetales has been shown to reduce susceptibility to a wide range of antimicrobials, including macrolides, chloramphenicol, tetracycline and aminoglycosides [8]. In *Mtb*, the regulon includes genes involved in drug efflux (*tap*), a putative macrolide exporter (Rv1473), the ribosomal methyltransferase *erm* and the aminoglycoside acetyltransferase *eis* [8].

Mtb and closely related animal strains, together with the leprosy bacilli are unusual insofar they constitute specialized pathogens of humans and other mammals (Figure 2). In contrast to most other mycobacteria, these bacilli readily transmit between mammalian hosts, a hallmark of true pathogens [9]. Despite the general perception that leprosy is a disease of the past, nearly a quarter million cases are reported yearly [10]. The ruminant-infecting *Mycobacterium avium subsp. paratuberculosis* also deserves to be mentioned as a host-specialized pathogen. However, even though this subspecies can only grow intracellularly, it can survive for long periods in the environment and transmits via the faecal-oral route and possibly also via nematodes and protozoa [11].

Up- and Down(sizing)

Genome-level comparison of *Mtb* and *M. marinum* revealed that *Mtb* has also undergone a process of genome downsizing on its path from an environmental ancestor to a specialized mainly intracellular pathogen [12], but on a far more moderate scale than the leprosy bacilli. The general downsizing has however also been accompanied by the acquisition of a number of genes, including genes involved in virulence [12]. There is strong evidence that recombination has been important in shaping the early evolution of *Mtb* as it evolved from an ancestor closely related to present-day **smooth tubercle bacilli (STB)** [13]. A number of strains adapted to

various mammalian hosts have evolved from *Mtb*. Together with *Mtb*, these animal strains have been grouped together in the so-called ***Mtb* complex (MBTC)**. The STB *M. canettii* (not yet accepted as a valid species name) is also generally included in the MTBC but evidence suggests that this species might be mainly environmental [14]. The human and animal adapted MTBC strains represent a clonal expansion rooted in the extensively recombining and genetically diverse STB. STB strains can cause TB in immunocompetent individuals, but are significantly less virulent than *Mtb*, and do not seem to transmit between humans [9, 15]. They have only been isolated from sporadic human cases in East Africa, which has led to the suggestions that this region of the world is where the MTBC originated. The evolutionary history of the MTBC seems to be analogous to the clonal expansion of animal-adapted *M. avium* strains from a more diverse environmental group (Box 1).

No consensus has been reached to date on whether modern *Mtb* has retained the ability to undergo recombination [16, 17], but the sum of evidence suggests that recombination is exceedingly rare. It is plausible that some analyses pointing to relatively high rates of recombination in *Mtb* had been misled by convergent evolution at a relatively high number of sites, many of which are likely due to multiple independent emergence of resistance mutations following exposure to antimicrobial compounds [18]. Indeed, both convergent parallel evolution and recombination lead to conflicts between loci over the best-supported topology of a phylogenetic tree.

Distance Equals Rate Times Time

Comparing mutation rates across bacterial species is challenging. Fluctuation assays [19], despite often being regarded as the gold standard, only allow estimating mutation rates if all possible mutations yielding resistance to a given antimicrobial compound are known, which is generally not the case. Even when comparisons are restricted to experiments using the same antimicrobial compound, estimated mutation rates can vary due to variation in the number of mutations that can potentially bring about resistance in different species. Additionally, the same mutation could yield variable levels of resistance in different species

An alternative approach to estimate mutation rates relies on Bayesian phylogenetic analyses of whole-genome sequences from clinical isolates sampled over several years and for which isolation dates are precisely known. Such data is available for all the species illustrated in Figure 1, and mutation estimates from independent studies are highly congruent for *Mtb* [20-22]. In this comparison *Mtb* has the lowest rate of all, with *Salmonella enterica* next on the list. This comparison is by no means perfect, due to the variation in sampling and methodology between studies. However, the approach offers the major advantage that such mutation rate estimates are scaled over unit time in natural conditions (rather than per generation). As such, these phylogenetic mutation rate estimates capture the capacity of different bacteria to adapt to antimicrobials in the wild. The generation time of *Mtb* is indisputably very slow compared to the vast majority of clinically important bacteria. As a result, despite what seems to be a relatively unremarkable mutation rate per generation compared to *e.g. Escherichia coli* [23, 24], *Mtb* evolves at a very slow pace compared to most other AMR threats.

An extrapolation of these short term-term mutation rates to more ancient times points to a fairly recent origin of *Mtb* less than 6,000 years ago [25, 26], which is incompatible with scenarios of ancient origin and a joint colonization of the globe of *Mtb* and anatomically modern humans some 40,000-70,000 years ago that have been suggested [27, 28]. These age estimates also fit somewhat uncomfortably with direct evidence for ancient MTBC infection from PCR and the detection of lipid biomarkers targeting the unusual and highly stable components of the cell wall [29]. These include the detection of TB in human remains from Syria some 11,000 years ago [30] and in a bison from Wyoming dated to ~17,000 years ago [31]. Also of note is the detection of DNA harboring the *Mtb*-specific TbD1-deletion in a women and child from Israel some 9,000 years ago [32]. At this time, it seems fair to accept that the jury is still out on the age of *Mtb* and additional ancient DNA genome sequences will be required to obtain better long-term calibration of mutation rates; the oldest *Mtb* genomes generated to date being only about 250 years old [26].

Coming Back to Life

Mtb has a very unusual life cycle with a long latency period. About 90% of infected people never develop active transmissible TB but stay healthy and asymptomatic [33], possibly because humans have adapted to control TB quite efficiently even though immune activation does not lead to sterilization. About two billion people are estimated to be latently infected with TB [34], constituting a massive challenge for TB eradication efforts. A ten-year follow-up study of TB contacts found that the majority of people that do develop active TB do so within the first three years of exposure [33]. However, old age is also known to be a risk factor for active TB [35].

If *Mtb* has co-evolved with humans since the dawn of our species, or at least for millennia, a long latency period could be regarded as an adaptation to the small and isolated populations of our ancestors. This adaptation might have allowed *Mtb* to transmit between host generations as active TB developed in the elderly and was transmitted to the next generations, without burning through and killing off small and isolated bands of hunter-gatherers and thus extinguishing its population of hosts [36]. A plausible and worrisome scenario is that modern TB strains are evolving towards shorter latency periods. There are now more than seven billion humans on the planet and potential human TB hosts frequently travel and migrate between countries and continents. As such, accelerated progression to active disease and hence possible transmission, could be selected for. In this context, it is interesting to note that the Beijing TB lineage which has expanded globally in recent decades seems to be associated with accelerated progression to active TB relative to other TB strains and *Mycobacterium africanum* [37].

Space Oddity

Globally, 3.3 % of new TB cases were estimated to be MDR in 2014. This relatively low rate might come as a surprise to many, and does conceal significant variation between and within countries and regions. In many western European countries the burden is low, as exemplified by the situation in the UK, where 1.6% of all cases were MDR-TB in 2013 https://www.gov.uk/government/publications/tuberculosis-in-england-annual-report. This figure in itself does not capture the full extent of the problem as 7.1% of strains in the country

are INH resistant in the (Figure 3), which is unusually high for a western European country. Again, this figure for INH resistance does only very imperfectly capture the reality on the ground. INH resistant strains in the UK are primarily found in London and are patchily distributed even at that scale.

