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Global control of tuberculosis: from extensively drug-resistant to untreatable tuberculosis

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KD conceived the idea and mainly wrote the abstract, introduction, conclusions, and sections about pathogenesis, medical and surgical management, outcomes, medicolegal aspects, health-care workers, and prophylaxis. GBM mainly wrote the sections about definitions and terminology, RW wrote the section about molecular epidemiology, MM wrote the section about clinical epidemiology, ZU wrote the section about drug-resistant tuberculosis in India and China, and NRG wrote the section about management of patients infected with HIV. TG cocontributed to the section about pathogenesis, and GT cocontributed to the section about diagnostics and pathogenesis. All authors edited and proofread the Review, and cocontributed to other sections as appropriate.

Declaration of interests

We declare that we have no competing interests.

Abstract

Extensively drug-resistant tuberculosis is a burgeoning global health crisis mainly affecting economically active young adults, and has high mortality irrespective of HIV status. In some countries such as South Africa, drug-resistant tuberculosis represents less than 3% of all cases but consumes more than a third of the total national budget for tuberculosis, which is unsustainable and threatens to destabilise national tuberculosis programmes. However, concern about drug-resistant tuberculosis has been eclipsed by that of totally and extremely drug-resistant tuberculosis —ie, resistance to all or nearly all conventional first-line and second-line antituberculosis drugs. In this Review, we discuss the epidemiology, pathogenesis, diagnosis, management, implications for health-care workers, and ethical and medicolegal aspects of extensively drug-resistant tuberculosis and other resistant strains. Finally, we discuss the emerging problem of functionally untreatable tuberculosis, and the issues and challenges that it poses to public health and clinical practice. The emergence and growth of highly resistant strains of tuberculosis make the development of new drugs and rapid diagnostics for tuberculosis—and increased funding to strengthen global control efforts, research, and advocacy—even more pressing.

Introduction

Although the incidence of tuberculosis is decreasing in several parts of the world (eg, the Americas, Europe, western Pacific, and southeast Asia), in other regions (eg, Africa) tuberculosis is out of control, and is responsible for about 1·5 million deaths annually.[1] The incidence of multidrug-resistant tuberculosis (ie, resistance to at least isoniazid and rifampicin) has increased during the past decade in many parts of the world.[2] More recently, this concern has been overtaken by the threat of extensively drug-resistant tuberculosis (resistance to isoniazid, rifampicin, one fluoroquinolone, and one second-line injectable drug; figure 1).[3–5] Appropriate recognition, prevention, and treatment of this new threat is crucial for several reasons. Drug-resistant tuberculosis and its progressive forms have poorer outcomes than does drug-susceptible tuberculosis; drug-resistant disease frequently affects economically active young adults, and although multidrug-resistant and extensively drug-resistant tuberculosis represent a very small fraction of the total tuberculosis burden in resource-poor settings, these strains consume a disproportionate percentage of resources in tuberculosis programmes. For example, in South Africa multidrug-resistant and extensively drug-resistant strains of tuberculosis represent less than 3% of the total tuberculosis caseload, but consume about 35% of the total budget of the national tuberculosis programme; a substantial proportion of this spending is attributable to extensively drug-resistant tuber culosis.[6] In this Review, we discuss several important aspects of extensively drug-resistant tuberculosis and resistance beyond extensively drug-resistant tuberculosis.

Definitions and terminology

Drug-resistant tuberculosis encompasses multidrug-resistant, extensively drug-resistant, extremely drug-resistant,[7] and totally drug-resistant strains (panel 1).[3–5] Extremely drug-resistant tuberculosis was first described in two Italian women who died after 422 and 625 days spent in hospital, and 94 and 60 months of treatment, respectively.[7] Totally drug-resistant tuberculosis has been described in Iran, India, and South Africa.[3–5] Resistance

beyond extensively drug-resistant tuberculosis has not been defined by WHO, and the comparative severity of each form is unclear.

Multidrug-resistant and extensively drug-resistant tuberculosis are defined by international consensus.[22–25] Indeed, in a European tuberculosis cohort study[25] of multidrug-resistant and extensively drug-resistant strains, the accepted definition of extensively drug-resistant tuberculosis had both clinical value (ie, outcomes for extensively drug-resistant tuberculosis were worse than were those for multidrug-resistant tuberculosis) and programmatic value (ie, emphasising that suboptimum adherence drives acquired resistance to second-line drugs). By contrast, the international community still needs to agree a definition for resistance beyond extensively drug-resistant tuberculosis, including which drugs should compose the panel defining this strain, whether new drugs (eg, delamanid, bedaquiline [Janssen Pharmaceuticals, NJ, USA], and PA-824 [TB Alliance, NY, USA]) should be included (panel 2), and how often should this panel be reassessed to allow inclusions of new compounds. Whether resistance beyond extensively drug-resistant tuberculosis is associated with a worse prognosis than is extensively drug-resistant tuberculosis is not clear.

Recently published data support the WHO recommendation not to use the term totally drug-resistant tuberculosis, but rather to define new categories of resistance beyond extensively drug-resistant tuberculosis.[21] In a study[21] of 405 patients with extensively drug-resistant tuberculosis divided into 4 groups on the basis of susceptibility data, the rate of cure was significantly lower in patients with extensively drug-resistant tuberculosis and resistance to all second-line drugs than for patients with extensively drug-resistant tuberculosis alone (19% *vs* 43%), whereas the risk of treatment failure and death was higher for patients with additional resistance (≥48% *vs* 35%). These data suggest that patients with additional resistance beyond extensively drug-resistant tuberculosis might have worse outcomes than do those with multidrug-resistant or extensively drug-resistant tuberculosis alone. Findings from another study of extensively drug-resistant tuberculosis in South Africa[16] showed that the number of drugs that a *Mycobacterium tuberculosis* isolate was resistant to was an independent predictor of mortality. Thus, internationally agreed definitions based on outcomes are needed to inform public health efforts to monitor and prevent further emergence of drug resistance.

Molecular epidemiology of extensively and totally drug-resistant tuberculosis

The development of methods to genotypically classify strains of *M tuberculosis* has provided insight into the movement of strains through space and time.[27] On the basis of spacer oligonucleotide typing,[28] IS6110 DNA fingerprinting,[29] or typing with mycobacterial inter spersed repetitive unit-variable number tandem repeats,[30] strains can be identified as genotypically identical to (clustered) or genotypically distinct from other strains. Accordingly, clustering suggests recent spread or transmission of tuberculosis, regardless of the presence or absence of primary drug resistance. By contrast, for drug-sensitive tuberculosis, identification of distinct genotypes suggests endogenous reactivation of latent infection (ie, the patient was infected in the past and subsequently developed disease).[31]

Distinct genotypes among cases of drug-resistant tuberculosis could result from either endogenous reactivation or new acquisition of drug resistance if patients were previously treated with antituberculosis drugs (ie, secondary resistance acquired in the course of treatment in an individual patient).[32] Application of these methods has addressed important knowledge gaps,[33–38] and was key to the description of the transmission of extensively drug-resistant tuberculosis in Tugella Ferry, KwaZulu-Natal, South Africa.[36] In that study,[36] despite acquiring resistance to at least four drugs, the strains retained both their fitness to spread and to exogenously re-infect previously treated patients.[33]

Of the 84 countries that reported extensively drug-resistant tuberculosis in 2011 (figure 1),[1] only 14 had reported genotyping of isolates by the end of 2012 (appendix). South Africa remains the only country where comprehensive molecular epidemiological investigations have been done, and these investigations have informed several WHO and local policy decisions about emergence, transmission, and amplification of drug-resistant tuberculosis (appendix).

Studies done in Iran[5] and South Africa[3] provide the only available data about the molecular epidemiology of totally drug-resistant tuberculosis. Findings from the Iranian study[5] showed that all of the strain genotypes of totally drug-resistant tuberculosis were unique; the investigators concluded that these cases emerged through the use of second-line drugs to treat respiratory diseases other than tuberculosis.[5] By contrast, findings from the South African study[3] showed that the genotypes of the totally drug-resistant strains were mostly clustered (84%) and were clustered with genotypes of extensively drug-resistant strains. The investigators concluded that the acquisition of additional resistance in these cases resulted from inadequate treatment of extensively drug-resistant tuberculosis because of the small number of effective drugs available.[3] These findings emphasise the need for improved laboratory capacity in association with universal use of rapid diagnostic testing, improved patient management, and strengthened treatment regimens for multidrug-resistant tuberculosis to halt the emergence and subsequent spread of extensively or totally drug-resistant tuberculosis.

Clinical epidemiology and future trends

Findings from several studies have shown that previous tuberculosis treatment is the strongest risk factor for multidrug-resistant tuberculosis and that, among previously treated patients, previous treatment failure, the number of previous regimens, and the quality of previous tuberculosis care predict drug resistance.[39–44] Other individual risk factors vary across settings, but often include young age,[41,42] male sex,[41] history of incarceration,[42] history of previous admission to hospital,[39,45] alcohol misuse,[42,45] poverty,[40,44] HIV infection,[41] and smoking.[42] Few studies have differentiated between risk factors for primary and acquired (or secondary) resistance. Therefore, whether these determinants are proxies for exposure to patients with drug-resistant tuberculosis, or instead are associated with suboptimum care that selects for drug resistance, is unclear. Molecular epidemiology has been used to identify multidrug resistance as a risk factor for clustering of cases,[46,47] and has shown that primary resistance can occur in patients who have been recently treated for tuberculosis (especially in the context of hospital admission), suggesting nosocomial

reinfection with a drug-resistant strain.[39,48] As expected, extensively drug-resistant tuberculosis is strongly predicted by previous use of second-line drugs in almost all studies that have assessed this strain.[39,40,49] In some settings, previous treatment that did not conform to national guidelines,[40] the purchase of fluoroquinolones before diagnosis of tuberculosis,[50] lack of adherence to a previous regimen, and poverty[44] were also independent predictors of extensively drug-resistant tuberculosis.

Mathematical models have been developed to explore the epidemic behaviour of drug-resistant tuberculosis and the potential effectiveness of interventions. Most of these models use deterministic compartmental simulations to capture both the acquisition of resistance by a small proportion of treated patients and the further spread of drug-resistant tuberculosis after emergence.[51,52] Epidemic theory posits that the number of people infected by a single infectious host depends not only on the infectiousness of the pathogen but also the duration of infectiousness, which in turn depends on the timing and effectiveness of diagnosis and treatment of the disease. Although multidrug-resistant strains of tuberculosis have often been assumed to have an evolutionary fitness cost that could reduce their transmissibility,[53] models have shown that this possible reduction is counterbalanced by the much longer than expected duration of the infectious period in patients with drug resistance who receive first-line drugs to which they respond more slowly than would a person with drug-sensitive disease.[54] These models suggest that early diagnosis and effective treatment of drug-resistant tuberculosis, either through improved access to culture and drug-sensitivity testing[54,55] or through rapid diagnostics,[54,56] could have a profound effect on reduction of the burden of resistant tuberculosis in high-prevalence settings. Other models that link dynamic epidemic simulations with economic analysis show that these interventions can be highly cost effective, because the interventions provide not only potentially lifesaving care but also prevent further spread of drug-resistant disease.[57] In view of the substantial burden of drug-resistant tuberculosis in Asia, India and China deserve special mention (appendix).

Pathogenesis and mechanisms of drug-resistant tuberculosis

Selection of drug-resistant mutants—conventional thinking

The conventional understanding of the mechanisms for emergence of multidrug-resistant and extensively drug-resistant tuberculosis (eg, in India, China, and South Africa) is based on research by Luria and Delbrück[58] and David.[59] The Luria-Delbrück experiment showed that genetic mutations in *M tuberculosis* that confer resistance arise independently of selection pressure.[58] In the 1970s, David[59] used Luria and Delbrück's tests to show very low average rates of mutation ($2{\cdot}6 \times 10^{-8}$ mutations per *M tuberculosis* bacterium per generation for isoniazid, $2{\cdot}3 \times 10^{-10}$ for rifampicin, and 1×10^{-7} for ethambutol).[59] Thus, tuberculosis resistance arises spontaneously but at a low and predictable de-novo rate, and not by horizontal gene transfer (figure 2).[59] In view of the large bacterial burdens of up to 10^9 colony-forming units per patient and bacterial replication, pre-existing *M tuberculosis* subpopulations resistant to one drug might be expected to occur in some patients, although the probability of pre-existing drug resistance to two or three drugs (as calculated by multiplication of mutation rates) is infinitesimally small. In the classic understanding of emergence of drug resistance with monotherapy, initial therapy kills off most of the

susceptible bacterial subpopulation, but allows the pre-existing drug-resistant subpopulation to continue to replicate, eventually replacing the drug-susceptible population (figure 2). This scenario could occur with fragmented treatment (panel 3) or lack of adherence to treatment regimens.

For combination treatments, pharmacokinetic mismatch could occur—ie, a drug in the combination treatment that has a short pharmacokinetic half-life disappears, leaving a companion drug with a longer half-life to act as monotherapy.[63] This situation causes sequential acquisition of drug-resistant mutations, with multidrug-resistant and extensively drug-resistant tuberculosis due to an accumulation of mutations acquired one at a time. In this conventional view, the main determinant of drug resistance is gene mutations, either in the genes converting pro-drugs to active drugs, or in the genes of drug targets that are selected because of lack of adherence. However, findings from experimental and clinical studies have challenged this traditional understanding.

Drug concentrations, efflux pumps, and evolution based understanding of acquired drug resistance

Drug resistance can develop even when adherence to treatment is excellent, and multidrug-resistant tuberculosis still occurs even under stringent supervision of patients.[35] Several mechanisms might account for this finding, some of which were established with use of the hollow-fibre-system model of tuberculosis, a preclinical tool in which different metabolic populations of *M tuberculosis* are exposed to concentration–time profiles of antituberculosis drugs. 1–2 years before the description of extensively drug-resistant tuberculosis by Gandhi and colleagues,[36] findings from studies using the hollow-fibre-system model of tuberculosis showed that quinolone resistance could easily occur even with drug concentrations that maximised microbial killing.[64,65] Acquired drug resistance occurred within 2 weeks of monotherapy, leading to a biphasic kill curve, especially when ciprofloxacin and ofloxacin are used with the less effective second-line drugs.[64,65] Findings from subsequent studies using the hollow-fibre-system model with isoniazid, rifampicin, pyrazinamide, and ethambutol showed the same rapid acquisition of resistance, and suggested two further related notions.[66–70] First, efficacy and acquired drug resistance were strongly associated with the area-under-the-curve and peak drug concentrations, indexed to minimum inhibitory concentration—ie, there were drug concentrations below which drug resistance was amplified and microbial killing failed. Second, the acquired drug resistance was either accompanied by, or preceded by, very early induction of many low-level-resistance efflux pumps, which also conferred multidrug resistance or tolerance.[70,71] These efflux pumps could have protected the bacilli during several rounds of replication, allowing for the eventual generation of chromosomal mutations associated with high-level acquired drug resistance in a process termed the “antibiotic resistance arrow of time” (figure 3).[70,72] In this “antibiotic resistance arrow of time” model in the laboratory, subtherapeutic drug concentrations initiated a one-directional sequence of events during long-term therapy that induced efflux pumps as a first step towards multidrug resistance and genetic mutations as a final step.

Findings from clinical studies lend support to the role of efflux-pump induction in emergence of multidrug resistance. Several researchers have examined drug-resistant clinical isolates (including multidrug-resistant tuberculosis), and reported the coexistence of genetic mutations and multidrug efflux pumps.[73,74] Repetitive sputum samples from the same patient were reported to have the same early induction of efflux pumps, and later establishment of chromosomal mutations often associated with acquired drug resistance.[75] However, findings from studies using whole-genome sequencing would not be expected to show this effect; because the induced efflux pumps are already encoded by the *M tuberculosis* genome, the induction is epigenetic.

Although acquired drug resistance has clearly been shown to occur even under strict supervision of patients, many public health researchers and doctors believe that poor adherence accounts for most cases of acquired drug resistance. Studies using the hollow-fibre-system model have directly examined the role of lack of adherence in emergence of drug resistance during standard short-course chemotherapy.[76] Although therapeutic failure occurred only when more than 60% of doses were missed, no multidrug-resistant isolates were reported in patients with any lack of adherence, except transient monoresistance that disappeared with time.[76] Findings from a follow-up study[77] that used a murine tuberculosis model also showed no emergence of multidrug-resistant tuberculosis with high rates of non-adherence. These findings, together with reports of acquired drug resistance even under strict supervision,[35] and the emergence of drug resistance below particular drug threshold concentrations, suggest that pharmacokinetic variability could be a proximate cause for acquired drug resistance.