TB drug resistance in the UK and in other high income countries is a serious public health issue incurring a significant financial burden on public health services, even though the rate of resistance is relatively low compared to those found in some other resistance threats (Figure 3). The extraordinarily rapid population-level response to antibiotics seen for example in *Staphylococcus aureus* and *Enterococcus faecium* is striking when compared to *Mtb*, and probably partly reflects its low mutation rate and lack of recombination and of resistance-determinants on mobile genetic elements (Figure 1). However, *Mtb* resistance rates in high-income countries are not representative of the frequency of MDR/XDR-TB strains in other parts of the world, and the burden due to AMR in TB resistance is crippling in some hotspots (Box 2 and Figure 4).

Road to Resistance

Although intrinsically resistant to many drugs, there is little evidence to suggest that the *Mtb* genome should be especially prone to evolving additional drug resistance (Figure 1). In fact, rates of RIF resistance in *M. leprae*, which is not generally regarded as a major resistance threat, seem to mirror the rates found in *Mtb*. The inability to culture *M. leprae*, and thus to perform phenotypic **drug susceptibility testing (DST)** complicates analyses of resistance in this bacterium. However, available data from India, South-East Asia and Colombia all found about 3% of all cases to be RIF resistant [38-40], a rate that is similar to recent estimates of *Mtb* RIF resistance globally at 3.3%.

The first outbreaks of MDR-TB were largely restricted to HIV co-infected patients and often confined to hospitals [41-43]. This can easily be explained by HIV infection triggering the development to active TB. To make matters worse, HIV and TB medications have been shown to negatively interfere with each other. However, strains with extensive resistance profiles have

recently been shown to have emerged and transmitted well before the HIV epidemic took off in the 1980s [2, 3] and there is currently little evidence to suggest a causal relationship between HIV co-infection and increased emergence or circulation of MDR-TB strains [44].

In former Soviet Eastern Europe states, the massive rates of drug resistant TB have been attributed to the collapse of health systems following the fall of the Soviet Union [45]. Similar forces might be at play in lower-middle income countries such as India today where anti-TB medication is available to most, but the health system infrastructure is often weak and antimicrobial stewardship is lacking [46]. Another possible explanation for the regional differences in resistance burden could be due to phenotypic differences between strains. If the dominant lineage in a region were more prone to develop resistance than other lineages, this could exacerbate the resistance burden in the region.

The Beijing lineage (Lineage 2) is the dominating lineage in large parts of Asia and eastern Europe and is often associated with drug-resistance [47]. Whether members of the lineage are actually more prone to develop resistance-conferring mutations as recently suggested by Ford and colleagues [48] remains unclear. In their study, Ford *et al.* relied heavily on lab strains [48] and an earlier similar study with slightly larger sample size did not point to a higher rate of acquisition of mutations of Lineage 2 strains [49]. It is possible that the Beijing lineage simply happened to be at the right place at the right time with the collapse of the Soviet Union, leading to the emergence of resistant strains still circulating in large numbers today. The observation that Beijing isolates are associated with accelerated progression to active TB [37] could be part of the explanation for the relatively recent and ongoing global expansion of the lineage.

To summarize, worldwide distribution of MDR-TB is extremely heterogeneous and has undoubtedly been shaped by past failings in public health infrastructure in various parts of the world. This heterogeneity might have been further exacerbated by an intrinsic propensity of certain lineages to acquire resistance more readily. However, lineage-specific factors are difficult to quantify because *Mtb* lineages are themselves patchily distributed.

My Body Is a Cage

The first clinical stage of TB infection is termed primary TB and typically involves the production and spread of granulomas systemically and to regional lymph nodes [50]. Within a few weeks, immunity develops and the infection regresses, but is not sterilized [50].TB is generally more virulent in animals than in humans, and common animal models such as mice, guinea pigs, rabbits and monkeys all develop aggressive primary TB that is not transmissible and often results in death [51]. Humans are special in that primary TB is generally not associated with serious illness. In humans however, *Mtb* enters a latent stage following regression of the primary infection. Upon re-activation of the dormant organisms or reinfection with new organisms from the environment, softened and fragmented lung tissue is coughed up leaving cavities that harbor small numbers of bacilli. This early stage of cavity formation can erode arteries to produce heavy bleeding, a classical sign of TB [50]. Upon maturation, cavities develop a thin fibrous wall. The inner surface is covered with fluid caseum with no viable cells. *Mtb* grows extracellularly on the surface of such cavities as a pellicle (biofilm) [52]. *Mtb* can grow in massive numbers on the surface of cavities where it can be coughed into the environment while the host remains in health except for the coughing [50]. This form of clinical TB is obviously extremely transmissible as billions of bacteria can be produced each day [53].

In addition to the role of cavities in the transmission of TB, they constitute a significant complication for successful antimicrobial therapy as different drugs penetrate cavities with varying efficiency: The fluoroquinolone moxifloxacin seem to penetrate well, whereas the first-line drugs INH, RIF and pyrazinamide (PZA) are less efficient [54]. The more experimental drug linezolid has been shown to be effective against cavitary TB, albeit often with quite serious side-effects [55]. Mathematical modelling has revealed that using drugs with different penetration profiles leads to spatial monotherapy and rapid evolution of multidrug resistance [56]. It is no surprise then, that cavitary TB is associated with treatment failure [57] and is a major risk factor for acquired resistance to second-line drugs [58, 59]. In fact, a recent study from Georgia found additional resistance to emerge in 58% of cavitary MDR-TB cases treated with second line

drugs, but in 'only' 16% of such patients not presenting with cavities [58]. Efforts to optimize regimens for progressed cavitary TB minimizing resistance development are warranted.

The emergence of drug resistant TB is most often attributed to poor patient adherence to drug treatment schemes, a problem that is ameliorated by **directly observed treatment (DOT)**. Patients are typically enrolled on anti-TB therapy for 6 to 24 months, depending on response and the resistance phenotype of the infection. In light of this one cannot expect the problem of imperfect patient compliance to go away anytime soon. Yet, based on a hollow-fiber model, pharmacokinetic variability alone was estimated to result in acquired multidrug-resistance in about 1% of patients, irrespective of adherence [60]. Many bacteria exhibit increased drug tolerance when growing in biofilms and this phenomenon has also been observed in *Mtb*. Bacilli in cavities are also separated from the host's immune defenses by the wall of the cavity that prevents penetration of viable cells. When allowed to form biofilms *in vitro*, a small but possibly clinically important subpopulation emerges, which is able to tolerate very high doses of antimicrobials [61]. *Mtb* biofilm formation was recently shown to depend on keto-mycolic acids and when co-cultured with a wild-type strain, even drug sensitive biofilm-defective mutants were found to become drug tolerant [62]. The biological relevance of biofilm formation within patients remains to be determined, but the biofilm-like growth of *Mtb* within and on the surface of cavities [52] suggest that this growth mode could be clinically very important.

Chemical Warfare

Recent studies utilizing deep-sequencing of patient isolates have revealed a surprising degree of *Mtb* genetic diversity within patients [63-67]. Resistance mutations have been found to emerge multiple times within a single patient, generally followed by selective sweeps resulting in one clone replacing the whole within-host population [63, 66, 67]. Large *Mtb* population sizes and significant genetic diversity upon diagnosis surely play important roles in the emergence of resistance, as a diverse population is more likely to encompass mutants with decreased susceptibility to anti-TB therapeutic drugs. The importance of within-host *Mtb* population size

297 and genetic diversity in resistance development is a research avenue that deserves further
298 attention.