To investigate this hypothesis, we simulated the pharmacokinetics of patients with tuberculosis from the Western Cape, South Africa, in computer-aided models of clinical trials. We noted that multidrug-resistant tuberculosis would still occur in 0·68% of patients during the first 2 months of treatment, despite 100% adherence,[76] because drug metabolism rates for rifampicin or isoniazid were high in some patients due to between-patient pharmacokinetic variability; these patients would effectively receive monotherapy for long periods of time. Such pharmacokinetic variability is driven mainly by genetic polymorphisms in xenobiotic metabolism and drug transporters.[78] These simulation findings have since been replicated in three clinical studies.[79–81] The first was a meta-analysis[79] that showed higher rates of acquired drug resistance among fast isoniazid acetylators than among slow acetylators in multidrug regimens. A second meta-analysis[80] of prospective studies showed that, despite higher rates of non-adherence, self administered therapy did not lead to increased rates of acquired drug resistance compared with directly observed short-course treatment. The third study[81] was a prospective study of 142 patients with tuberculosis in Western Cape, South Africa, in which the pharmacokinetics of pyrazinamide, rifampicin, and isoniazid were measured and patients were followed up for up to 2 years. Between-patient pharmacokinetic variability was wide; although the highest ratio of the highest dose to lowest dose for all three drugs was 2·7, the ratios of the highest-to-lowest peak concentrations were more than 102 for rifampicin, more than 31 for isoniazid, and more than 63 for pyrazinamide. The peak and area-under-the-curve concentrations of pyrazinamide, rifampicin, and isoniazid predicted more than 91% of therapy failure; 0·7% of all cases of acquired drug resistance were reported by the third month of therapy, and was preceded by

low peak and area-under-the-curve drug concentrations in all patients who developed resistance despite 100% adherence;[81] non-adherence did not predict acquired drug resistance. Thus, low drug concentrations due to between-patient pharmacokinetic variability is a proximate cause of acquired drug resistance. Pharmacokinetic variability can also be caused by differential penetration of drugs into the lung microcompartments (figure 2). The finding of isolates from different lesions in the same patient that have different resistance profiles (ie, heteroresistance), lends support to the hypothesis of differential drug penetration or exposure.[82] Collectively, these data from laboratory experiments, clinical trial simulations, and prospective clinical studies provide support for the notion that biological variability (eg, pharmacokinetic variability) is the main culprit for emergence of multidrug-resistant tuberculosis. Therefore, innovative and new methods are needed to optimally dose patients. These methods could include a pharmacogenetic approach, measurement of drug concentrations in patients, improved targeting of drugs to the disease site when given orally, or targeting of drugs through the inhalational route.[83]

Genotype, strain type, and compensatory mutations

Biological variability within the *M tuberculosis* genotype is an important cause of emergence of multidrug resistance in tuberculosis. The typical mutation rates identified by David[59] were established with use of the *M tuberculosis* laboratory strain, H37Rv. Findings from a 2013 study[84] showed that among different *M tuberculosis* genotypes, as defined by large-sequence polymorphism phylogenetic analyses, lineage 2 (to which the Beijing strain belongs) had much higher mutation rates than did lineage 4 (common in Western Europe and the USA, to which H37Rv belongs). In view of this finding, emergence of multidrug-resistant tuberculosis is 22 times more likely for lineage 2 than for lineage 4, and multidrug resistance could even preexist in some patients originally infected with a drug-susceptible strain before therapy initiation.[84] Calculations of average mutation rates that suggested that acquired drug resistance would not easily result from therapy with more than one drug are therefore probably not applicable to the most common *M tuberculosis* genotypes associated with multidrug-resistant tuberculosis. In addition to differences in mutation rates between different genotypes, the mutation rate also varies between strains of the same genotype. However, the reasons for this variability are still unclear. Although some evidence suggests that certain strains (eg, the Beijing strain) are associated with increased likelihood of emergence of multidrug resistance,[85] little evidence supports the contention that some strains are more likely to mutate as a result of dysfunctional DNA repair mechanisms.[85] Additionally, interaction between hypermutable bacterial lineages and patients who rapidly metabolise first-line drugs might account for the high rates of multidrug-resistant tuberculosis reported in some settings despite patients receiving directly observed short-course treatment.[86]

Findings from studies of whole-genome sequencing have shown that resistance-encoding mutations are associated with compensatory mutations elsewhere in the tuberculosis genome.[87,88] Similar to compensatory mutations in pseudomonas species that can modulate virulence and transmissibility,[87,88] compensatory mutations in *M tuberculosis* could be associated with physiological and structural changes (panel 3, figure 2). Collectively, these

findings raise the possibility that drug resistance could affect micro-anatomical structure and antigenic specificity, and hence perhaps the T-cell immune response.

Potential role for immune-modulating therapy

Data about the immunology of drug-resistant tuberculosis are scarce, but there have been some reports of immune-mediated clearance.[89–91] Using an in-vitro killing assay, we showed that depletion of peripheral blood CD4+/FOXP3+ regulatory T cells in patients with drug-sensitive[92] and drug-resistant (unpublished) tuberculosis augmented mycobacterial containment, suggesting an important subversive biological role for these cells. This finding suggests that immunomodulation by manipulation of regulatory T cells (eg, targeting of antagonists for interleukin 2 receptors, autologous transfusion after expansion, infusion of mesenchymal stromal cells,[93] or blocking of other regulatory T-cell functions)[94] might affect disease progression. Effective tuberculosis treatment might therefore require not only drug therapy, but also realignment or redirection of the immune system to deal more effectively with mycobacteria. Roughly 20% of tuberculosis cases in the prechemotherapeutic era spontaneously resolved, which supports the notion of immune-mediated clearance. Several immunotherapeutic interventions have been proposed for the treatment of drug-resistant tuberculosis—eg, *M vaccae*, vitamin D, and intravenous immunoglobulin—and many of these therapies modulate regulatory T cells.[95] An alternative approach could be to use immunosuppressive drugs (eg, steroids or TNF antagonists) to enhance the chemotherapeutic effect by driving the replication of long-term persisters, thus exposing these bacteria by disruption of the protective shelter of the granuloma.[95] Thus, counterintuitively, immunosuppressive drugs might enhance bacterial clearance.

Transmission dynamics

Several clinical, bacterial, behavioural, and environmental factors probably drive transmission (figure 2),[96–98] but most cases of tuberculosis result from transmission by a small proportion of patients (so-called super-spreaders).[99,100] The determinants of this super-spreader status are poorly characterised, but culturable bacterial load is important,[101] and epidemiological modelling studies suggest that infection-control interventions[96] (especially those aimed at health-care workers and the small number of patients who probably bring about most transmission[100,102]) will probably reduce the probability of epidemics of drug-resistant tuberculosis emerging. The high transmissibility[36] and virulence (ie, high mortality and extensive clinical lung immunopathology)[16] of highly drug-resistant strains, including extensively drug-resistant tuberculosis, and their extensive spread even in regions with low HIV prevalence suggests that resistance-conferring mutations could be associated with compensatory mutations that result in normal or possibly even enhanced evolutionary fitness levels.[87] Epistatic interactions (in which the phenotypic effect of a mutation such as fitness changes dependent on the presence or absence of other mutations in the same genome[103]) between several resistance-conferring and non-resistance-conferring mutations, the genotype or strain type, and associated compensatory mutations might modulate fitness costs associated with drug resistance.[103,104]

Diagnostic aspects

The rollout of the Xpert MTB/RIF assay[105] (Cepheid, Sunnyvale, CA, USA)—a semi-nested quantitative real-time PCR assay that simultaneously detects tuberculosis and resistance to rifampicin—in many countries with a high burden of tuberculosis is projected to substantially reduce the tuberculosis epidemic (figure 4).[57] Xpert MTB/RIF is validated for use with sputum, although evidence is emerging to support its use with extrapulmonary samples.[106] Xpert MTB/RIF increases the number of tuberculosis cases detected through increased sensitivity and improves time to treatment initiation.[107] Through increased detection of rifampicin resistance compared with GeneXpert and earlier detection than for culture, the assay is expected to enable early initiation of appropriate therapy and thereby reduce transmission of drug-resistant tuberculosis.[57] However, substantial operational challenges need to be overcome; in South Africa, even with the availability of a rapid line-probe assay, initiation of second-line treatment still took a median of 55 days.[108]

The Xpert MTB/RIF assay can be done by minimally trained health-care workers in primary care facilities, which suggests that similar assays could be used to tackle tuberculosis in prisons and clinical hotspots, especially where rates of drug-resistant tuberculosis are high.[107] However, the Xpert MTB/RIF assay does not detect isoniazid monoresistance, which has a frequency of about 10–15% in high-burden settings.[109,110] Furthermore, in countries such as South Africa where almost 10 000 cases of drug-resistant tuberculosis are treated annually and the proportion of drug-resistance in new tuberculosis cases is about 5–6%,[111] the positive predictive value for rifampicin resistance is suboptimum (about 70%)—ie, about 1 in 3 or 4 rifampicin-resistant results will probably be false positive. However, where recommended algorithmically (eg, in South Africa) or where patients are unwell or true multidrug-resistant disease is clinically plausible, patients with a positive Xpert MTB/RIF assay are often started on treatment for multidrug-resistant tuberculosis while confirmatory test results are awaited.

Other diagnostic methods include the line-probe assays (eg, the MTBDR*plus* assay [Hain Lifesciences, Nehren, Germany]); version 2 of the MTBDR*plus* assay offers similar performance to Xpert MTB/RIF for tuberculosis detection directly from patient specimens, and has excellent performance for the detection of multidrug-resistant tuberculosis.[112,113] Thus, molecular assays such as Xpert MTB/RIF and MTBDR*plus* can offer a rapid and accurate diagnosis of multidrug-resistant tuberculosis within days of patient contact. More recently, the MTBDR*sl* assay (Hain Lifesciences, Nehren, Germany) has been introduced, which assesses resistance to second-line injectable drugs (mutations on the *rrs* gene), fluoroquinolones (mutations on the *gyrA* gene), and ethambutol (mutations on the *embB* gene).[114] However, this assay is not recommended for use directly on smear-negative specimens because of decreased accuracy,[115] meaning that culture isolates often need to be used to rule-in resistance. Sensitivity for resistance to second-line injectable drugs and fluoroquinolones is about 90% and 95%, respectively.[115–117] Alternative testing platforms that could be used in resource-poor settings and have excellent accuracy include microscopic observation drug susceptibility, the nitrate reductase assay, and thin-layer agar (appendix).[118]

Medical and surgical management of extensively drug-resistant tuberculosis and treatment failure

In patients who do not respond to treatment for multidrug-resistant tuberculosis (generally culture positive after at least 6 months of treatment) and who persistently remain susceptible to fluoroquinolones and aminoglycosides, treating physicians should consider non-compliance, malabsorption, drug quality, and other factors.[95,119–121] Surgery or a regimen of alternative second-line drugs and injectables should be considered for these patients if appropriate (panel 4).

Construction of a treatment regimen for extensively drug-resistant tuberculosis is more challenging than for multidrug-resistant tuberculosis (panels 2, 4). Because rates of previous multidrug resistance in patients with extensively drug-resistant tuberculosis are high,[16] capreomycin resistance often occurs because of cross-resistance with aminoglycosides—in 178 isolates of extensively drug-resistant tuberculosis, the rate of capreomycin resistance was about 80% (K Dheda, unpublished)[124]—and drugs such as ethionamide and cycloserine are often not an option. Other drug options include high-dose isoniazid (which can be successful for tuberculosis strains with *inhA* promoter mutations, and has been used to treat extensively drug-resistant tuberculosis in South Africa),[125] clofazimine, and other drugs from group 3 or 4 (panel 2). However, some of these drugs are of doubtful value. Moxifloxacin is often added to the regimen despite documented resistance to fluoroquinolones.[126] Where appropriate, meropenem can be useful,[127] and if mutational screening is used rifabutin can be an option.[128] Linezolid, although not available to national tuberculosis programmes worldwide, can substantially improve culture-con version outcomes in patients with extensively drug-resistant tuberculosis, but longer-term outcomes are unknown and cost and toxic effects are major concerns.[129] The recent approval by the US Food and Drug Administration of TMC-207 (bedaquiline) is encouraging, but this drug is not yet available in resource-poor settings. In South Africa the registration of the drug was turned down by the Medicines Control Council, in favour of a clinical registry to document its safety and effectiveness in a regimen to treat some forms of drug-resistant tuberculosis. Concerns have been raised about high mortality in the placebo arm of the trial of TMC-207.[130] Delamanid is also promising, although it is still undergoing approval.[131,132]

Collectively, this evidence suggests that patients with extensively drug-resistant tuberculosis and more resistant strains of tuberculosis are functionally untreatable. The poor prognosis (20% culture conversion during the study period and 40% 1-year mortality) of extensively drug-resistant tuberculosis in high-burden settings such as South Africa is therefore not surprising.[16–18,133] Resistance beyond extensively drug-resistant tuberculosis is difficult to interpret because of the poor reliability of susceptibility testing for capreomycin, para-aminosalicylic acid, cycloserine, and other drugs, and because there are insuffi cient data about whether resistance beyond extensively drug-resistant tuberculosis affects outcomes.[21,134] A further complication is the high rates of adverse events in patients with extensively drug-resistant tuberculosis, with potentially fatal results (panel 4).[135]

Definition of treatment failure in patients with extensively drug-resistant tuberculosis is contentious but important, because it defines when treatment should potentially be

withdrawn because of futility. In South Africa, treatment is regarded as being unlikely to produce a favourable outcome if culture conversion has not occurred within 12 months. The median time to culture conversion in this patient population is about 6 months.[16] However, prospective data from larger cohorts are needed to clarify the predictive value of this suggested definition. About 25% of converters can subsequently revert to culture positivity.[124]

Management of patients with HIV

With the emergence of the epidemic of drug-resistant tuberculosis in southern Africa, and the increasing prevalence of HIV in eastern Europe, large numbers of patients with multidrug-resistant and extensively drug-resistant tuberculosis also have HIV.[16,136–139] Mortality rates are substantially increased for these patients.[16,134,140–142] Reduction of mortality needs prompt treatment, although few data are available to guide therapeutic approaches to treat both diseases. Concerns exist, as for treatment of drug-susceptible tuberculosis and HIV, that concurrent treatment of drug-resistant tuberculosis and HIV will lead to drug–drug interactions, increased toxic effects, reduced adherence, and increased incidence of immune reconstitution inflammatory syndrome.[143]

Few studies have prospectively examined each of these issues in the setting of drug-resistant tuberculosis and HIV. No data are available for drug–drug interactions between second-line tuberculosis drugs and anti-retroviral therapy, although interactions are suspected.[144] Small retrospective studies about over lapping toxic effects with treatment for both multidrug-resistant tuberculosis and HIV have been reported;[135,145–147] however, although adverse events were common in these studies, they were no more frequent for combination with antiretroviral therapy than for treatment for multidrug-resistant tuberculosis alone. Adherence to treatment of multidrug-resistant tuberculosis alone is probably low, in view of the complexity, duration, and toxic effects of the drugs used, but this has not been well studied. The addition of antiretroviral therapy is likely to further worsen adherence. The frequency of immune reconstitution inflammatory syndrome (defined as clinical deterioration despite appropriate treatment, caused by restoration of pathogen-specific immune responses) in the setting of concurrent treatment of drug-resistant tuberculosis and antiretroviral therapy is also unknown. Whether immune reconstitution inflammatory syndrome is more frequent or severe in patients with both HIV and drug-resistant tuberculosis is not clear, because bacterial loads will probably be increased, the potency of second-line tuberculosis drugs is reduced, and time to culture conversion is lengthened.[143,148,149]

In the absence of high-quality data, guidelines for the management of drug-resistant tuberculosis and HIV are based mostly on expert opinion and data from studies of drug-susceptible tuberculosis and HIV.[150] Findings from several well designed clinical trials[151–154] and prospective observational studies[155–158] have shown that concurrent tuberculosis treatment and anti-retroviral therapy is associated with improved treatment outcomes and low rates of adverse events and immune reconstitution inflammatory syndrome. Patients receiving concurrent treatment for drug-sensitive tuberculosis and HIV in the SAPiT study[151] also had lower mortality than did patients for whom antiretroviral

therapy was deferred until after tuberculosis treatment was completed.[151] On the basis of these data, concurrent treatment is recommended for all patients co-infected with drug-resistant tuberculosis and HIV, irrespective of baseline CD4 cell count.[150] Tuberculosis treatment guidelines are the same for patients with drug-resistant strains co-infected with HIV as for patients without HIV.[150]

Outcomes for multidrug-resistant tuberculosis compared with extensively and totally drug-resistant tuberculosis