299 Even more worrisome than resistance evolving in individual patients is the transmission of
300 resistant strains with little or no apparent fitness cost to the bacterium. The overall robustness
301 of *Mtb* when challenged with antimicrobials led to standardized drug treatment schemes
302 including a cocktail of four drugs. Unfortunately, these schemes are not always paired with
303 robust drug-susceptibility testing. It has been argued that standardized treatment schemes for
304 susceptible and MDR-TB in the absence of phenotypic resistance testing has been a direct
305 driver of the evolution of XDR-TB in South Africa [68]. It is well documented that the most
306 commonly transmitted RIF-resistance mutation *rpoB* S450L in combination with secondary
307 compensatory mutations in polymerase subunits is associated with little or no fitness cost [69,
308 70] whereas the picture is less clear for INH-resistance. It has however been shown that the
309 most common INH-resistance mutation, namely *katG* S315T retains residual catalase-
310 peroxidase activity, is virulent in mice, and importantly, transmits well between people [71, 72].

311 Recent studies have documented that MDR-TB strains have been in circulation for decades [2,
312 3]. The four-drug anti-TB regimen currently in use includes drugs that have all been used
313 continuously against TB for 40-60 years. It may thus come as no surprise that this has selected
314 for highly transmissible MDR-TB strains, and we might perhaps consider ourselves lucky that
315 the problem is not yet worse than it is.

316 **Concluding Remarks: Know Your Enemy**

317 Essentially irrespectively of the feature under scrutiny, *Mtb* stands out from all other bacteria
318 considered as AMR threats. Some of these peculiarities should constitute major chinks in its
319 armor making it a tractable target for a rare success in stemming the rise of AMRs. In particular,
320 *Mtb* has a low mutation rate and limited genetic diversity, lacks any mechanism for extensive
321 HGT and does not benefit from any hiding place outside its human host, such as an
322 environmental or zoonotic reservoir. It remains to be defined what exact form a determined
323 assault against AMR-TB (and TB more generally) should take. However, it is clear that any

successful public health strategy will have to be informed by robust fundamental scientific evidence and be multipronged to be successful. We have learned a lot about *Mtb* and TB, in particular since the advent of fast and affordable sequencing technologies. However, it would be foolish to assume that the current knowledge is sufficient to vanquish *Mtb* (see Outstanding Questions), as it remains a deadly and surprisingly adaptable foe despite its apparent inherent weaknesses.

Acknowledgements

The authors acknowledge support from the Norwegian Research Council (grant 221562), the ERC (grant ERC 260801 – BIG_IDEA) and the National Institute for Health Research University College London Hospitals Biomedical Research Centre. We are grateful for the help and information provided by Helen Donoghue and Robert L. Hunter.

References

1 Achtman, M. (2008) Evolution, Population Structure, and Phylogeography of Genetically Monomorphic Bacterial Pathogens. *Annu. Rev. Microbiol.* 62, 53-70

2 Cohen, K.A.*, et al.* (2015) Evolution of Extensively Drug-Resistant Tuberculosis over Four Decades: Whole Genome Sequencing and Dating Analysis of *Mycobacterium tuberculosis* Isolates from KwaZulu-Natal. *PLoS Med* 12, e1001880

3 Eldholm, V.*, et al.* (2015) Four decades of transmission of a multidrug-resistant *Mycobacterium tuberculosis* outbreak strain. *Nat Commun* 6

4 Smith, S.E.*, et al.* (2012) Comparative genomic and phylogenetic approaches to characterize the role of genetic recombination in mycobacterial evolution. *PLoS One* 7, e50070

5 Reva, O.*, et al.* (2015) Role of the horizontal gene exchange in evolution of pathogenic Mycobacteria. *BMC Evol Biol* 15 Suppl 1, S2

6 Gray, T.A.*, et al.* (2013) Distributive conjugal transfer in mycobacteria generates progeny with meiotic-like genome-wide mosaicism, allowing mapping of a mating identity locus. *PLoS Biol* 11, e1001602

7 Mortimer, T.D. and Pepperell, C.S. (2014) Genomic signatures of distributive conjugal transfer among mycobacteria. *Genome biology and evolution* 6, 2489-2500

8 Morris, R.P.*, et al.* (2005) Ancestral antibiotic resistance in *Mycobacterium tuberculosis*. *Proceedings of the National Academy of Sciences of the United States of America* 102, 12200-12205

9 Veyrier, F.J.*, et al.* (2011) The rise and fall of the Mycobacterium tuberculosis genome. *Trends Microbiol* 19, 156-161

10 World Health Organisation (2013) Global leprosy: Update on the 2012 situation. *Wkly Epidemiol Rec* 88, 365-379

11 Rowe, M.T. and Grant, I.R. (2006) Mycobacterium avium ssp. paratuberculosis and its potential survival tactics. *Letters in applied microbiology* 42, 305-311

12 Stinear, T.P.*, et al.* (2008) Insights from the complete genome sequence of Mycobacterium marinum on the evolution of Mycobacterium tuberculosis. *Genome Res* 18, 729-741

13 Gutierrez, M.C.*, et al.* (2005) Ancient origin and gene mosaicism of the progenitor of *Mycobacterium tuberculosis*. *PLoS Pathog* 1, e5

14 Koeck, J.L.*, et al.* (2011) Clinical characteristics of the smooth tubercle bacilli '*Mycobacterium canettii*' infection suggest the existence of an environmental reservoir. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 17, 1013-1019

15 Supply, P.*, et al.* (2013) Genomic analysis of smooth tubercle bacilli provides insights into ancestry and pathoadaptation of *Mycobacterium tuberculosis*. *Nat Genet* 45, 172-179

16 Pepperell, C.S.*, et al.* (2013) The Role of Selection in Shaping Diversity of Natural *M. tuberculosis* Populations. *Plos Pathogens* 9

17 Namouchi, A.*, et al.* (2012) After the bottleneck: Genome-wide diversification of the *Mycobacterium tuberculosis* complex by mutation, recombination, and natural selection. *Genome Res.* 22, 721-734

18 Farhat, M.R.*, et al.* (2013) Genomic analysis identifies targets of convergent positive selection in drug-resistant *Mycobacterium tuberculosis*. *Nat Genet* 45, 1183-1189

19 Luria, S.E. and Delbrück, M. (1943) Mutations of bacteria from virus sensitivity to virus resistance. *Genetics* 28, 491-511

20 Eldholm, V.*, et al.* (2015) Four decades of transmission of a multidrug-resistant *Mycobacterium tuberculosis* outbreak strain. *Nat Commun* 6, 7119