Survival and treatment outcomes in tuberculosis are associated with the degree of drug resistance. Generally, patients with drug-susceptible tuberculosis have higher survival and cure rates than do those with multidrug-resistant tuberculosis; similarly, success rates are higher in multidrug-resistant tuberculosis than in extensively drug-resistant tuberculosis.[134,159,160] Treatment success rates in drug-susceptible tuberculosis generally vary from 70% to 95%,[161] whereas in multidrug-resistant tuberculosis, success rates are usually between 40 and 70%.[160,162] Treatment success rates in extensively drug-resistant tuberculosis have been reported to be as high as 60%;[142] however, in most settings, the success rates typically range from 20% to 50%.[16,18,160] Treatment success is linked not only to drug-resistance category, but also to resistance to additional first-line and second-line drugs. Patients with multidrug-resistant tuberculosis with only resistance to isoniazid and rifampicin have better survival than do those with additional resistance to ethambutol or streptomycin (figure 5).[134] Additional resistance to fluoroquinolones or second-line injectable drugs results in worse odds of treatment success in a stepwise fashion than does multidrug resistance alone, multidrug resistance plus fluoroquinolone resistance, multidrug resistance plus resistance to second-line injectable drugs, or extensively drug-resistant tuberculosis.[163] Resistance to second-line and third-line drugs beyond the minimum definition of extensively drug-resistant tuberculosis is also associated with further reductions in the likelihood of treatment success. Findings from a meta-analysis[21] showed that patients with the minimum definition of extensively drug-resistant tuberculosis had higher cure rates (43%) than did patients with more drug-resistant tuberculosis; cure rates were lower for extensively drug-resistant tuberculosis with resistance to all other injectables (30%), resistance to all injectables and at least one group 4 drug (34%), and resistance to all injectables, one group 4 drug, and either pyrazinamide or ethambutol (19%).

On the basis of the available evidence, the factors associated with improved odds of treatment success are use of a large number of active drugs,[16,142,164] concurrent use of antiretroviral therapy in patients co-infected with HIV,[16,17,40,165] use of community based models of treatment,[141,166–168] and use of a later-generation fluoroquinolone.[16] Further rigorous studies are needed to establish the best regimens and treatment protocols to optimise treatment success, dependent on the degree of drug resistance.

The emergence of untreatable tuberculosis—ethical and medicolegal aspects

No effective drugs are available to treat patients with extensively drug-resistant tuberculosis who do not respond to therapy (ie, no culture conversion after 12–18 months of intensive inpatient treatment) and who are unsuitable for surgical lung resection, rendering this patient population functionally untreatable. Large numbers of these patients have been reported in South Africa, and present a management, medicolegal, and ethical challenge. In the Western Cape Province of South Africa, about 100 of these patients have been discharged back into the community since 2008 because of treatment futility and insufficient bed space.[124] The median time to death in the community after discharge was just under 2 years, and almost a third of patients were smear positive at discharge and therefore pose a high risk of transmission.[124] At present, no systematic programme in South Africa is available to ensure that these patients have appropriate infection control in their home environments, and there are no reasonable alternatives (eg, long-stay community facilities) where they can live. Both long-term community facilities (where multi disciplinary care can be delivered, and patients stay on a voluntary basis) and palliative care facilities for patients who are terminally ill are needed.[169] Often, these patients reside in single-room dwellings and are employed as casual labourers; can their sending home be justified? The need to support their families means that engaging in employment, use of public transport, and visiting public places is unavoidable, which has serious implications for the uncontrolled spread of highly drug-resistant forms of tuberculosis.

There are several other unresolved questions.[119,120,123] Should these patients be left on treatment if they are deemed to be non-responsive to treatment for extensively drug-resistant tuberculosis? Should treatment be withdrawn for patients who do not adhere to regimens, to prevent the evolution of higher-grade drug resistance? What should be done with the small number of patients who continue to default their treatment, usually in the setting of substance abuse, and are a danger to fellow patients and health-care workers? Should there be facilities to incarcerate such patients in appropriate cases? What about the rights of communities and individuals to be protected from a virtually untreatable and potentially fatal disorder? In countries such as the USA and the Netherlands, incarceration has been used highly selectively to prevent unchecked spread of drug-resistant tuberculosis, and to mitigate the danger to the community.[170] In a recent international survey,[170] only 17% of respondents were against incarceration in principle. Mothers with extensively drug-resistant tuberculosis who are pregnant also present difficulties in management, particularly in resource-poor settings where social services are not developed and relatives might not be available to accept care of the child. The beds in many tuberculosis facilities in South Africa are occupied by patients with extensively drug-resistant tuberculosis who have not responded to treatment and have nowhere to go. Although some patients have relatively short lifespans after treatment failure, others live for months to years, can continue to raise families, and engage in employment and use of public transport. The issue is not confined to high-burden settings, but is also becoming problematic in Russia, eastern Europe, and other parts of the world. Urgent and cohesive action is needed from WHO and governments to deal with this growing problem.

Drug-resistant tuberculosis in health-care workers and infection control

Several reports have documented very high rates of tuberculosis in health-care workers in high-burden settings, with an incidence of more than 1000 per 100 000 workers.[171] Recently, reports of drug-resistant tuberculosis in health-care workers have started to emerge. In the province of KwaZulu-Natal, South Africa,[172] we identified more than 300 health-care workers with multidrug-resistant and extensively drug-resistant tuberculosis; these rates were about six times higher than were those of the general population, suggesting that drug-resistant tuberculosis was probably nosocomially acquired. We have also documented drug-resistant tuberculosis in health-care workers (eg, nurses, medical students, medical officers, and senior doctors) in Western Cape Province, South Africa.[173] Fears about contraction of drug-resistant tuberculosis could further exacerbate the shortage of health-care workers in settings where they are needed the most. Thus, health-care workers in appropriate settings should be screened at least annually (with symptom questionnaires and chest radiography), should have access to rapid diagnostics, and appropriate measures for environmental control (ie, adequate ventilation) and personal protection (ie, availability of 1 μm filter masks with fit testing) should be available and implemented.[174] Health-care facilities should have a written administrative plan about how patients with tuberculosis should be triaged, and how health-care workers should be screened and managed for drug-resistant tuberculosis.[174] However, comprehensive measures to prevent tuberculosis in health-care workers have yet to be widely implemented in many resource-poor settings.[174]

Prophylaxis for people exposed to patients with drug-resistant tuberculosis

How people who are in close contact with patients with highly drug-resistant forms of tuberculosis should be managed remains controversial. The risk of substantial toxic effects should be balanced against the less than 5–10% likelihood of household contacts developing active disease from airborne transmission.[175,176] The list of suitable drugs for chemoprophylaxis is small, but could include combinations of clofazimine and moxifloxacin, clofazimine and para-aminosalicylic acid, and linezolid and clofazimine. All these drugs have potential serious toxic effects, including cardiac arrhythmias (clofazamine and moxifloxacin), skin discoloration (clofazamine), and neurological and bone-marrow toxicity (linezolid). Appropriate counselling with regular follow-up and imaging is often the route followed by most clinicians.

Conclusions

Multidrug-resistant tuberculosis emerged in several high-burden settings in the early 1980s.[19] Roughly 20 years later, the first cases of extensively drug-resistant tuberculosis emerged,[19] and more than a decade later functionally untreatable cases of tuberculosis have been reported.[124] While existing patients are appropriately managed, health-systems infrastructure and capacity need to be drastically strengthened with a multidisciplinary approach to curb the development of further drug-resistant tuberculosis. Several new approaches to study the pathogenesis and mechanisms driving resistance will probably inform new strategies for prevention and management of this disease, including identification of new drug-specific mutations, epistasis and compensatory evolution, and

delineation of the fundamental biology of transmission. Rapid diagnostics urgently need to be rolled out, and the research community needs to be supported by international funding agencies to develop comprehensive, rapid, and point-of-care diagnostics for drug-resistant tuberculosis. Because new diagnostic tests have been implemented in resource-poor, high-burden settings where they were previously unavailable, treatment capacity for new cases of drug-resistant tuberculosis needs to be urgently scaled up. New drugs could shorten regimens for multidrug-resistant tuberculosis to less than 1 year without requiring an injectable drug, and several multicentre trials (including studies of optimum combinations of antiretroviral therapy in patients with drug-resistant tuberculosis) are underway. However, specific efforts for tuberculosis control will not succeed if broader issues—eg, reduction of poverty, overcrowding, political stability, and rates of HIV—are not addressed. There is also an urgent and comprehensive need to scale up protective measures and strategies against tuberculosis in health-care workers. The large-scale emergence of functionally untreatable tuberculosis needs governments and international agencies to take serious steps to reform the global economy to reduce poverty, make existing drugs available in resource-poor settings, and accelerate drug development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key messages

- Extensively drug-resistant and more drug-resistant strains of tuberculosis pose a serious threat to global health, result in high mortality, and are extremely costly to treat
- Data from molecular epidemiological studies show gradual acquisition and emergence of extensively drug-resistant tuberculosis followed by transmission and amplification of the epidemic
- Modelling studies suggest that early diagnosis and effective treatment of drug-resistant tuberculosis could profoundly reduce the burden of disease
- Several new insights (eg, within-person pharmacokinetic variability, efflux pumps, intrapulmonary drug gradients, adaptive mutations that differentially affect fitness, and the notion of disease superspreaders) challenge the present understanding of the pathogenesis of drug-resistant tuberculosis
- Several new technologies (eg, the GeneXpert MTB/RIF assay) enable the rapid diagnosis of drug-resistant tuberculosis
- Treatment outcomes for patients with extensively drug-resistant tuberculosis (particularly in endemic settings) are dismal because of a lack of effective drugs
- Absence of effective drugs and scarce resources mean that patients with incurable tuberculosis are discharged into the community where they are at high risk of transmitting disease
- Several new effective antimicrobials (eg, delamanid, bedaquiline, and linezolid) have recently become available for clinical use, but a combination regimen for drug-resistant tuberculosis needs urgent validation in a prospective clinical study in intermediate-burden and high-burden settings

Panel 1

Established and emerging definitions of drug-resistant tuberculosis

Multidrug-resistant tuberculosis

Resistance to at least isoniazid and rifampicin, the two most effective first-line antituberculosis drugs; treatment is more costly and toxic, and has worse cure rates (40–80%) than for drug-susceptible strains (cure rates of >90%) in effective programmes[8–15]

Extensively drug-resistant tuberculosis

Multidrug-resistant tuberculosis plus resistance to any fluoroquinolone and any second-line injectable drug (ie, kanamycin, amikacin, or capreomycin); cure and survival rates are worse for extensively drug-resistant tuberculosis than for multidrug-resistant tuberculosis[16–20]

Resistance beyond extensively drug-resistant tuberculosis

Preliminary data suggest that cure and survival rates could be worse for resistance to all second-line injectable drugs, all WHO group 4 drugs, ethambutol, and pyrazinamide in addition to extensively drug-resistant tuberculosis,[21] but needs confirmation in prospective studies; two definitions (extremely and totally drug-resistant tuberculosis), not endorsed by WHO, have been proposed to describe the most severe forms of drug resistance

Extremely drug-resistant tuberculosis

Reported in two patients with strains resistant to all known first-line and second-line drugs, including all group 3 drugs (fluoroquinolones), and to additional group 5 drugs of uncertain efficacy (rifabutin, clofazimine, dapsone, clarithromycin, and thioacetazone; linezolid was not available at the time of testing);[9] both patients died after protracted illness

Totally drug-resistant tuberculosis

Reported in Iran, where 15 isolates were resistant to all first-line or second-line drugs tested,[5] India, where 4 cases were resistant to all first-line and second-line drugs tested (cycloserine was not tested),[4] and South Africa, where resistance was defined by high-confidence genotypic mutations (with the exception of para-aminosalicylic acid);[3] treatment outcomes were poor with a high death rate in all studies

Panel 2

First-line and second-line drugs for tuberculosis as classified by WHO[26]

Group 1—first-line oral drugs

- Isoniazid
- Pyrazinamide
- Ethambutol
- Rifampicin
- Rifabutin

Group 2—second-line injectable drugs

- Kanamycin
- Amikacin
- Capreomycin
- Streptomycin

Group 3—fluoroquinolones

- Levofloxacin
- Moxifloxacin
- Ofloxacin
- Gatifloxacin

Group 4—oral bacteriostatic second-line drugs

- Para-aminosalicylic acid
- Cycloserine
- Terizidone
- Ethionamide
- Prothionamide

Group 5—drugs with unclear efficacy or role in treatment of drug-resistant tuberculosis

- Clofazimine
- Linezolid
- Amoxicillin and clavulanate
- Thioacetazone
- Clarithromycin
- Imipenem and cilastatin

- Meropenem clavulanate
- High-dose isoniazid
- Bedaquiline
- Delaminid

Based on guidelines by WHO and the Stop TB initiative.[26]

Panel 3

Pathogenesis and mechanisms of the development of drug-resistant mutations

Poor drug regimens and non-adherence select resistant mutants

- Continuation phase in patients with sputum-culture-positive isoniazid-resistant strains (20% of tuberculosis cases in many parts of South Africa) is effectively rifampicin monotherapy, leading to rifampicin resistance
- Social factors including interruptions (figure 2) all interact to increase the risk of non-adherence and further acquired drug resistance

Subtherapeutic drug concentrations drive resistance despite excellent adherence

- Induction of efflux pumps
- Variability in drug metabolic rates, causing subtherapeutic serum concentrations
- Variability in drug absorption rates, leading to subtherapeutic peak concentrations
- Exposure of mycobacteria to suboptimum drug concentrations in fibro-cavitary lesions

Increased transmission of drug-resistant *Mycobacterium tuberculosis*

- High bacterial burden
- *M tuberculosis* genotype
- Metabolic status of organisms in aerosolised sputum
- Frequency and intensity of cough and sputum viscosity
- Cavitary disease and number of cavities
- Degree of ventilation, length of exposure, humidity, and other factors

Effects of genotype and compensatory mutations

- Epistatic interactions might modulate fitness (increased, decreased, or unchanged)
- Compensatory mutations affect structural and physiological pathways because of changes in the proteome[60]
- Strain and types of mutations associated with different cell-wall characteristics, thickness, and budding characteristics[61,62]
- Changes to structure and bacterial proteome in drug-resistant isolates affect host response

Panel 4

Management of extensively drug-resistant tuberculosis and more drug-resistant strains

Principles for medical management of multidrug-resistant tuberculosis

- Regimens based, when possible, on proven or probable susceptibility to at least four drugs
- Regimen generally based on a later-generation fluoroquinolone (eg, moxifloxacin or levofloxacin), plus an injectable drug, usually an aminoglycoside (ie, either amikacin or kanamycin), any first-line drug to which the isolate is susceptible (appendix), and addition of group 4 drugs (eg, cycloserine, terizidone, or ethionamide), such that at least four drugs to which the isolate is probably susceptible are used
- Injectable drugs are used for 6–8 months or longer, and the total duration of treatment is suggested to be 24 months[122]
- Drugs that patients have previously received for 3 months or longer are generally avoided
- Psychological factors should be addressed to ensure compliance
- Patients should be monitored for adverse drug reactions, which are common[103]
- A single drug should not be added to a failing regimen

Principles for management of extensively drug-resistant tuberculosis

- Regimens should be constructed on the basis of prevailing patterns in drug susceptibility testing
- One injectable is chosen from group 2, any drug that the isolate is susceptible to from group 1, and any remaining available drugs from group 3 or 4
- In view of the high background rates of tuberculosis and multidrug resistance in several countries, regimens are often constructed around a backbone of capreomycin and para-aminosalicylic acid
- Patients should be carefully monitored for adverse drug reactions, particularly when receiving capreomycin (eg, renal failure, hypokalaemia, and hypomagnesaemia), which are common[103]
- Patients receiving capreomycin should have urea and electrolytes monitored weekly for the first 8 weeks, and then monthly thereafter; attention should be paid to correction of risk factors for renal failure (ie, dehydration, nausea, vomiting and diarrhoea, and avoidance of other nephrotoxic drugs [co-trimoxazole and nevirapine]), and early identification of underlying renal disease (eg, diabetes and HIV-associated nephropathy)

Surgical management of extensively drug-resistant tuberculosis

- Candidates include patients with unilateral disease (or apical bilateral diseases in selected cases) with adequate lung function who have not responded to treatment for multidrug-resistant tuberculosis, any patient with extensively drug-resistant tuberculosis, or any patient who has not responded to treatment for extensively drug-resistant tuberculosis[119,123]
- Facilities for surgical lung resection are scarce in high-burden settings and, even when available, surgery is an option only for very few patients
- Patients with contralateral disease are usually not surgical candidates, but establishment of whether contralateral disease is due to old tuberculosis or fibrosis rather than active disease is often difficult; the role of PET and CT scanning for these patients needs further investigation

Search strategy and selection criteria

We searched PubMed for articles published in all languages between January, 1995, and November, 2013, with the terms “tuberculosis”, “drug-resistant”, “MDR-TB”, and “XDR-TB”. We also identified relevant articles through searches of the authors’ personal files, review articles, landmark papers, and other relevant search engines (eg, Google Scholar).