21 Roetzer, A*., et al.* (2013) Whole Genome Sequencing versus Traditional Genotyping for Investigation of a *Mycobacterium tuberculosis* Outbreak: A Longitudinal Molecular Epidemiological Study. *PLoS Med* 10, e1001387
22 Walker, T.M*., et al.* (2013) Whole-genome sequencing to delineate *Mycobacterium tuberculosis* outbreaks: a retrospective observational study. *Lancet Infect Dis* 13, 137-146
23 Ford, C.B*., et al.* (2013) *Mycobacterium tuberculosis* mutation rate estimates from different lineages predict substantial differences in the emergence of drug-resistant tuberculosis. *Nat Genet* advance online publication **45**, 784–790
24 Krašovec, R*., et al.* (2014) Mutation rate plasticity in rifampicin resistance depends on Escherichia coli cell–cell interactions. *Nat Commun* 5, 3742
25 Bos, K.I*., et al.* (2014) Pre-Columbian mycobacterial genomes reveal seals as a source of New World human tuberculosis. *Nature* 514, 494-497
26 Kay, G.L*., et al.* (2015) Eighteenth-century genomes show that mixed infections were common at time of peak tuberculosis in Europe. *Nat Commun* 6, 6717
27 Wirth, T*., et al.* (2008) Origin, Spread and Demography of the *Mycobacterium tuberculosis* Complex. *PLoS Pathog* 4, e1000160
28 Comas, I*., et al.* (2013) Out-of-Africa migration and Neolithic coexpansion of *Mycobacterium tuberculosis* with modern humans. *Nat Genet* 45, 1176-1182
29 Lee, O.Y.C*., et al.* (2015) Lipid biomarkers provide evolutionary signposts for the oldest known cases of tuberculosis. *Tuberculosis* 95, Supplement 1, S127-S132
30 Baker, O*., et al.* (2015) Human tuberculosis predates domestication in ancient Syria. *Tuberculosis (Edinburgh, Scotland)* 95 Suppl 1, S4-s12
31 Lee, O.Y.C*., et al.* (2012) *Mycobacterium tuberculosis* Complex Lipid Virulence Factors Preserved in the 17,000-Year-Old Skeleton of an Extinct Bison, *Bison antiquus. PLoS ONE* 7, e41923
32 Hershkovitz, I*., et al.* (2008) Detection and molecular characterization of 9,000-year-old *Mycobacterium tuberculosis* from a Neolithic settlement in the Eastern Mediterranean. *PLoS One* 3, e3426
33 Sloot, R*., et al.* (2014) Risk of Tuberculosis after Recent Exposure. A 10-Year Follow-up Study of Contacts in Amsterdam. *American Journal of Respiratory and Critical Care Medicine* 190, 1044-1052
34 Barry, C.E*., et al.* (2009) The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. *Nat Rev Micro* 7, 845-855
35 Tocque, K*., et al.* (1998) Long-term Trends in Tuberculosis. *American Journal of Respiratory and Critical Care Medicine* 158, 484-488
36 Gagneux, S. (2012) Host-pathogen coevolution in human tuberculosis. *Philos Trans R Soc Lond B Biol Sci* 367, 850-859
37 de Jong, B.C*., et al.* (2008) Progression to Active Tuberculosis, but Not Transmission, Varies by *Mycobacterium tuberculosis* Lineage in The Gambia. *Journal of Infectious Diseases* 198, 1037-1043
38 Guerrero, M.I*., et al.* (2014) Is drug-resistant *Mycobacterium leprae* a real cause for concern?: First approach to molecular monitoring of multibacillary Colombian patients with and without previous leprosy treatment. *Biomedica : revista del Instituto Nacional de Salud* 34 Suppl 1, 137-147
39 Lavania, M*., et al.* (2015) Emergence of primary drug resistance to rifampicin in *Mycobacterium leprae* strains from leprosy patients in India. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*
40 Matsuoka, M*., et al.* (2007) The frequency of drug resistance mutations in *Mycobacterium leprae* isolates in untreated and relapsed leprosy patients from Myanmar, Indonesia and the Philippines. *Leprosy review* 78, 343-352
41 Ritacco, V*., et al.* (1997) Nosocomial Spread of Human Immunodeficiency Virus-Related Multidrug-Resistant Tuberculosis in Buenos Aires. *J Infect Dis* 176, 637-642

42 Small, P.M.*, et al.* (1993) Exogenous reinfection with multidrug-resistant *Mycobacterium tuberculosis* in patients with advanced HIV infection. *The New England journal of medicine* 328, 1137-1144
43 Wells, C.D.*, et al.* (2007) HIV Infection and Multidrug-Resistant Tuberculosis—The Perfect Storm. *J Infect Dis* 196, S86-S107
44 Suchindran, S.*, et al.* (2009) Is HIV Infection a Risk Factor for Multi-Drug Resistant Tuberculosis? A Systematic Review. *PLoS ONE* 4, e5561
45 Merker, M.*, et al.* (2015) Evolutionary history and global spread of the *Mycobacterium tuberculosis* Beijing lineage. *Nat Genet*
46 Udwadia, Z.F.*, et al.* (2010) Tuberculosis management by private practitioners in Mumbai, India: has anything changed in two decades? *PLoS One* 5, e12023
47 Bifani, P.J.*, et al.* (2002) Global dissemination of the *Mycobacterium tuberculosis* W-Beijing family strains. *Trends Microbiol* 10, 45-52
48 Ford, C.B.*, et al.* (2013) *Mycobacterium tuberculosis* mutation rate estimates from different lineages predict substantial differences in the emergence of drug-resistant tuberculosis. *Nat Genet* 45, 784-790
49 Werngren, J. and Hoffner, S.E. (2003) Drug-Susceptible *Mycobacterium tuberculosis* Beijing Genotype Does Not Develop Mutation-Conferred Resistance to Rifampin at an Elevated Rate. *J. Clin. Microbiol.* 41, 1520-1524
50 Hunter, R.L. (2011) Pathology of post primary tuberculosis of the lung: an illustrated critical review. *Tuberculosis (Edinburgh, Scotland)* 91, 497-509
51 Hunter, R.L.*, et al.* (2006) Multiple Roles of Cord Factor in the Pathogenesis of Primary, Secondary, and Cavitary Tuberculosis, Including a Revised Description of the Pathology of Secondary Disease. *Annals of Clinical & Laboratory Science* 36, 371-386
52 Hunter, R.L.*, et al.* (2014) Pathogenesis of Post Primary Tuberculosis: Immunity and Hypersensitivity in the Development of Cavities. *Annals of Clinical & Laboratory Science* 44, 365-387
53 Osler, W. (1892) Tuberculosis. In *In: The Principles and Practice of Medicine*, pp. 184–255, Appleton
54 Kjellsson, M.C.*, et al.* (2012) Pharmacokinetic evaluation of the penetration of antituberculosis agents in rabbit pulmonary lesions. *Antimicrob Agents Chemother* 56, 446-457
55 Schecter, G.F.*, et al.* (2010) Linezolid in the treatment of multidrug-resistant tuberculosis. *Clin Infect Dis* 50, 49-55
56 Moreno-Gamez, S.*, et al.* (2015) Imperfect drug penetration leads to spatial monotherapy and rapid evolution of multidrug resistance. *Proc Natl Acad Sci U S A* 112, E2874-2883
57 Kritski, A.L.*, et al.* (1997) Retreatment tuberculosis cases. Factors associated with drug resistance and adverse outcomes. *Chest* 111, 1162-1167
58 Kempker, R.R.*, et al.* (2015) Acquired Drug Resistance in *Mycobacterium tuberculosis* and Poor Outcomes among Patients with Multidrug-Resistant Tuberculosis. *Emerg Infect Dis* 21, 992-1001
59 Shin, S.S.*, et al.* (2010) Development of extensively drug-resistant tuberculosis during multidrug-resistant tuberculosis treatment. *Am J Respir Crit Care Med* 182, 426-432
60 Srivastava, S.*, et al.* (2011) Multidrug-resistant tuberculosis not due to noncompliance but to between-patient pharmacokinetic variability. *J Infect Dis* 204, 1951-1959
61 Ojha, A.K.*, et al.* (2008) Growth of *Mycobacterium tuberculosis* biofilms containing free mycolic acids and harbouring drug-tolerant bacteria. *Mol Microbiol* 69, 164-174
62 Sambandan, D.*, et al.* (2013) Keto-mycolic acid-dependent pellicle formation confers tolerance to drug-sensitive Mycobacterium tuberculosis. *MBio* 4, e00222-00213
63 Eldholm, V.*, et al.* (2014) Evolution of extensively drug-resistant *Mycobacterium tuberculosis* from a susceptible ancestor in a single patient. *Genome Biol* 15, 490
64 Merker, M.*, et al.* (2013) Whole genome sequencing reveals complex evolution patterns of multidrug-resistant *Mycobacterium tuberculosis* Beijing strains in patients. *PLoS One* 8, e82551