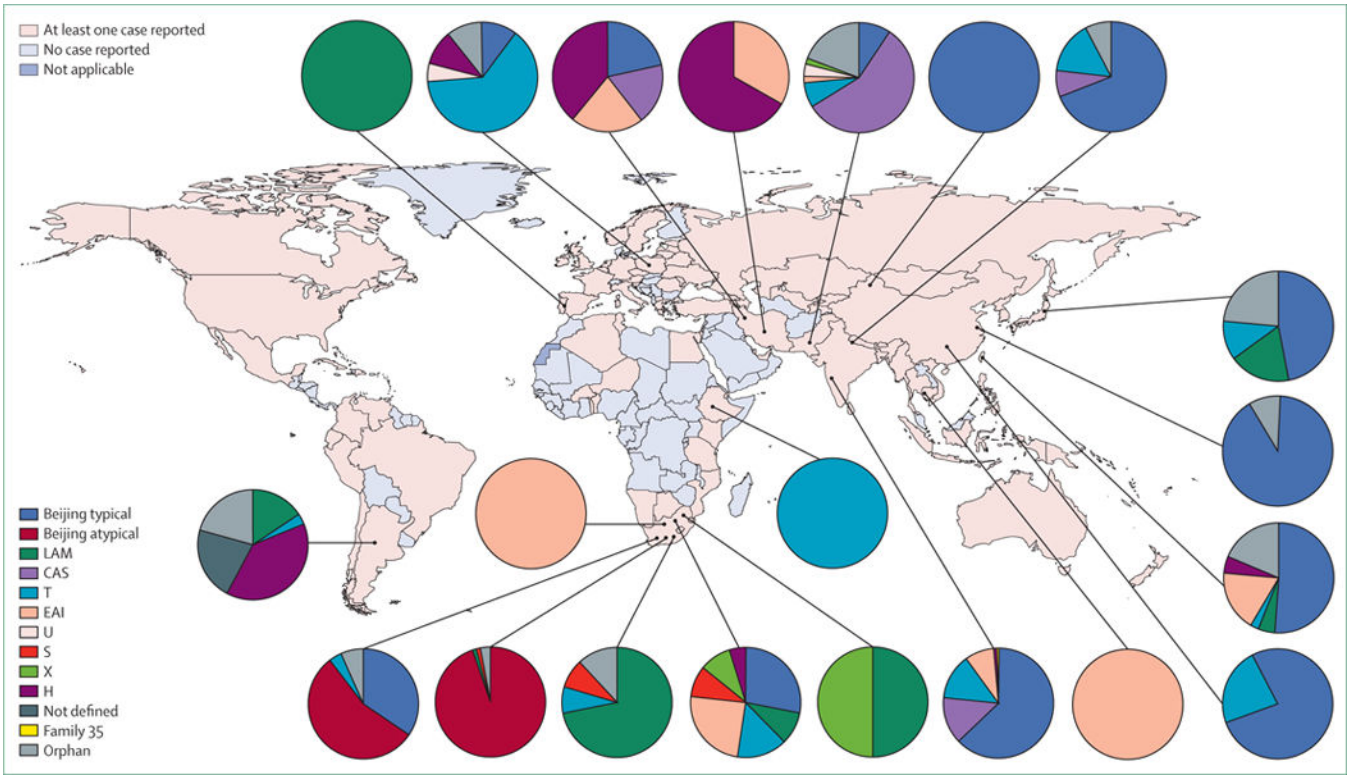

Figure 1. Global distribution of extensively drug-resistant tuberculosis by genotype and country

The proportion of isolates with a defined genotype are shown for South Africa, Ethiopia, Argentina, Portugal, Poland, Iran, Pakistan, India, Nepal, Cambodia, China, Taiwan, and Japan. We classified Beijing genotype strains from South Africa as typical or atypical to show regional differences in the population structure of extensively drug-resistant tuberculosis. Data sources provided in the appendix. LAM=Latino-American-Mediterranean family. CAS=Central-Asian family. T=T family. EAI=East-African-Indian family. U=U family. S=S family. X=X family. H=Haarlem family.

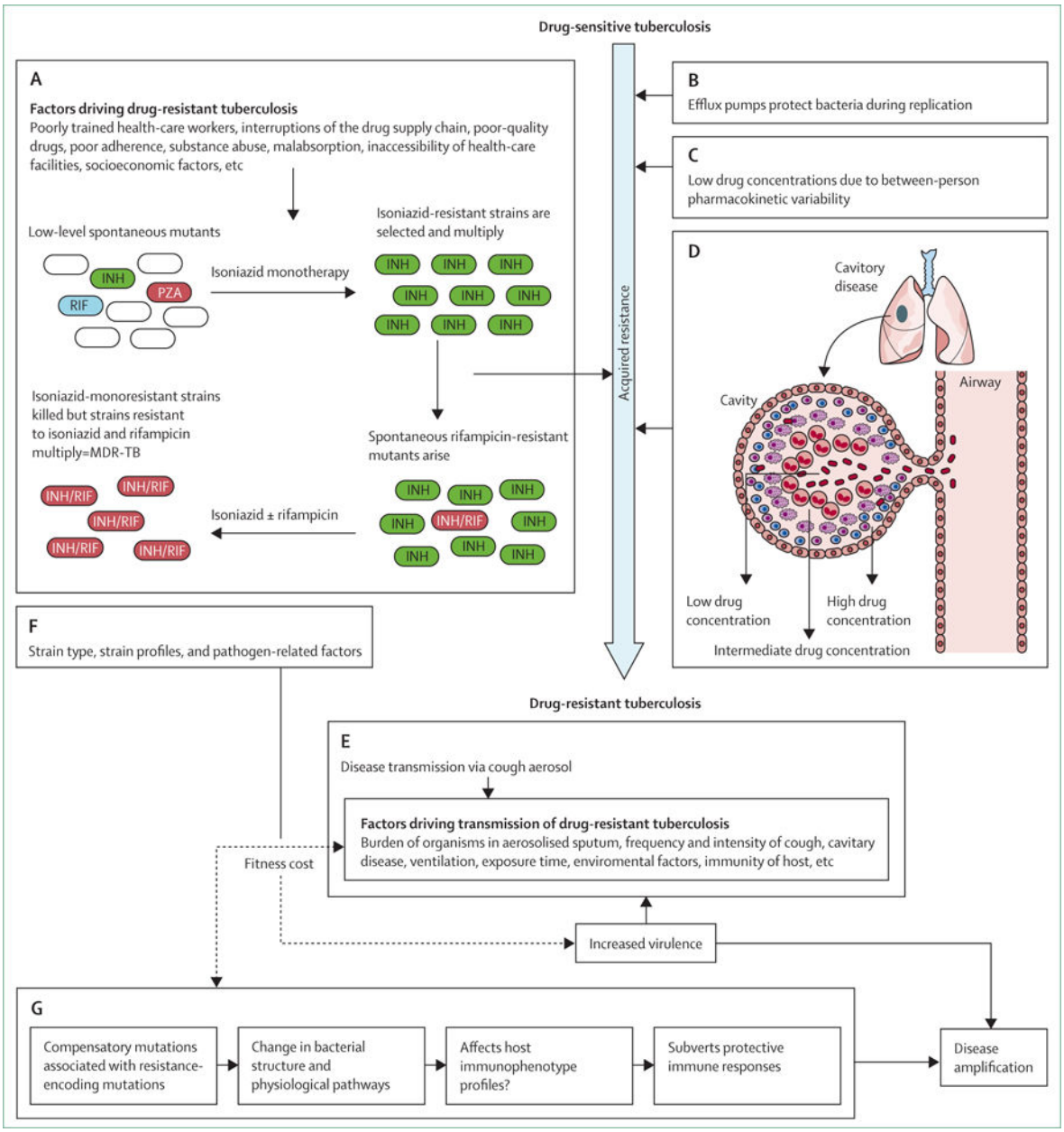

Figure 2. The pathogenesis of drug-resistant tuberculosis

The traditional interpretation of resistance development is that sequential drug resistance develops through fragmented treatment (A), which can be fuelled by several programmatic and socioeconomic factors. However, resistance can develop despite excellent adherence. Several factors, including efflux pumps (B), between-person pharmacokinetic variability (C), and extensive immunopathology in the lung resulting in differential drug penetration into granulomas and cavities (D) might all drive site-specific drug concentrations below minimum inhibitory concentrations, thus probably enabling drug resistance. After acquired drug resistance develops, person-to-person transmission might constitute the major route of spread (E). Strain-specific genotype, newly acquired drug-encoding mutations, and compensatory mutations that can affect fitness cost (and hence transmission) might also

interact (F). Compensatory mutations could be associated with changes in structure and physiological pathways, which could affect host immune response and thereby potentially subvert protective responses and drive progressive disease (G). INH=isoniazid. RIF=rifampicin. PZA=pyrazinamide. MDR-TB=multidrug-resistant tuberculosis.