65 O'Neill, M.B.*, et al.* (2015) Diversity of *Mycobacterium tuberculosis* across evolutionary scales. *PLoS Pathog* 11: e1005257
66 Perez-Lago, L.*, et al.* (2014) Whole genome sequencing analysis of intrapatient microevolution in *Mycobacterium tuberculosis*: potential impact on the inference of tuberculosis transmission. *J Infect Dis* 209, 98-108
67 Sun, G.*, et al.* (2012) Dynamic Population Changes in *Mycobacterium tuberculosis* During Acquisition and Fixation of Drug Resistance in Patients. *Journal of Infectious Diseases* 206, 1724-1733
68 Pillay, M. and Sturm, A.W. (2007) Evolution of the extensively drug-resistant F15/LAM4/KZN strain of *Mycobacterium tuberculosis* in KwaZulu-Natal, South Africa. *Clin Infect Dis* 45, 1409-1414
69 Brandis, G. and Hughes, D. (2013) Genetic characterization of compensatory evolution in strains carrying rpoB Ser531Leu, the rifampicin resistance mutation most frequently found in clinical isolates. *The Journal of antimicrobial chemotherapy* 68, 2493-2497
70 Comas, I.*, et al.* (2012) Whole-genome sequencing of rifampicin-resistant *Mycobacterium tuberculosis* strains identifies compensatory mutations in RNA polymerase genes. *Nat Genet* 44, 106-110
71 Gagneux, S.*, et al.* (2006) Impact of Bacterial Genetics on the Transmission of Isoniazid-Resistant *Mycobacterium tuberculosis*. *PLoS Pathog* 2, e61
72 Pym, A.S.*, et al.* (2002) Effect of katG Mutations on the Virulence of *Mycobacterium tuberculosis* and the Implication for Transmission in Humans. *Infection and Immunity* 70, 4955-4960
73 MRC Cardiothoracic Epidemiology Group Unit (1987) National survey of tuberculosis notifications in England and Wales in 1983: Characteristics of disease. *Tubercle* 68, 19-32
74 Marks, J. (1961) Drug resistance in untreated pulmonary tuberculosis in England and Wales during 1960. A survey by the Public Health Laboratory Service. *Tubercle* 42, 308-313
75 Kruijshaar, M.E.*, et al.* (2008) Increasing antituberculosis drug resistance in the United Kingdom: analysis of National Surveillance Data. *BMJ (Clinical research ed.)* 336, 1231-1234
76 Rose, A.M.C.*, et al.* (2001) Tuberculosis at the end of the 20th century in England and Wales: results of a national survey in 1998. *Thorax* 56, 173-179
77 Reacher, M.H.*, et al.* (2000) Bacteraemia and antibiotic resistance of its pathogens reported in England and Wales between 1990 and 1998: trend analysis. *BMJ (Clinical research ed.)* 320, 213-216
78 Johnson, A.P. and James, D. (1997) Continuing increase in invasive methicillin-resistant infection. *Lancet* 350, 1710
79 Speller, D.C.*, et al.* (1997) Resistance to methicillin and other antibiotics in isolates of Staphylococcus aureus from blood and cerebrospinal fluid, England and Wales, 1989-95. *Lancet* 350, 323-325
80 Skrahina, A.*, et al.* (2013) Multidrug-resistant tuberculosis in Belarus: the size of the problem and associated risk factors. *Bulletin of the World Health Organization* 91, 36-45
81 D'Souza D, T.*, et al.* (2009) High levels of multidrug resistant tuberculosis in new and treatment-failure patients from the Revised National Tuberculosis Control Programme in an urban metropolis (Mumbai) in Western India. *BMC public health* 9, 211
82 Coovadia, Y.M.*, et al.* (2013) Rifampicin mono-resistance in *Mycobacterium tuberculosis* in KwaZulu-Natal, South Africa: a significant phenomenon in a high prevalence TB-HIV region. *PLoS One* 8, e77712
83 Massyn, N.*, et al.* (2014) District Health Barometer 2013/2014. Health System Trust
84 Nessar, R.*, et al.* (2012) Mycobacterium abscessus: a new antibiotic nightmare. *The Journal of antimicrobial chemotherapy* 67, 810-818
85 Bryant, J.M.*, et al.* (2013) Whole-genome sequencing to identify transmission of *Mycobacterium abscessus* between patients with cystic fibrosis: a retrospective cohort study. *Lancet* 381, 1551-1560
86 Nash, K.A.*, et al.* (2009) A Novel Gene, erm(41), Confers Inducible Macrolide Resistance to Clinical Isolates of *Mycobacterium abscessus* but Is Absent from Mycobacterium chelonae. *Antimicrobial Agents and Chemotherapy* 53, 1367-1376

87 Turenne, C.Y.*, et al.* (2008) *Mycobacterium avium subsp. paratuberculosis* and *M. avium subsp. avium* Are Independently Evolved Pathogenic Clones of a Much Broader Group of M. avium Organisms. *Journal of Bacteriology* 190, 2479-2487
88 Doig, K.D.*, et al.* (2012) On the origin of *Mycobacterium ulcerans*, the causative agent of Buruli ulcer. *BMC genomics* 13, 258
89 Guenin-Macé, L.*, et al.* (2011) Mycolactone impairs T cell homing by suppressing microRNA control of L-selectin expression. *Proceedings of the National Academy of Sciences* 108, 12833-12838
90 Fyfe, J.A.M.*, et al.* (2010) A Major Role for Mammals in the Ecology of *Mycobacterium ulcerans*. *Plos Neglect. Trop. Dis.* 4
91 Dalal, A.*, et al.* (2015) Resistance Patterns among Multidrug-Resistant Tuberculosis Patients in Greater Metropolitan Mumbai: Trends over Time. *PLoS ONE* 10, e0116798
92 Isaakidis, P.*, et al.* (2014) Alarming Levels of Drug-Resistant Tuberculosis in HIV-Infected Patients in Metropolitan Mumbai, India. *PLoS ONE* 9, e110461
93 Skrahina, A.*, et al.* (2012) Alarming levels of drug-resistant tuberculosis in Belarus: results of a survey in Minsk. *European Respiratory Journal* 39, 1425-1431

Figures

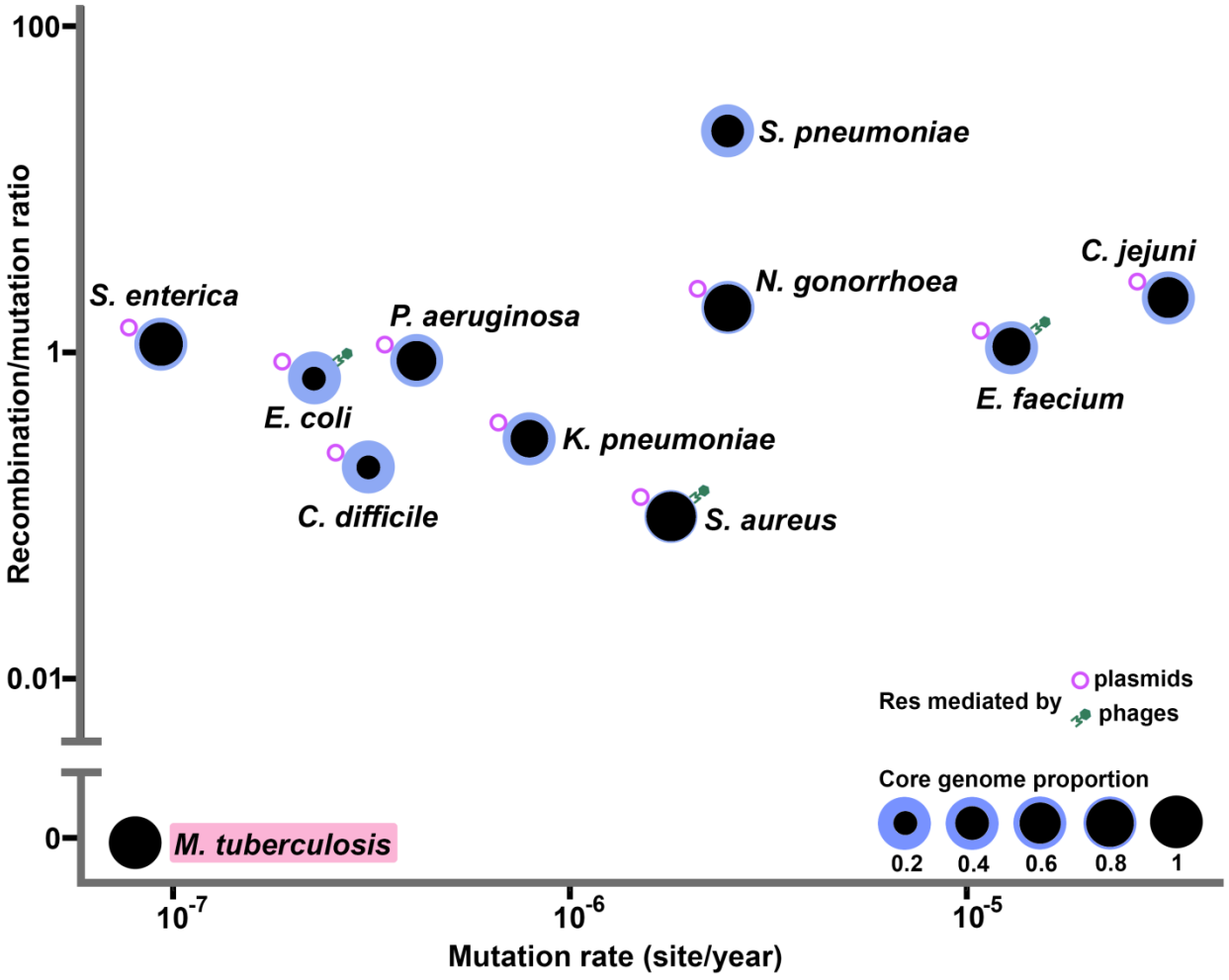


Figure 1. Basic Genomic Features of the Main Bacterial Resistance Threats. The core genome proportion indicates the proportion of typical individual genomes that are common to all members of the species. Mutation rates are based on whole-genome phylogenetic analyses of clinical isolates. The presence of plasmid- or phage-mediated resistance is indicated in the figure. In this figure we considered *M. tuberculosis* to have entirely lost the ability to recombine (see main text for a brief discussion on possible recombination in *M. tuberculosis*). A*cinetobacter baumanni* was not included in the figure as we could not find relevant estimates for mutation rates and recombination/mutation ratio.