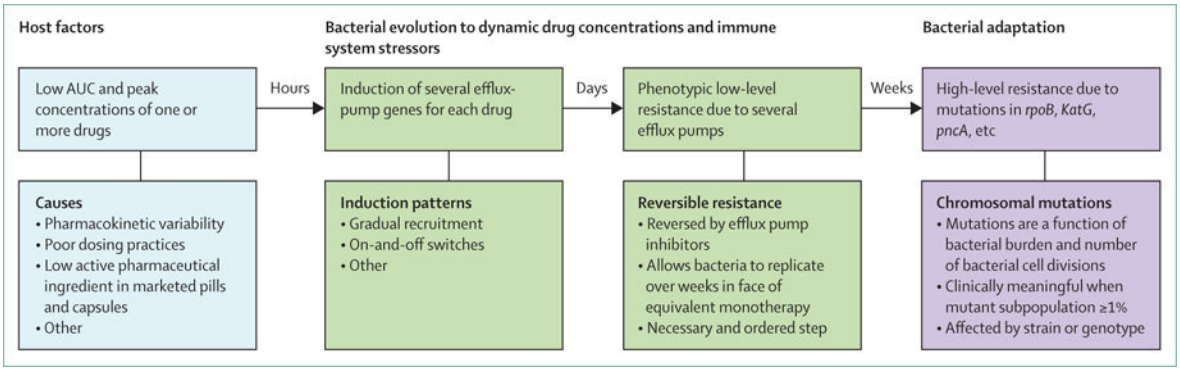

Figure 3. The arrow of time of antibiotic resistance

Several factors initiate the process, the most important of which is low drug concentrations due to pharmacokinetic variability. Variability is encountered at each step of drug absorption, distribution, metabolism, and elimination, and could be due to different single-nucleotide polymorphisms in enzymes for drug transport and xenobiotic metabolism, comorbid conditions (eg, AIDS), or increased patient weight. This variability leads in some patients to low drug concentrations of one or more drugs in the regimen (effectively equivalent to monotherapy). Bacteria then adapt to these concentrations, initially through epigenetic mechanisms (eg, induction of several efflux pumps). These efflux pumps are associated with low-level multidrug resistance and enable several rounds of bacterial replication, allowing for development of mutations in canonical genes associated with drug resistance. AUC=area under the curve.

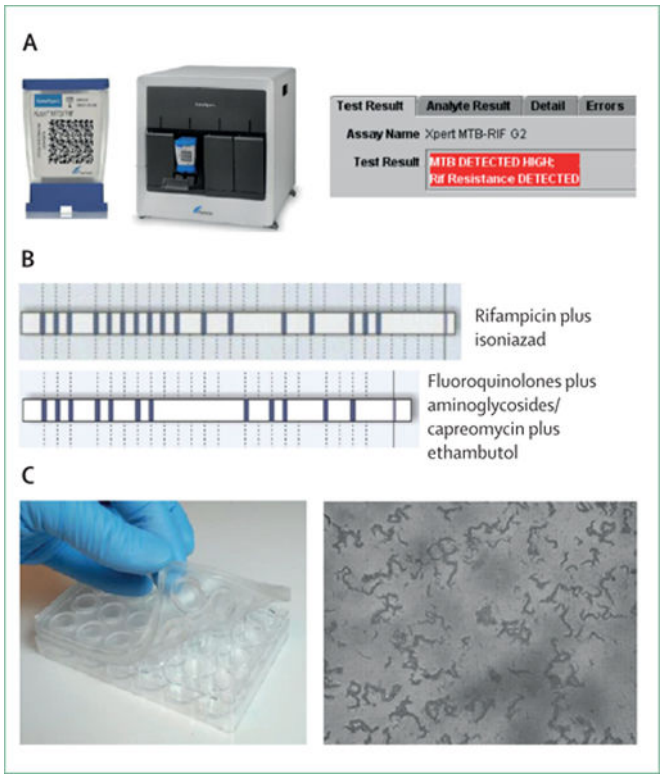

Figure 4. Molecular (A, B) and culture-based (C) tests for diagnosis of drug resistance

(A) The Xpert MTB/RIF assay, an automated real-time PCR assay that can detect genotypic resistance to rifampicin and *Mycobacterium tuberculosis* complex DNA. A test cartridge, four-module machine, and an example of a tuberculosis-positive rifampicin-resistant result are shown. (B) Line-probe hybridisation patterns, resulting from the detection of specific DNA fragments containing mutations associated with drug resistance, for the Hain Lifesciences MTBDR*plus* (top row) and Hain Lifesciences MTBDR*sl* (bottom row) test. (C) An example of a commercially available kit for microscopic observation drug susceptibility, in which bacteria are cultured in a microtitre-well-plate format, and visually inspected with light microscopy for distinctive cording patterns that are indicative of *M tuberculosis* growth in the presence of the drug of interest.

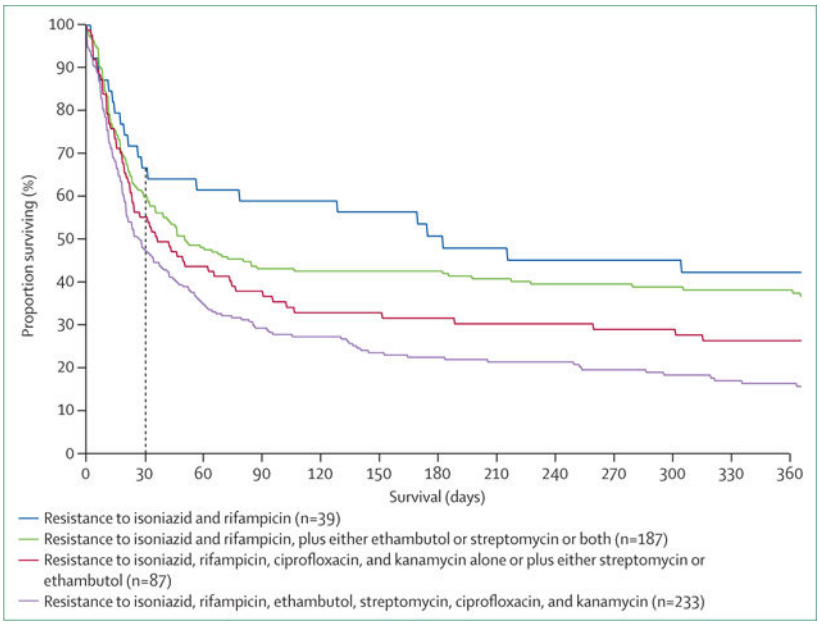

Figure 5. Kaplan-Meier survival plot by drug-resistance pattern for patients with multidrug-resistant and extensively drug-resistant tuberculosis, 2005–07 (log-rank p<0·0001)

Median survival was 182 days (95% CI 31–395) for patients with tuberculosis resistant to isoniazid and rifampicin; 50 days (95% CI 35–106) for patients with tuberculosis resistant to isoniazid and rifampicin, plus either ethambutol or streptomycin or both; 36 days (95% CI 23–74) for patients with tuberculosis resistant to isoniazid, rifampicin, ciprofloxacin, and kanamycin, alone or plus either streptomycin or ethambutol; and 27 days (95% CI 20–38) for patients with tuberculosis resistant to isoniazid, rifampicin, ethambutol, streptomycin, ciprofloxacin, and kanamycin. Figure reproduced from Gandhi and colleagues[134] by permission of the American Thoracic Society.