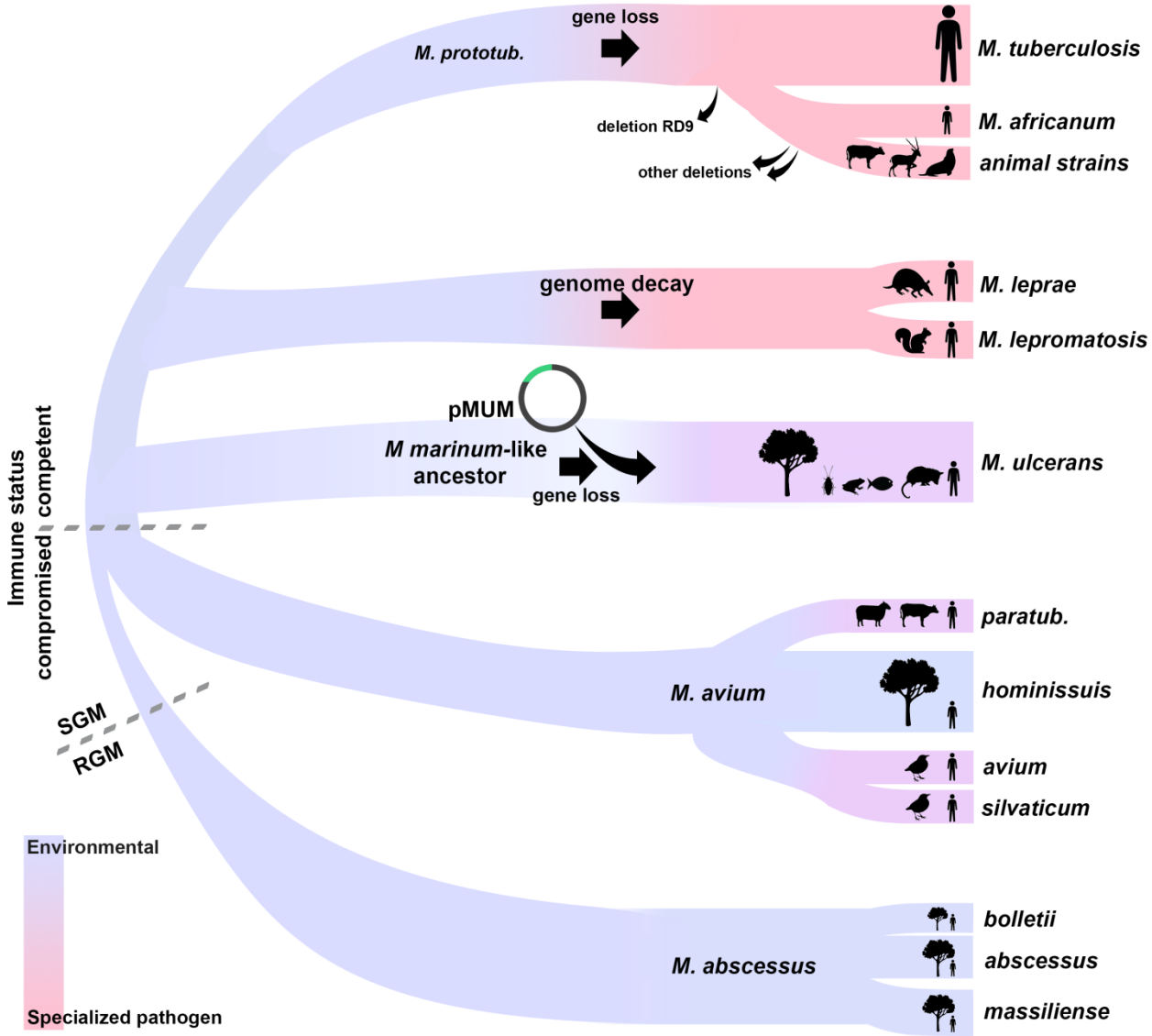


Figure 2. History and Host Range of Important Mycobacterial Pathogens. The figure includes the most important pathogens in the genus *Mycobacterium*. SGM = slow-growing mycobacteria; RGM = rapid-growing mycobacteria. *M. tuberculosis* and related species are descendants from a hypothetical environmental ancestral species termed *M. prototuberculosis*, whereas *M. ulcerans* evolved from a *M. marinum*-like ancestor. The acquisition of the pMUM mega-plasmid containing genes required for the synthesis of mycolactone was central in the evolution of this pathogen. Pictograms indicate host range, whereas the presence of a tree specifically indicates that the species is environmental. *M. avium* and *M. abscessus* are separated into subspecies.

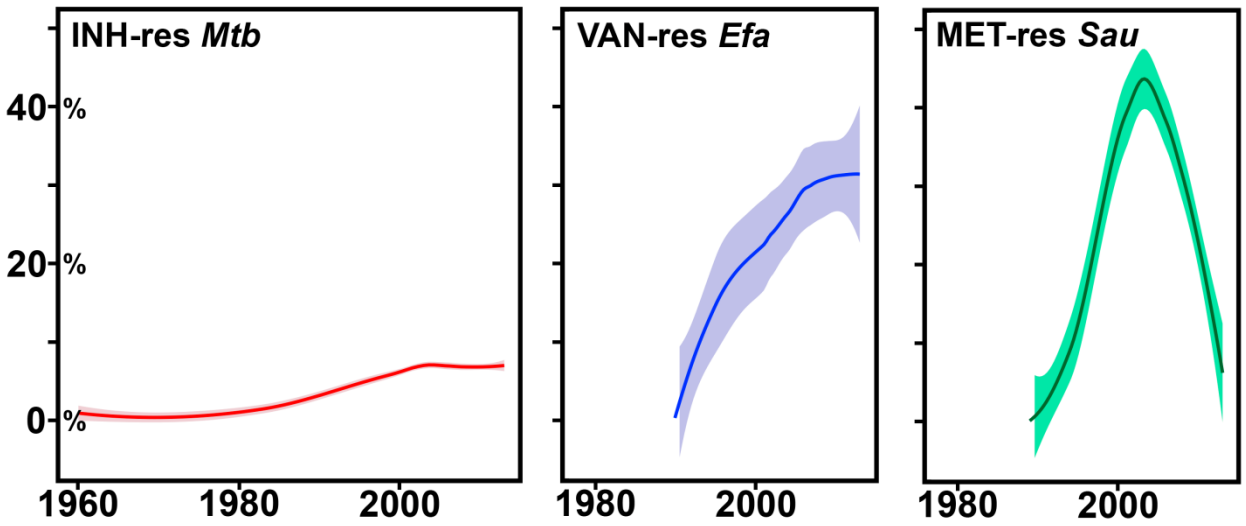
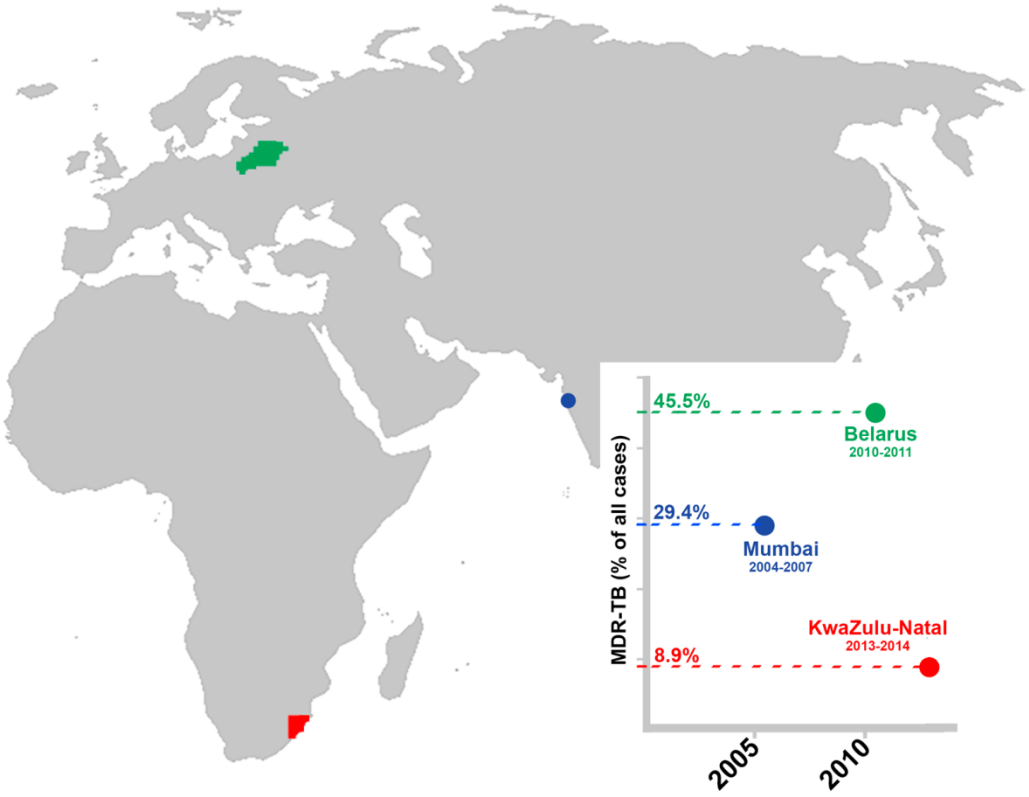


Figure 3. Population-level Response to Antimicrobials of Selected Pathogens, United Kingdom
From left: Percentage of *Mtb* clinical isolates resistant to isoniazid (INH-res *Mtb*) (From 1960-1983 only new cases [73, 74], from 1988-2013 all cases [75, 76]; Invasive *E. faecium* isolates resistant to vancomycin (VAN-res *Efa*) [77]; Invasive *S. aureus* isolates resistant to methicillin (MET-res *Sau*). [78, 79]. For *E. faecium* and *S. aureus*, data for the years 2001-2013 was retrieved from http://www.bsacsurv.org/**.** Shaded areas correspond to 95% confidence intervals based on local regression. *Mtb* data from England and Wales 1960-1999; England, Wales and Northern Ireland 2000-2005; UK 2005-2013. *E. faecium* data from England and Wales 1990-1998; UK and Ireland 2001-2013. *S. aureus* data from England and Wales 1989-1996; UK and Ireland from 2001-2013.



572

573 **Figure 4. Hotspots of MDR-TB.** Data on MDR-TB incidence was collected from [80] (Belarus),
574 [81] (Mumbai) and [82] (KwaZulu-Natal). Note that the MDR-TB frequencies reported here for
575 Mumbai are significantly higher than those reported nation-wide from the World Health
576 Organization. The reported MDR-TB incidence in KwaZulu-Natal represents RIF-resistant
577 isolates as identified by TB Xpert [83].

Text Boxes

Box 1 You all look the same to me

M. abscessus is a rapidly growing environmental species but also a relatively common source of soft tissue infections, disseminated infections in immunocompromised individuals and pulmonary infection in cystic fibrosis patients. Treating *M*. *abscessus* infections with antimicrobials is challenging, as the group is intrinsically resistant to most available drugs [84]. The species harbors three subspecies, namely *abscessus*, *bolettii* and *masilliense*. The macrolide clarithromycin has been used frequently to treat infections, but the relatively recent discovery that nearly all *abscessus* and *bolettii* strains, but not *masilliense*, can induce resistance to the drug by activation of the *erm*(41) gene [85], encoding a ribosome methylase [86], highlights critical differences within this species complex and the need for improved taxonomic assignment tools for effective treatment.

M. avium is a species consisting of four main subspecies ranging from environmental to more specialized pathogens. *M. avium subsp. homoinissuis*, an environmental species causing opportunistic infection in immune-compromised people, is a diverse group undergoing frequent recombination events. In contrast, the subspecies *silvaticum*, *avium* and *paratuberculosis* represent clonal lineages radiating out of the *hominisuis* group that have adapted to various animal hosts [87](Fig. 2).

M. ulcerans falls somewhere in the middle of the spectrum between environmental and host-specialized mycobacteria. The bacterium can be considered as a semi-specialized pathogen mainly due to the acquisition of the pMUM plasmid by a *M. marinum*-like ancestor [88]. The plasmid encodes the genes necessary for the synthesis of mycolactone, a polyketide-derived macrolide that serves both as a toxin, triggering tissue damage, and an immunomodulatory compound inhibiting the host immune response. Analogous to *Mtb* and the leprosy bacilli, *M. ulcerans* has undergone significant gene loss and contains 771 pseudogenes, in stark contrast to *M. marinum*, where only 65 inactivated genes have been identified [88]. This pseudogenization seems to have been partially driven by the expansion of insertion sequence *IS2404* that was

acquired after the split between *M. marinum* and *M. ulcerans*. Insertion sequences seem to have played important roles also in the host-adaption of other pathogenic mycobacteria. *M. ulcerans* has been identified in a wide range of environments, including soil, water, frogs, fish, mosquitos, water bugs and mammals. However, the finding that mycolactone specifically inhibits T-cell controlling mammalian microRNAs [89] combined with a very close genetic relationship between *M. ulcerans* isolates in humans and opossums in southwest Australia [90] do suggest that the bacterium has evolved to accommodate a mammalian niche.

Box 2 Terrible Places (for MDR-TB)

In India, 4.3 % of notified TB cases were estimated to be MDR-TB in 2013, but within the country there are significant regional differences in resistance burden. A survey of four municipal wards in Mumbai revealed rates of MDR-TB close to 30% in the years 2004-2007 [81] (Figure 4) and subsequent studies have confirmed very high rates of drug resistance [91, 92]. A significant proportion of the MDR-isolates are resistant to additional drugs, exemplified by a study from 2013 that found 10.6 % of all MDR-TB isolates to qualify for XDR-TB status [91].

Results from first-line diagnostics reported annually in South Africa found 6.6 % of all TB cases in South Africa to be RIF resistant in 2013/2014 [83]. However, in the province of KwaZulu-Natal which is home to 30% of all TB cases in the country, and the region with the highest incidence of MDR-TB in the world, 8.9% of TB cases were RIF resistant in the same period. RIF-resistance is often used as a proxy for MDR, an assumption that is correct in more than 90% of the cases in KwaZulu-Natal [82], but does not hold for instance in the UK. These numbers suggest that the WHO estimates from 2013 of rates of around 2.1 % of MDR-TB are overly optimistic.

A study conducted in Minsk (Belarus) in 2009-2010 revealing that almost half of all TB cases were MDR raised a few eyebrows [93]. However, these figures were confirmed by a follow-up country-wide study one year later that confirmed that 45.5% of all isolates in Belarus are indeed MDR-TB. Possibly, even more shocking was the observation that among MDR-TB isolates, 11.9% were XDR [80].

Glossary Box

Ancient DNA (aDNA): is DNA isolated from any ancient specimen. It is generally loosely used to describe any DNA recovered from biological material that has not been preserved specifically for later DNA sequencing. Examples include DNA recovered from archaeological and historical skeletal material, mummified tissues and archival collections of non-frozen specimens.

Antimicrobial resistance (AMR): is resistance of a microorganism to an antimicrobial compound to which it was originally sensitive. Resistant organisms (bacteria, fungi, viruses and some parasites) are able to withstand exposure to antimicrobial drugs, so that standard treatments become ineffective and infections persist increasing the risk of transmission to other hosts. The evolution of resistant strains is a natural phenomenon generally induced by exposure to antimicrobial drugs.

Directly observed therapy (DOT): case management that helps to ensure that patients adhere to treatment. DOT is considered the most effective strategy for making sure patients take their medicines.

Drug susceptibility testing (DST): the various procedures to find out which drugs a bacterial strain is resistant to. This represents an essential step for rapid identification of resistant strains so that patients carrying such strain can be put on adequate drug treatment as soon as possible.

Isoniazid (INH): an antibiotic used as a first-line agent together with rifampicin (RIF) in the prevention and treatment of both latent and active TB.

Multi-drug-resistant tuberculosis (MDR-TB): defined as a form of TB infection caused by bacteria that are resistant to treatment with at least two of the first-line anti-TB drugs, isoniazid (INH) and rifampicin (RIF).

***Mycobacterium tuberculosis* complex (MTBC):** a group of closely related strains and species including pathogens of humans and animals as well as the highly diverse probably environmental *Mycobacterium canettii*.

Rifampicin (RIF): an antibiotic used to treat a number of bacterial infections. It constitutes one of the two first-line agents together with isoniazid (INH). It is on the World Health Organization's List of Essential Medicines, the most important drugs needed in a functional basic public health system.

Smooth tubercle bacilli (**STB):** a group of mycobacteria found in Eastern Sub-Saharan Africa and are considered as the putative ancestors of *Mtb*. They include in particular the species *M. canettii* that can cause TB but does not seem to transmit directly between human hosts.

Tuberculosis (TB): a bacterial infection caused by some species in the genus *Mycobacterium*, with the main agent being *M. tuberculosis*. The infection generally resides in the lungs but can spread through the lymph nodes and bloodstream to any organ. Most people who are infected by *Mtb* remain healthy and asymptomatic and do not transmit the bacterium to others.

Extensively drug-resistant TB (XDR-TB): a type of multidrug-resistant tuberculosis (MDR-TB) that is resistant to isoniazid and rifampin, plus any fluoroquinolone and at least one of the three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin).