Respiratory Medicine (2009) **103**, 1777–1790

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/rmed

REVIEW

Recent advances in the diagnosis and treatment of multidrug-resistant tuberculosis

Suhail Ahmad*, **Eiman Mokaddas**

Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait

Received 30 March 2009; accepted 19 July 2009
Available online 5 August 2009

KEYWORDS
MDR-TB;
Rapid diagnosis;
Treatment;
Management

Summary
Tuberculosis (TB) is a major infectious disease killing nearly two million people, mostly in developing countries, every year. The increasing incidence of resistance of *Mycobacterium tuberculosis* strains to the most-effective (first-line) anti-TB drugs is a major factor contributing to the current TB epidemic. Drug-resistant strains have evolved mainly due to incomplete or improper treatment of TB patients. Resistance of *M. tuberculosis* to anti-TB drugs is caused by chromosomal mutations in genes encoding drug targets. Multidrug-resistant (resistant at least to rifampin and isoniazid) strains of *M. tuberculosis* (MDR-TB) evolve due to sequential accumulation of mutations in target genes. Emergence and spreading of MDR-TB strains is hampering efforts for the control and management of TB. The MDR-TB is also threatening World Health Organization's target of tuberculosis elimination by 2050. Proper management of MDR-TB relies on early recognition of such patients. Several diagnostic methods, both phenotypic and molecular, have been developed recently for rapid identification of MDR-TB strains from suspected patients and some are also suitable for resource-poor countries. Once identified, successful treatment of MDR-TB requires therapy with several effective drugs some of which are highly toxic, less efficacious and expensive. Minimum treatment duration of 18–24 months is also long, making it difficult for health care providers to ensure adherence to treatment. Successful treatment has been achieved by supervised therapy with appropriate drugs at institutions equipped with facilities for culture, drug susceptibility testing of MDR-TB strains to second-line drugs and regular monitoring of patients for adverse drug reactions and bacteriological and clinical improvement.

* Correspondence to: Suhail Ahmad, Department of Microbiology, Faculty of Medicine, AMAR Health Sciences Center, Kuwait University, P. O. Box 24923, Safat 13110, Kuwait. Tel.: +965 2498 6503; fax: +965 2531 8454.
E-mail addresses: suhail_ah@hsc.edu.kw, suhail_ah2000@yahoo.com (S. Ahmad).

0954-6111/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2009.07.010

Contents

Introduction

The morbidity and mortality associated with tuberculosis (TB) continue to remain high despite intense worldwide efforts. Two major factors that are sustaining the current TB epidemic include expanding human immunodeficiency virus (HIV) infection and its association with active TB disease and increasing resistance of *Mycobacterium tuberculosis* strains to the most-effective (first-line) anti-TB drugs.[1] Other factors that have also contributed include population expansion, poor case detection and cure rates in impoverished countries, active transmission in overcrowded hospitals, prisons and other public places, immigration from high incidence countries, drug abuse and homelessness. Active disease patients with sputum-positive pulmonary TB are the main source of infection in communities. Although an effective immune response arrests multiplication of *M. tuberculosis* soon after infection in majority of cases, only ~10% individuals eradicate the pathogen completely while ~90% only succeed in containment of infection and thus remain latently infected.[2] The World Health Organization (WHO) has estimated that nearly a third of the world population is now infected with *M. tuberculosis* and 5–10% of the infected individuals will develop active TB disease during their lifetime. However, the risk of developing active disease is 5–15% every year and lifetime risk is ~50% in HIV-coinfected individuals.[3]

In 2006, 9.2 million new active disease cases (4.1 million being sputum smear-positive) corresponding to an estimated incidence of 139 per 100,000 population occurred throughout the world.[1] Only half of these cases were actually reported while the rest were based on assessments of effectiveness of surveillance systems. The highest incidence rate (363 per 100,000 population) was recorded for the African region, mainly due to high prevalence of HIV infection. The 22 high burden (11 Asian, 9 African, 1 South American and 1 East European) countries accounted for >84% of all active TB cases worldwide. The lowest incidence rates (<20 cases per 100,000) were recorded for some developed, rich countries. Nearly 700,000 (7.6%) of 9.2 million TB patients were coinfected with HIV and ~0.5 million cases of multidrug-resistant TB (MDR-TB, defined as infection with *M. tuberculosis* strains resistant at least to rifampin, RIF and isoniazid, INH) occurred in 2006. Globally, the total prevalent TB cases in 2006 were 14.4 million that resulted in 1.7 million deaths (~95% occurring in developing countries).[1]

Emergence of drug-resistant TB

The problem of drug-resistant TB is not new. In 1948, the British Medical Research Council reported that the mortality rates in pulmonary TB patients treated or not treated with streptomycin (SM) were the same, however, most patients in the treated group had died of relapse with SM-resistant strains.[4] Standard short-course chemotherapy with multiple drugs developed in 1960s and 1970s effectively kills all bacilli localized in different environments (e.g. pulmonary cavities, pus, solid caseous material, immune cells) including naturally occurring monodrug-resistant strains (as they are killed by other drugs) and controls the emergence of multidrug-resistant strains.[5,6]

Successful treatment of fully susceptible TB depends on combination of drugs and duration of therapy, cost, and drug's side effects. Conversion of culture-positive to culture-negative sputum within 2 months and subsequent clearing of filtrates on chest radiograph are positive signs while positive sputum cultures after 4 months of multidrug therapy indicate treatment failure.[7] Treatment failure is due to multiple factors (including, among other, incomplete or inappropriate therapy) and results in persistence of the disease mainly due to emergence of drug resistance (acquired resistance). Primary resistance occurs when the resulting *M. tuberculosis* strain is transmitted to a new host, as it causes TB that is already resistant to the indicated drug(s).[1,2] Major factors associated with drug resistance development include presence of cavitation together with smear-positive sputum and positive sputum culture, non-adherence to therapy due to multiple factors (such as cost of drugs, long duration and multiple drug combination used in treatment regimens and drug related adverse

reactions), treatment failures, coinfection with HIV, inadequate resources in high burden countries, and prior history of treatment with anti-TB drugs.[1,7–9] The *M. tuberculosis* W/Beijing genotype has strong association with MDR phenotype.[10] Simultaneous treatment of HIV-TB coinfection may lead to malabsorption and suboptimal therapeutic blood levels of RIF and INH (despite adherence to therapy) that facilitate the development of drug-resistant TB and MDR-TB.[3,9] The malabsorption of anti-TB drugs may also occur in patients with some other underlying diseases.[11,12]

Global epidemiology of MDR-TB

Four successive reports of Global Projects on Antituberculosis Drug Resistance Surveillance sponsored by WHO were published in 1997, 2001, 2006 and 2008. The latest report collected drug susceptibility testing (DST) data for INH, RIF, SM and ethambutol (EMB) from 93 settings in 81 countries during 2002–2007 and showed that resistance to at least one anti-TB drug (any resistance) among new TB cases was lower (varying from 0% to 56%) than in previously treated TB patients (varying from 0% to 86%).[9] The worldwide average for any resistance, INH resistance and MDR among all TB cases were estimated to be 20%, 13.3% and 5.3%, respectively. The rates of resistance for any drug, INH and MDR were higher in retreatment versus new TB cases. The data showed that 489139 cases of MDR-TB occurred in 2006 representing 4.8% of all TB cases. The highest percentage of MDR-TB cases were estimated for countries of Eastern Europe (19.2%) followed by Western Pacific region (7%) and Southeast Asia (4.3%). The data also showed that compared to the previous report,[13] the burden of MDR-TB cases declined in USA and Hong Kong, remained stable in several European countries and increased in some developing countries.[9]

The MDR-TB is a major threat to global public health as it is difficult to treat and often results in relapse or treatment failure.[14,15] It is also a risk factor for the emergence of XDR-TB (defined as infection with MDR-TB strains additionally resistant either to a new-generation fluoroquinolone and an injectable agent or to three of the six main classes of second-line drugs).[16] In 2006, nearly 10% of all MDR-TB cases in the former Soviet Union countries (ranging from 4% in Armenia to 24% in Estonia) were XDR-TB.[9] In South Africa, 996 of 17615 (5.6%) of MDR-TB cases were also XDR-TB. Although 50 countries have already reported one or more cases of XDR-TB, there is no information for several other countries that have a high incidence of MDR-TB.[17] The emergence of XDR-TB is extremely worrisome as it is more difficult to treat than MDR-TB in developed countries and is virtually untreatable in developing countries. An outbreak of XDR-TB in South Africa was highly lethal.[18]

Anti-TB drugs: mechanism of action and resistance

A better understanding of drug resistance mechanisms in *M. tuberculosis* is crucial for the development of rapid methods for drug resistance detection and new anti-TB drugs to treat MDR-TB/XDR-TB patients. Early molecular studies identified *katG* (encoding catalase–peroxidase) and *rpoB* (encoding β-subunit of RNA polymerase) genes as major targets conferring resistance of *M. tuberculosis* to INH and RIF, respectively.[19,20] The current knowledge of the genes and their target regions involved in mediating resistance of *M. tuberculosis* to important anti-TB drugs is summarized in Table 1.

Isoniazid (INH), a pro-drug, has the highest activity (MIC <0.05 μg/ml) against actively dividing *M. tuberculosis*.[19] It is activated by catalase–peroxidase encoded by *katG*. Activated INH mainly targets NADH-specific enoyl-acyl carrier protein (ACP) reductase (encoded by *inhA*) and β-ketoacyl ACP synthase (encoded by *kasA*) involved in mycolic acid synthesis. Depletion of mycolic acids results in bacterial killing.[19,21]

Table 1 Important genes (and their encoded products) of *M. tuberculosis* conferring resistance to first-line and some second-line anti-TB drugs.

Anti-TB drug(s)	Gene target	Encoded protein or RNA product	Major target region in encoded product	Level of resistance
RIF	*rpoB*	β-Subunit of RNA polymerase	81-bp HSR	High
INH	*katG*	Catalase/peroxidase	Entire gene	High
INH/ETH	*inhA*	Enoyl-acyl carrier protein reductase	Upstream regulatory region	Low
PZA	*pncA*	Pyrazinamidase	Several codons	High
EMB	*embB*	Arabinosyltransferase	Several codons	High
EMB	*embA*	Arabinosyltransferase	Several codons	High
EMB	*iniA*	Isoniazid-inducible gene	Few codons	High
SM	*rrs*	16S rRNA	Several nucleotides	Low
SM	*rpsL*	Ribosomal Protein S12	Codons 43 and 48	High
FQs	*gyrA*	A subunit of DNA gyrase	Few codons	High
FQs	*gyrB*	B subunit of DNA gyrase	Few codons	High
KAN/AMI	*rrs*	16S rRNA	Position 1401 or 1484	High
CAP/VIO	*rrs*	16S rRNA	Position 1401 or 1484	High
CAP/VIO	*tlyA*	Cytotoxin/haemolysin homologue	Several codons	High

RIF, rifampin; INH, isoniazid; ETH, ethionamide; PZA, pyrazinamide; EMB, ethambutol; SM, streptomycin; FQs, fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin); KAN, kanamycin; AMI, amikacin; CAP, capreomycin; VIO, viomycin; and HSR, hot-spot region.

The molecular basis of resistance to INH involves a variety of mutations (insertions, deletions and point mutations) in several genes. The main targets include *katG* and the regulatory and coding region of *inhA*.[19,21,22] Mutations in other genes are either compensatory resulting from the loss of catalase–peroxidase activity or have a minor role.[23] Despite the complexity, high- and low-level resistance to INH is mainly due to mutations within the *katG* and *inhA* regulatory region.[19,21–24] The *katG* gene mutations occur frequently between codons 138 and 328, particularly at codon 315 (*katG315)*. The mutations in *inhA* regulatory region occur mostly at positions −15 and −8 (relative to *mabA* translational start site). The frequency of *katG315* (~50–95%) and *inhA* regulatory region (~6–30%) mutations vary considerably depending upon the ethnicity of the infected TB patient/geographical location where *M. tuberculosis* strain is isolated (Table 2).[24–28] Some INH-resistant strains do not have an identifiable mutation implying that additional targets are involved.[23,24]

Rifampin (RIF), a lipophilic rifamycin derivative, binds to β-subunit of RNA polymerase (encoded by *rpoB*) and inhibits RNA transcription and consequently, protein synthesis in *M. tuberculosis*.[20,23] Mutations in *rpoB* gene confer RIF resistance by affecting drug binding. Monoresistance to RIF is rare except in HIV-coinfected patients and RIF resistance is often a surrogate marker for MDR-TB since ~85–90% RIF-resistant strains are also resistant to INH.[3,20,29–32] Nearly 90–95% of RIF-resistant strains carry missense mutations or small in-frame insertions/deletions within an 81-bp hot-spot region (HSR) mainly involving *rpoB* codons 531, 526 and 516 (Table 2).[20,30–34] Resistance in ~5–10% RIF-resistant isolates is due to mutations in N-terminal or other regions of the *rpoB* gene or due to some unknown mechanism.[20,35,36] Specific mutations in HSR and N-terminal region also show variations depending upon the ethnicity of TB patient/geographical location where *M. tuberculosis* strain is isolated.[30–34,36]

Missense mutations at *rpoB* codon 511, 518 or 522 cause low-level while those at codon 516, 526 or 531 cause high-level RIF resistance.[35,37] Also, a relationship exists between specific *rpoB* mutations and cross-resistance of *M. tuberculosis* isolates to other rifamycins. Isolates with mutations at codon 511 or 533 exhibit low-level RIF resistance but remain susceptible to rifabutin (RBU) while those with mutations at codon 514, 515, 516, 522 or 533 become RIF-resistant but remain susceptible or exhibit low-level resistance to RBU. However, *M. tuberculosis* isolates with *rpoB* mutations in N-terminal region or at codon 513, 526 or 531 exhibit high-level resistance to both RIF and RBU.[37,38] Thus, RBU may be used as a second-line drug for the treatment of some patients infected with RIF-resistant strains carrying specific *rpoB* mutations.

Pyrazinamide (PZA), a pro-drug, is highly effective against semidormant bacilli in acidic environment (like macrophages).[22,23] The PZA is activated by pyrazinamidase encoded by *pncA* gene to pyrazinoic acid which, by lowering intracellular pH, inactivates a vital fatty acid synthase. The PZA is highly specific against *M. tuberculosis*, other mycobacteria are intrinsically resistant to PZA due to lack of an efficient pyrazinamidase. Similar to INH, most PZA-resistant *M. tuberculosis* strains also contain mutations in *pncA* gene that activates the pro-drug.[39,40] Nearly 20–30% of PZA-resistant *M. tuberculosis* strains contain wild-type *pncA*, suggesting the existence of other resistance conferring mechanism(s).[23,39,40]

Ethambutol (EMB) is now used in place of SM in combination therapy with INH, RIF and PZA since resistance of *M. tuberculosis* strains to EMB is much less compared to SM.[1,9,13] The molecular basis of resistance to EMB is not fully defined. The EMB mainly targets enzymes participating in synthesis and polymerization of cell wall arabinan, and interacts with three homologous, membrane associated arabinosyltransferases encoded by *embC–embA–embB* genes.[41] It also affects proteins encoded by isoniazid-inducible genes like *iniA* and acyl carrier proteins and other proteins regulating their expression.[41,42] Mutations in *embB*, particularly at *embB306*, *embB406* and *embB497* occur more frequently and confer resistance to EMB.[43,44] The frequency of *embB306* mutations also varies (20–70%) in EMB-resistant strains from different geographical locations (Table 2).[41,42,45]

Streptomycin (SM) is nearly as effective as EMB, however, several factors have discouraged its use in more recent times. Oral formulations in multidrug regimens are not feasible requiring frequent patient's visit to health care facilities. Globally, highest level of resistance to an anti-TB drug is also observed for SM.[9,13] The SM may be used as a first-line or second-line drug for treating patients with failing therapy or MDR-TB provided the *M. tuberculosis* strain is susceptible to SM.[7] The SM binds to a ribosomal protein and 16S rRNA (encoded by *rpsL* and *rrs*, respectively) causing misreading of mRNA and faulty protein

Table 2 Frequency of mutations in main target region and most commonly mutated codon/nucleotide in target gene conferring resistance to anti-TB drugs in *M. tuberculosis*.

Anti-TB agent(s)	Gene target	Main target region	Relative frequency in resistant strains	Most common mutation at	
				Codon/nucleotide	Frequency
RIF	*rpoB*	81-bp HSR	90–95%	Codon 531	30–75%
INH	*katG*	Entire gene	50–95%	Codon 315	50–95%
INH/ETH	*inhA*	Regulatory region	6–30%	Nucleotide (-)15[a]	6–30%
PZA	*pncA*	Several codons	62–97%	No dominant mutation	
EMB	*embB*	Several codons	47–89%	Codon 306	20–70%
SM	*rpsL*	Codons 43 and 48	40–95%	Codon 43	40–90%

RIF, rifampin; INH, isoniazid; ETH, ethionamide; PZA, pyrazinamide; EMB, ethambutol; SM, streptomycin; and HSR, hot-spot region.
[a] Nucleotide situated relative to the start of *mabA* open reading frame of *mabA-inhA* operon.

synthesis. Since *M. tuberculosis* genome contains a single *rrs* gene, nearly 30% of SM-resistant *M. tuberculosis* isolates contain mutations in the *rrs* gene.[46,47] The remaining SM-resistant *M. tuberculosis* strains either contain mutations in *rpsL* (at *rpsL43* or *rpsL88*) or in other genes.[23,46,47]

Other aminoglycosides, kanamycin (KAN) and amikacin (AMI) also inhibit protein synthesis (peptide chain elongation) in *M. tuberculosis* and are used as second-line injectable drugs for actively dividing bacteria.[2,7] Resistance to KAN and AMI is mainly associated with *rrs* mutations. Although cross-resistance between KAN and AMI has been reported, cross-resistance between SM and either KAN or AMI is not reported, hence KAN/AMI may be used for SM-resistant strains.[48] However, cross-resistance between KAN/AMI and other injectable agents such as capreomycin (CAP) and viomycin (VIO) (cyclic peptides) has been described. The *rrs* mutation A1401 G is associated with high-level KAN and AMI resistance but usually causes low-level resistance to CAP and no resistance to VIO while C1402 T is associated with high-level CAP and VIO resistance but usually causes low-level resistance to KAN and no resistance to AMI in *M. tuberculosis*.[48] However, *rrs* mutation G1484 T is associated with high-level resistance to CAP, VIO, KAN and AMI.[48] Another mechanism conferring resistance to CAP and VIO involves mutations in the *tlyA* gene.[49]

Fluoroquinolones (FQs) such as ofloxacin (OFX) and levofloxacin (LFX) are important second-line drugs for treating MDR-TB.[1,9,15] The new-generation FQs (moxifloxacin, MFX and gatifloxacin, GFX) have excellent bactericidal activity against *M. tuberculosis*.[50] The FQs inactivate DNA gyrase (composed of two A and two B subunits encoded by *gyrA* and *gyrB* genes, respectively) and inhibit DNA replication.[51] In *M. tuberculosis*, resistance to FQs is mainly associated with mutations at codons 90, 91 and 94 of *gyrA* gene.[31,51–53] Some FQ-resistant *M. tuberculosis* strains contain mutations at *gyrB464* or *gyrB495*.[52]

Ethionamide (ETH), a structural analog of INH, is used as a second-line drug for MDR-TB and shares the target with INH. Similar to INH, ETH is also a pro-drug, however, it is activated by a monooxygenase (encoded by *ethA*).[54] The *ethA* catalyses a two step activation of ETH to its active form (4-ethyl-4-amidopyridine). Similar to INH and PZA, majority of ETH-resistant *M. tuberculosis* strains contain mutations in *ethA* that abolish activation of pro-drug.[54] Similar to INH, the main cellular target of activated ETH is *inhA* and mutations in *inhA* regulatory region that are associated with INH resistance also cause cross-resistance to ETH.[23,54]

Diagnosis of active TB disease

The diagnosis of active disease (including pulmonary as well as extrapulmonary TB) is usually based on clinical suspicion, chest radiographs, smears for acid-fast bacilli (AFB), solid and liquid culture and, more recently, hybridization- and PCR amplification-based detection of *M. tuberculosis* nucleic acid in clinical specimens. A brief outline for the diagnosis of active TB disease is described here. Several review articles are available for a more detailed description.[6,55–57]

Microscopic examination of smears for acid-fast bacilli for all clinical specimens is a rapid and inexpensive test, however, it is positive in only 34–80% of expectorated sputum samples and is often negative in HIV-coinfected TB patients due to atypical presentation.[1,55] The sensitivity for non-sputum samples is even less. Also, the test does not differentiate TB from infections caused by non-tuberculous mycobacteria (NTM). The gold standard for TB diagnosis is culture of *M. tuberculosis*. The culture also allows species-specific identification, DST to guide therapy and fingerprinting of isolates during epidemics. Culture on solid media takes 4–6 weeks while liquid media-based culture systems yield faster (7–12 days) growth of *M. tuberculosis*.[6] Phage-based assays developed more recently offer rapid detection of *M. tuberculosis* in resource-poor settings.[56]

Molecular methods have provided rapid diagnosis of TB in smear-negative specimens particularly when the organism cannot be grown in culture and also to rapidly differentiate culture-grown *M. tuberculosis* from NTM.[6,57] Two molecular tests are also approved by FDA for respiratory specimens.[57] In-house developed PCR assays vary in their choice of amplification target, detection platforms, sensitivity and reliability of the results.[6,57,58] Two reverse hybridization-based assays allow identification of *M. tuberculosis* and several NTM species, both in cultured isolates and clinical specimens.[59–61] However, molecular tests have not been able to replace microscopy and culture, but are rather used to confirm the presence of *M. tuberculosis* in positive sputum and non-sputum smears.

Diagnosis of MDR-TB

Rapid detection of drug resistance of *M. tuberculosis* and MDR-TB strains ensures effective treatment of TB patients and limits further development of resistance to additional drugs. The CDC strongly encourages reporting of DST results within 30 days of specimen collection.[7] Recent developments have considerably improved the diagnosis of drug-resistant TB and MDR-TB by both, conventional (phenotypic) and molecular (genotypic) methods.

Conventional (phenotypic) methods

Conventional (phenotypic) DST methods require culture and detect the growth of *M. tuberculosis* in the presence of anti-TB drugs by one of three (proportion, resistance ratio or absolute concentration) methods on solid media.[6] The broth-based semiautomated, radiometric BACTEC 460 TB system (Becton Dickinson) is regarded as the gold standard for culture and DST of *M. tuberculosis* to both first- and second-line anti-TB drugs.[62,63] The 460 TB system reports DST results within 4–12 days from primary cultures, while solid media-based methods require nearly 3 weeks. More recently nonradioactive, fully automated liquid culture systems have been developed for culture and DST of *M. tuberculosis*.[63–65] Of these, BACTEC MGIT 960 TB system has been more extensively used and is considered equivalent to BACTEC 460 TB system in recovery and DST of *M. tuberculosis*.[63–65] Susceptibility cut-off values for both, first-line and second-line anti-TB drugs have been determined.[6,62–65] Although MGIT 960 system may be used directly on sputum samples, additional time and labor is needed for identification of *M. tuberculosis*. The automated liquid culture systems are expensive for developing

countries. Other cost-effective methods have been developed for resource-poor settings.

The liquid media-based microscopic observation drug susceptibility (MODS) test is a low-cost, rapid and direct assay for simultaneous detection and DST of *M. tuberculosis* in sputum specimens. The test requires skills similar to those for smear microscopy, detects *M. tuberculosis*-specific cording growth and results are available within 2 weeks in most cases.[66–68] The limitation of MODS test are indirect identification of *M. tuberculosis* and requirement for frequent (if not daily) microscopic observations. The colorimetric methods are another alternative for detecting drug-resistant *M. tuberculosis* strains.[69] They are based on reduction of redox indicators that are added to culture medium during *in vitro* growth of *M. tuberculosis*. The tetrazolium salt-based assay utilizes 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) which is yellow in color in its oxidized form but, is reduced to blue/purple colored compound during growth of *M. tuberculosis*. This method has been evaluated for direct detection of RIF resistance on smear-positive sputum samples with excellent results.[70] The resazurin microtitre assay is based on oxidation of resazurin by a growing culture of *M. tuberculosis* in the presence of anti-TB drugs.[71] The nitrate reductase assay depends on ability of viable *M. tuberculosis* to reduce nitrate to nitrite and the results are usually available within 10 days.[72] This simple assay provides rapid, accurate and cost-effective detection of MDR-TB and has performed well with smear-positive sputum samples.[69,72] Unlike MODS, a limitation of nitrate reductase assay is the possibility of false-positive detection of *M. tuberculosis* due to growth of other organisms.

The phage-based assays use mycobacteriophages to infect live *M. tuberculosis* in the absence and presence of anti-TB drugs and detect the bacilli using either the phage amplification assay or production of light.[56] The method based on detection of light uses luciferase reporter mycobacteriophages (e.g. phAE142). Viable *M. tuberculosis* growing in presence of anti-TB drug and infected with phage phAE142 emit light in the presence of luciferin and are identified as drug-resistant strains.[73] The FAST Plaque TB-Response (Biotec Laboratories Ltd.) assay detects drug resistance of *M. tuberculosis* directly in sputum specimens. The method provides rapid (within 2 days) and accurate results when compared with the proportion method and the BACTEC radiometric method.[74]

Molecular (genotypic) methods

Molecular (genotypic) DST methods detect resistance associated mutations in target genes of *M. tuberculosis*, provide results within 1–2 days and can be performed directly on smear-positive sputum and other clinical samples.[57,75,76] Although genotypic methods have been developed for all first-line and many second-line drugs, detection of RIF resistance is more practical and a priority, since 90–95% RIF-resistant strains contain mutations in a small (81-bp) region (HSR) of a single (*rpoB*) gene and RIF resistance is a surrogate marker for MDR-TB.[20,75–77] For other anti-TB drugs, the sensitivity of resistance detection varies more widely due to the number of gene loci involved and the sheer diversity of mutations.[23] Still, drug-resistant *M. tuberculosis* strains isolated from TB patients of different ethnic background or from different geographical locations may exhibit few dominant mutations.[26] Hence, prior knowledge about the nature and frequency of resistance conferring mutations is critical to develop region-specific molecular diagnostic methods. Alternatively, assays targeting entire gene or multiple gene loci in a single test may be utilized.[40,78–80] Several methods have been developed and evaluated for the detection of resistance conferring mutations in MDR-TB strains.

The PCR-restriction fragment length polymorphism (PCR-RFLP) analysis is a simple, rapid and inexpensive method to detect polymorphism at a single or few codons that are mutated in drug-resistant strains.[81–83] The PCR-RFLP has been mostly used for the detection of *katG315* and *embB306* mutations for rapid detection of INH and EMB resistance, respectively.[45,83]

The DNA sequencing provides unambiguous detection of mutations. Results of resistance detection by other methods have traditionally been confirmed by DNA sequencing of target gene region/codon. Direct DNA sequencing of gene target is most practical if majority of drug-resistant strains contain mutations in a limited region of a single target gene. This is particularly true for RIF (>90% RIF-resistant strains contain mutations in HSR of *rpoB* gene) and PZA (due to small size of *pncA* gene).[20,39] Also, most INH-resistant strains from some geographical locations contain mutations at *katG315*.[26,83] Thus, targeted DNA sequencing has been applied for the detection of mutations conferring resistance to RIF, INH and PZA. The DNA sequencing is still considered as impractical in most developing countries for analyzing large number of specimens. However, recent technological advances may lead to rapid, accurate and cost-effective analysis of DNA sequences for detection of MDR *M. tuberculosis* strains.[84]

Real-time PCR assays have also been developed for rapid diagnosis of MDR-TB in smear-positive sputum specimens by detecting mutations in *rpoB*, *katG* and regulatory region of *inhA* gene.[85,86] A commercial real-time PCR-based assay has now become available for RIF resistance detection in both smear-positive and smear-negative sputum samples (Xpert MTB, Cepheid). Since RIF resistance is a surrogate marker for MDR-TB, the positive test will also detect ~90% of MDR-TB strains from most geographical locations.

The reverse hybridization-based line probe assays (macroarrays) and microarray-based assays have also been developed for detection of MDR-TB strains directly in clinical specimens. There are two commercially available DNA macroarray assays for detection of MDR *M. tuberculosis*. The INNO-LiPA Rif. TB assay (Innogenetics) detects mutations in HSR of *rpoB* gene.[75,87] Since RIF resistance is a surrogate marker for MDR-TB, the positive result also predicts MDR status of ~90% *M. tuberculosis* strains. The GenoType MTBDR*plus* (Hain Lifesciences) assay detects MDR-TB by simultaneously detecting mutations in HSR of *rpoB* gene for RIF resistance and mutations at *katG315* and *inhA* regulatory region for INH resistance.[76,88,89] Although expensive, these assays have performed satisfactorily in high incidence countries for detection of MDR *M. tuberculosis* in clinical samples within one working day.[87,89] Recently, line probe assays have also been developed for detecting resistance of *M. tuberculosis* to PZA and FQs.[40,90]

Other hybridization-based assays involve microarrays and have been tested for detecting resistance to RIF, INH, and EMB by using several probes simultaneously.[80,91] Some microarray-based assays are now commercially available (Autogenomics).

Treatment and management of TB and MDR-TB

The drugs currently being used for the treatment of TB and MDR-TB are listed in Table 3. First-line drugs (INH, RIF, PZA and EMB) are mainly bactericidal, highly efficacious and relatively less toxic. Second-line drugs are mainly bacteriostatic, less effective, costly and have more drug-associated adverse effects.[7] These include aminoglycosides (kanamycin, KAN; amikacin, AMI and streptomycin, SM), the polypeptides (capreomycin, CAP and viomycin, VIO), the thioamides (ethionamide, ETH and prothionamide, PTH), several fluoroquinolones (FQs) (such as ofloxacin, OFX; levofloxacin, LFX; moxifloxacin, MFX; and gatifloxacin, GFX), *para*-amino salicylic acid (PAS) and D-cycloserine (CS).[7,9] The SM is now regarded as a second-line drug due to higher rates of resistance of *M. tuberculosis* isolates to SM and the availability of more effective anti-TB drugs.[7] Rifabutin (RBU) may be used as a second-line drug for some RIF-resistant *M. tuberculosis* strains. The new-generation FQs (MFX and GFX) are bactericidal and are being considered as first-line agents.[50,92] Some other drugs that are not *M. tuberculosis*-specific but exhibit significant *in vitro* and/or *in vivo* activity are also being used for the treatment of MDR-TB and XDR-TB. These third-line or reinforcing agents (Table 3) include clarithromycin (CLR), clofazimine (CFZ), amoxicillin-clavulanic acid (AMX-CLV) and linezolid (LZD).[17,93,94]

The first-line drugs, INH and RIF are the back bone of treatment for TB. If resistance develops to either of the two drugs, the patients can still be successfully managed with the available drugs, although treatment duration is increased, particularly for infections caused by RIF-resistant strains.[95] Among clinical *M. tuberculosis* isolates, monoresistance to INH is common while monoresistance to RIF occurs infrequently except in HIV-coinfected TB patients and is regarded as a surrogate marker for MDR-TB.[9,20] Although infections caused by INH monoresistant strains can be effectively managed with available first-line and other drugs, both treatment duration and treatment failure are slightly higher compared to TB caused by fully susceptible strains.[7,95] However, infections caused by RIF-resistant *M. tuberculosis* strains have poor prognosis compared to drug-susceptible TB mainly because RIF is most-effective drug against persisting bacilli that are mainly responsible for clinical relapse. Increasing treatment duration to 12 months with anti-TB drugs results in better prognosis.[7,95]

If *M. tuberculosis* strain has become resistant to both, INH and RIF (MDR-TB), treatment is much more difficult, particularly in HIV-TB coinfected patients. The treatment for MDR-TB requires more extensive usage of second-line drugs and lasts for nearly two years. The treatment for MDR-TB is ~100 times more costly, many of the second-line drugs are highly toxic and more likely to cause adverse drug reactions, undesirable interactions occur with antiretroviral drugs in HIV-coinfected TB patients and much higher relapse and mortality rates are observed compared to drug-susceptible TB.[7,95–97] Although initial outbreaks of MDR-TB resulted in very high mortality rates, subsequent advances in diagnosis and optimization of therapy regimens in industrialized countries greatly

Table 3 Some important anti-tubercular drugs and their mode of action.

Drug(s)	Chemical description	Route	Class	Mode of action	Biological process inhibited
INH	Nicotinic acid hydrazide	Oral	First-line	Bactericidal	Mycolic acid synthesis
RIF	Rifamycin derivative	Oral	First-line	Bactericidal	Protein synthesis
PZA	Nicotinamide derivative	Oral	First-line	Bactericidal	Unknown
EMB	Ethylene diimino di-1-butanol	Oral	First-line	Bacteriostatic	Lipid/cell wall synthesis
RBU	Rifamycin derivative	Oral	Second-line	Bactericidal	Protein synthesis
SM	Aminoglycoside	Injectable	Second-line	Bactericidal	Protein synthesis
KAN/AMI	Aminoglycoside	Injectable	Second-line	Bactericidal	Protein synthesis
CAP/VIO	Cyclic peptide	Injectable	Second-line	Bactericidal	Protein synthesis
CIP/OFX	Fluoroquinolone	Oral	Second-line	Bacteriostatic	DNA replication
LFX	Fluoroquinolone	Oral	Second-line	Likely bactericidal	DNA replication
MFX/GFX	Newer fluoroquinolone	Oral	Second-line	Bactericidal	DNA replication
ETH/PTH	Isonicotinic acid derivative	Oral	Second-line	Bacteriostatic	Mycolic acid synthesis
PAS	*Para*-amino salicylic acid	Oral	Second-line	Bacteriostatic	Unknown
CS	D-Cycloserine	Oral	Second-line	Bacteriostatic	Cell wall synthesis
TAC	Thiacetazone	Oral	Second-line	Bacteriostatic	Mycolic acid synthesis
CLR	Erythromycin derivative	Oral	Third-line	Bactericidal	Protein synthesis
AMX-CLA	β-lactam with β-lactamase inhibitor	Oral	Third-line	Bactericidal	Cell wall synthesis
CFZ	Iminopherazine derivative	Oral	Third-line	Bacteriostatic	Cell membrane function
LZD	Oxazolidinone derivative	Oral	Third-line	Bactericidal	Protein synthesis

INH, isoniazid; RIF, rifampin; PZA, pyrazinamide; EMB, ethambutol; RBU, rifabutin; SM, streptomycin; KAN, kanamycin; AMI, amikacin; CAP, capreomycin; VIO, viomycin; CIP, ciprofloxacin; OFX, ofloxacin; LFX, levofloxacin, MFX, moxifloxacin; GFX, gatifloxacin; PAS, *para*-amino salicylic acid; CS, D-cycloserine; TAC, thiacetazone; CLR, clarithromycin; AMX-CLA, amoxicillin β-lactam antibiotic with clavulanate β-lacatamase inhibitor; CFZ, clofazimine; LZD, linezolid.

improved clinical outcome, even in HIV-positive TB patients receiving antiretroviral drugs.[98–100] Recent developments in rapid diagnosis of MDR-TB, prompt institution of aggressive therapy with several active drugs, close radiological and bacteriological (for sputum smear and culture) monitoring of patients have resulted in further improvement in clinical outcome and lower mortality rates in both HIV-negative and HIV-coinfected TB patients.[101–103] The suggested therapy regimens and average duration of treatment for infections with MDR *M. tuberculosis* strains resistant to different combinations of anti-TB drugs are listed in Table 4.

Current guidelines for successful management of patients require treating centers to be equipped with rapid diagnostic procedures for MDR-TB detection and universal DST for first- and second-line drugs.[66,104–106] Due to poor prognosis, rapid diagnosis of MDR-TB is even more critical in HIV-coinfected individuals, however, conventional diagnostic methods lack sensitivity due to lower sputum-smear positivity and atypical presentation.[3,104,106] Molecular methods, if available, should be used for rapid detection and DST to guide therapy. The DST profile is crucial for tailoring individualized treatment regimens (ITRs) and for monitoring response to treatment during therapy for MDR-TB. Studies have shown that ITRs guided by DST results have better clinical outcome even in developing countries.[105,107,108] In developing countries that lack universal culture and DST facilities, epidemiological information, prior treatment history and poor response to current TB treatment may help in the diagnosis of MDR-TB. In some resource-poor countries with limited infrastructure, culture and DST are being performed by a regional/national laboratory.[109] It has also been observed that a proactive approach to identify MDR-TB cases optimizes therapy with more aggressive initial drug regimens. Early initiation of therapy regimens containing several (4–6 or even more) drugs, not previously taken and thus more likely to be effective, has a more favorable outcome for MDR-TB while delaying therapy results in higher mortality rates.[7,97,110] Therapy regimens that include a new-generation FQ (MFX or GFX), an injectable agent and bactericidal second-line drugs with manageable side effects are associated with better clinical outcome. Studies have also shown that therapy with insufficient number of second-line drugs while awaiting DST results may actually result in acquisition of resistance to additional drugs.[101,111]

In addition to aggressive therapy with 5–7 drugs in initial phase, other factors also influence rapid sputum conversion and improve treatment outcome. These include therapy for >3 months with 6 or more active drugs, use of an effective injectable agent for >6 months, oral treatment for >18 months and initial resistance to fewer drugs.[97,110,112] Regular (once a month) monitoring of sputum specimens for smear microscopy, culture and DST indicates response to treatment and should be carried out until sputum culture conversion has been achieved. Early sputum culture conversion (within 2 months of initiating therapy) is associated with better clinical outcome while patients who remain sputum culture-positive at three months are more likely to have poor prognosis.[110,112] Continued sputum smear and culture positivity without clinical improvement usually indicate ineffective therapy or poor adherence to treatment and warrant fresh DST and reassessment of treatment strategies.[101,110]

Directly observed therapy (DOT) support for MDR-TB patients ensures adherence to treatment regimens and rapid adjustments to avoid adverse drug reactions minimize the risk of default. This is best achieved in hospital settings, since inpatient management allows close monitoring for adverse drug reactions and also ensures patient's adherence to treatment.[113,114] However, recent studies have shown that successful treatment of MDR-TB patients can also be achieved in community settings even in developing countries.[115–117] Community-based treatment decreases the chances for nosocomial transmission of infection to other patients and health care workers.

The treatment of MDR-TB has been markedly less successful in developing countries due to lack of rapid diagnostic methods, universal culture and DST facilities and

Table 4 Potential regimens for the treatment of patients with MDR-TB and XDR-TB.

Different patterns of resistance to anti-TB drugs	Appropriate therapy regimens	Total duration of treatment, months	Desirable number of active drugs for favorable outcome
None	INH, RIF, PZA, EMB	6	4
INH	RIF, PZA, EMB and/or FQ	6–9	4
RIF	INH, PZA, EMB, SM ± FQ	9–12	4–5
INH, RIF	PZA, EMB, FQ, INJ ± SLD	18–24	5–6
INH, RIF, EMB	PZA, FQ, INJ + SLD	18–24	5–6
INH, RIF, PZA	EMB, FQ, INJ + SLD	24	5–7
INH, RIF, PZA, EMB	FQ, INJ + SLD	24	5–7
INH, RIF, PZA, EMB, FQ	INJ + SLD + TLD	>24	5–7
INH, RIF, PZA, EMB, INJ	FQ + INJ[a] + SLD + TLD	>24	5–7
INH, RIF, PZA, EMB, FQ, INJ	INJ[a] + SLD + TLD	>24	5–7

INH, isoniazid; RIF, rifampin; PZA, pyrazinamide; EMB, ethambutol; SM, streptomycin; FQ, ciprofloxacin or ofloxacin or levofloxacin or moxifloxacin or gatifloxacin; INJ, injectable agents like SM or kanamycin or amikacin or capreomycin or viomycin; SLD, second-line drugs like rifabutin, ethionamide, prothionamide, *para*-amino salicylic acid, D-cycloserine and thiacetazone; TLD, third-line drugs like CLR, clarithromycin; AMX-CLA, amoxicillin β-lactam antibiotic with clavulanate β-lacatamase inhibitor; CFZ, clofazimine; and LZD, linezolid.

[a] Capreomycin or viomycin may be used for kanamycin or amikacin or vise versa if no cross-resistance is observed.

availability of second-line drugs. Retreatment of patients under WHO's DOTS strategy in endemic countries for MDR-TB has been largely ineffective and has actually resulted in emergence of additional resistance.[95–97] The WHO and its international partners have now evolved DOTS-Plus strategy for better treatment of MDR-TB patients in developing countries.[118] The WHO's Green Light Committee (GLC) has subsidized drug prices but have also exerted strict control on availability of second-line drugs. However, only ~10% of MDR-TB cases in developing countries are currently being treated under the GLC program. Thus, greater distribution of quality-assured drugs, universal culture and DST availability for initial diagnosis and for monitoring response to therapy will be required to substantially decrease the overall burden of MDR-TB in resource-poor settings.

Successful treatment of XDR-TB is much more difficult than MDR-TB even in developed countries and involves therapy with all available first-, second- and third-line drugs, close monitoring of patients for adverse drug reactions and other interventions such as surgery.[93,94] In developing countries, XDR-TB is virtually an untreatable disease and all efforts should be made to minimize its emergence by successful treatment of the existing MDR-TB cases. Modeling studies have shown that emergence of XDR-TB can be avoided by proper management of MDR-TB.[17] However, treatment and management of MDR-TB can not be accomplished without local laboratory capacity for sputum culture, DST and close monitoring of patients for adherence to treatment regimens and for adverse drug reactions.

Promising new anti-TB drugs

Rifampin was the last TB-specific drug introduced in combination therapy regimens and was instrumental, due to its activity against non-replicating persistent bacteria, in reducing the duration of chemotherapy to six months which has remained unchanged for nearly forty years.[5,119] The incomplete eradication of non-replicating persistent bacilli is a major factor for relapse or treatment failure in TB patients not adhering to therapy.[119,120] To overcome this phenotypic drug resistance, strict monitoring of treatment under DOTS strategy with four first-line drugs for six months was instituted. However, this labor-intensive strategy for an extended period of time has been too difficult to implement on a nationwide scale for many countries resulting in development of drug-resistant and MDR *M. tuberculosis* strains.[1] The search for new anti-TB drugs aims to shorten the treatment duration of drug sensitive active TB to 2 months. Mathematical modeling studies have shown that nearly a fourth of new TB cases and TB-related deaths could be avoided with the introduction of a 2 month regimen in South-Southeast Asia alone.[121] The worldwide emergence of MDR-TB and XDR-TB has also accelerated the search for novel drugs against new targets that will be effective against MDR and XDR *M. tuberculosis* strains.

New anti-TB drugs, to be effective, must have potency greater than the most active drugs currently available against actively dividing and non-replicating persistent bacteria (exemplified by INH and RIF, respectively, in the current treatment regimens). The substitution of EMB by newer fluoroquinoloes (MXF and GFX) in the standard treatment regimens shows promise to shorten the duration of treatment from the current 6 months to 4 months.[122] Several new anti-TB drugs (Table 5) are at various stages of development for the treatment of drug sensitive active TB, MDR-TB and XDR-TB.

The PA-824, a nitroimidazo-oxazine pro-drug of nitroimidazole class is a promising new compound and is currently undergoing Phase II clinical trials for drug sensitive TB.[123,124] This drug requires bioreductive activation to exert its antitubercular effect. The PA-824 is equally active against drug sensitive as well as MDR *M. tuberculosis* strains and is also active against non-replicating persistent bacteria, making it an ideal drug candidate to shorten treatment duration.[123,124] The OPC-67683, a nitroimidazo-oxazone pro-drug of nitroimidazole class is another promising new compound and is currently undergoing Phase II clinical trials for the treatment of MDR-TB.[125] Although both PA-824 and OPC-67683 are activated by the same mechanism, their cellular targets are different. *In vitro* generated PA-824-resistant strains have identified a conserved hypothetical protein (Rv3547) of *M. tuberculosis* as the main target of PA-824. Although the exact function of the target protein is not known at present, the PA-824-resistant *M. tuberculosis* strains with mutations in Rv3547 remained sensitive to nitroimidazo-oxazines.[126]

The drug R207910 (also known as TMC207) is a diarylquinoline that inhibits adenosine triphosphate (ATP) synthase.[127] This novel compound has excellent activity against both, drug sensitive as well as MDR *M. tuberculosis* strains.[128] In animal models, R207910 has shown more potent early bactericidal activity than INH during early phase of infection and higher bactericidal activity late in infection than RIF alone.[127] The activity of R207910 against both, actively dividing and non-replicating persistent bacteria suggests that this anti-TB drug also has great potential to shorten the duration of treatment for drug sensitive as well as MDR-TB.[128] Phase II clinical trials are

Table 5 Some promising anti-tubercular drugs in various stages of development.

Potential drug	Active or Pro-drug	Description	Cellular process inhibited	Stage of development
PA-824	Pro-drug	Nitroimidazo-oxazine	Mycolic acid synthesis	Phase II
OPC-67683	Pro-drug	Nitroimidazo-oxazole	Mycolic acid synthesis	Phase II
R207910	Active	Diarylquinoline	ATP synthesis	Phase II
SQ109	Active	Ethylenediamine derivative	Lipid/cell wall synthesis	Phase I
Compound 5	Pro-drug	Quinoxaline-oxide derivative	Unidentified	Preclinical
Compound 7 g	Unknown	Quinoline-isoxazole derivative	Unidentified	Preclinical

currently being conducted to evaluate its safety and efficacy in combination therapy with standardized second-line treatment regimen for MDR-TB.

The 1,2-ethylenediamine (also known as SQ109) is structurally related to first-line drug, EMB.[129] However, its mechanism of action appears to be different than that of EMB as the drug has *in vitro* bactericidal activity against EMB-resistant *M. tuberculosis* strains. The drug also exhibits synergistic bactericidal action with INH and RIF and has now entered phase I clinical trials for dose-dependant efficacy studies.[129,130]

Several other new anti-TB drugs have also shown promising results for both, drug sensitive and drug-resistant *M. tuberculosis* strains. Notable among these are compound 5, a quinoxaline-oxide derivative and compound 7 g, a quinoline-isoxazole derivative (Table 5).[131,132] Both drugs have shown submicromolar and micromolar bactericidal activities against actively dividing and non-replicating persistent bacteria, respectively, under *in vitro* conditions. If these findings are also replicated under *in vivo* conditions, these drugs will add further to the promising leads for new anti-TB drug development. Hopefully, some of these drugs will be finally approved for the treatment and proper management of MDR-TB.

Conclusion

The current MDR-TB epidemic is the result of decades of neglect for an important infectious disease, lack of resources for national TB control programs, poor case detection and inadequate/inappropriate therapy in high-burden countries. Initial outbreaks of MDR-TB in developed countries were associated with very high mortality rates both in HIV-negative and HIV-coinfected patients. Optimization of treatment regimens together with rapid diagnosis and DST for first- and second-line drugs, greatly improved the clinical outcome. Recent advances in diagnosis of MDR-TB and aggressive empirical treatment of patients with several drugs in the initial phase of treatment have further improved the prognosis MDR-TB. However, in developing countries, the prognosis is still largely poor due to inadequate laboratory support that is critical for successful management of MDR-TB patients. Rapid and cost-effective methods for culture and DST of *M. tuberculosis* strains have recently been developed that are suitable for resource-poor countries. The new anti-TB drugs that are in various stages of development also offer hope that we will not soon run out of treatment options against TB and MDR-TB.

Acknowledgements

We thank Kuwait University for support through research grants MI116, MI06/02, MI02/04 and YM03/06.

Conflict of interest statement

The authors declare that we do not have any conflict of interest with any company/organization/people that could have influenced our opinion.

References

1. World Health Organization. *Global tuberculosis control: surveillance, planning and financing*. WHO report 2008. WHO/HTM/TB/2008.393. Geneva, Switzerland: WHO; 2008.
2. Frieden TR, Sterling TR, Munsiff SS, Watt CJ, Dye C. Tuberculosis. *Lancet* 2003;**362**:887—99.
3. Wells CD, Cegielski JP, Nelson LJ, et al. HIV infection and multidrug-resistant tuberculosis-the perfect storm. *J Infect Dis* 2007;**196**(Suppl. 1):S86—107.
4. Medical Research Council. Streptomycin treatment of pulmonary tuberculosis: a Medical Research Council Investigation. *Br Med J* 1948;**4582**:769—82.
5. Mitchison DA. The action of antituberculosis drugs in short-course chemotherapy. *Tubercle* 1985;**66**:219—25.
6. Mitchison DA. The diagnosis and therapy of tuberculosis during the past 100 years. *Am J Respir Crit Care Med* 2005; **171**:699—706.
7. American Thoracic SocietyCDCInfectious Disease Society of America. Treatment of tuberculosis. *MMWR Morb Mortal Wkly Rep* 2003;**52**:1—77.
8. Palaci M, Dietze R, Hadad DJ, et al. Cavitary disease and quantitative sputum bacillary load in cases of pulmonary tuberculosis. *J Clin Microbiol* 2007;**45**:4064—6.
9. World Health Organization. *Anti-tuberculosis drug resistance in the world: fourth Global report*. WHO/HTM/TB/2008.394. Geneva, Switzerland: WHO; 2008.
10. Drobniewski F, Balabanova Y, Ruddy M. Rifampin- and multi-drug-resistant tuberculosis in Russian civilians and prison inmates: dominance of the Beijing strain family. *Emerg Infect Dis* 2002;**8**:1320—6.
11. Kimerling ME, Phillips P, Patterson P, Hall M, Robinson CA, Dunlap NE. Low serum antimycobacterial drug levels in non-HIV-infected tuberculosis patients. *Chest* 1998;**113**: 1178—83.
12. Mokaddas E, Ahmad S, Abal AT, Al-Shami AS. Molecular fingerprinting reveals familial transmission of rifampin-resistant tuberculosis in Kuwait. *Ann Saudi Med* 2005;**25**:150—3.
13. Aziz MA, Wright A, Laszlo A, et al. for the WHO/International Union Against Tuberculosis and Lung Disease global project on anti-tuberculosis drug resistance surveillance. Epidemiology of antituberculosis drug resistance (the Global Project on Anti-tuberculosis Drug Resistance Surveillance): an updated analysis. *Lancet* 2006;**368**:2142—54.
14. Zignol M, Hosseini MS, Wright A, et al. Global incidence of multidrug-resistant tuberculosis. *J Infect Dis* 2006;**194**: 479—85.
15. Mitnick CD, Castro KG, Harrington M, Sacks LV, Burman W. Randomized trials to optimize treatment of multidrug-resistant tuberculosis. *PLoS Med* 2007;**4**:e292.
16. Centers for Disease Control and Prevention (CDC). Emergence of *Mycobacterium tuberculosis* with extensive resistance to second-line drugs-worldwide, 2000—2004. *MMWR Morb Mortal Wkly Rep* 2006;**55**:301—5.
17. Dye C. Doomsday postponed? Preventing and reversing epidemics of drug-resistant tuberculosis. *Nat Rev Microbiol* 2009;**7**:81—7.
18. Gandhi NR, Moll A, Sturn AW, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet* 2006;**368**:1554—6.
19. Zhang Y, Heym B, Allen B, Young D, Cole ST. The catalase—peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. *Nature* 1992;**358**:591—3.
20. Telenti A, Imboden P, Marchesi F, et al. Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*. *Lancet* 1993;**341**:647—50.

21. Vilcheze C, Wang F, Arai M, et al. Transfer of a point mutation in *Mycobacterium tuberculosis inhA* resolves the target of isoniazid. *Nat Med* 2006;**12**:1027–9.
22. Ramaswamy S, Musser JM. Molecular genetics basis of antimicrobial agent resistance in *Mycobacterium tuberculosis*: 1998 update. *Tuber Lung Dis* 1998;**79**:3–29.
23. Johnson R, Streicher E, Louw G, Warren RM, van Helden PD, Victor TC. Drug resistance in Mycobacterium tuberculosis. *Curr Issues Mol Biol* 2006;**8**:97–111.
24. Ramaswamy S, Reich R, Dou S, et al. Single nucleotide polymorphisms in genes associated with isoniazid resistance in Mycobacterium tuberculosis. *Antimicrob Agents Chemother* 2003;**47**:1241–50.
25. Abal AT, Ahmad S, Mokaddas E. Variations in the occurrence of the S315T mutation within the *katG* gene in isoniazid-resistant clinical *Mycobacterium tuberculosis* isolates from Kuwait. *Microb Drug Resist* 2002;**8**:99–105.
26. Mokrousov I, Narvskaya O, Otten T, Limeschencko E, Steklova L, Vyshnevskiy B. High prevalence of Ser315Thr substitution among isoniazid-resistant *Mycobacterium tuberculosis* clinical isolates of from Northwestern Russia, 1996–2001. *Antimicrob Agents Chemother* 2002;**46**:1417–24.
27. Silva MSN, Senna SG, Ribeiro MO, et al. Mutations in *katG*, *inhA*, and *ahpC* genes of Brazilian isoniazid-resistant isolates of *Mycobacterium tuberculosis*. *J Clin Microbiol* 2003;**41**: 4471–4.
28. Hazbon MH, Brimacombe M, del Valle MB, et al. Population genetics study of isoniazid resistance mutations and evolution of multidrug-resistant *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2006;**50**:2640–9.
29. Mokaddas E, Ahmad S, Samir I. Secular trends in susceptibility patterns of *Mycobacterium tuberculosis* isolates in Kuwait, 1996–2005. *Int J Tuberc Lung Dis* 2008;**12**:319–25.
30. Van Rie A, Warren R, Mshanga I, et al. Analysis for a limited number of gene codons can predict drug resistance of *Mycobacterium tuberculosis* in a high-incidence community. *J Clin Microbiol* 2001;**39**:636–41.
31. Siddiqi N, Shamim M, Hussain S, et al. Molecular characterization of multidrug-resistant isolates of *Mycobacterium tuberculosis* from patients in North India. *Antimicrob Agents Chemother* 2002;**46**:443–50.
32. Afanas'ev MV, Ikryannikova LN, Il'ina EN, et al. Molecular characteristics of rifampicin- and isoniazid resistant *Mycobacterium tuberculosis* isolates from Russian Federation. *J Antimicrob Chemother* 2007;**59**:1057–64.
33. Ahmad S, Mokaddas E, Fares E. Characterization of *rpoB* mutations in rifampin-resistant clinical *Mycobacterium tuberculosis* isolates from Kuwait and Dubai. *Diagn Microbiol Infect Dis* 2002;**44**:245–52.
34. Sheng J, Li J, Sheng G, et al. Characterization of *rpoB* mutations associated with rifampin resistance in *Mycobacterium tuberculosis* from eastern China. *J Appl Microbiol* 2008; **105**:904–11.
35. Heep M, Brandstatter B, Rieger U, et al. Frequency of *rpoB* mutations inside and outside the cluster I region in rifampin-resistant clinical *Mycobacterium tuberculosis* isolates. *J Clin Microbiol* 2001;**39**:107–10.
36. Ahmad S, Mokaddas E. The occurrence of rare *rpoB* mutations in rifampicin-resistant *Mycobacterium tuberculosis* isolates from Kuwait. *Int J Antimicrob Agents* 2005;**26**:205–12.
37. Yang B, Koga H, Ohno H, et al. Relationship between antimycobacterial activities of rifampicin, rifabutin and KRM-1648 and *rpoB* mutations of *Mycobacterium tuberculosis*. *J Antimicrob Chemother* 1998;**42**:621–8.
38. Cavusoglu C, Karaca-Derici Y, Bilgic A. *In-vitro* activity of rifabutin against rifampicin-esistant *Mycobacterium tuberculosis* isolates with known *rpoB* mutations. *Clin Microbiol Infect* 2004;**10**:662–5.
39. Scorpio A, Zhang Y. Mutations in *pncA*, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. *Nat Med* 1996;**2**:662–7.
40. Sekiguchi JI, Nakamura T, Miyoshi-Akiyama T, et al. Development and evaluation of a line probe assay for rapid identification of *pncA* mutations in pyrazinamide-resistant *Mycobacterium tuberculosis* strains. *J Clin Microbiol* 2007; **45**:2802–7.
41. Ramaswamy S, Amin AG, Koksel S, et al. Molecular genetic analysis of nucleotide polymorphisms associated with ethambutol resistance in human isolates of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2000; **44**:326–36.
42. Telenti A, Phillipp W, Sreevatsan S, et al. The *emb* operon, a unique gene cluster of *Mycobacterium tuberculosis* involved in resistance to ethambutol. *Nat Med* 1997;**3**:567–70.
43. Safi H, Sayers B, Hazbon MH, Alland D. Transfer of *embB* codon 306 mutations into clinical *Mycobacterium tuberculosis* strains alters susceptibility to ethambutol, isoniazid and rifampin. *Antimicrob Agents Chemother* 2008;**52**:2027–34.
44. Starks AM, Gumusboga A, Plikaytis BB, Shinnick TM, Posey JE. Mutations at *embB* codon 306 are an important molecular indicator of ethambutol resistance in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2009;**53**:1061–6.
45. Ahmad S, Jaber AA, Mokaddas E. Frequency of *embB* codon 306 mutations in ethambutol-susceptible and -resistant clinical *Mycobacterium tuberculosis* isolates in Kuwait. *Tuberculosis* 2007;**87**:123–9.
46. Sreevatsan S, Pan X, Stockbauer KE, Williams DL, Kreisworth BN, Musser JM. Characterization of *rpsL* and *rrs* mutations in streptomycin-resistant *Mycobacterium tuberculosis* isolates from diverse geographic locations. *Antimicrob Agents Chemother* 1996;**40**:1024–6.
47. Cooksey RC, Morlock GP, McQueen A, Glickman SE, Crawford JT. Characterization of streptomycin resistance mechanisms among *Mycobacterium tuberculosis* isolates from patients in New York City. *Antimicrob Agents Chemother* 1996;**40**:1186–8.
48. Maus CE, Pilkaytis BB, Shinnick TM. Molecular analysis of cross-resistance to capreomycin, kanamycin, amikacin and viomycin in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2005;**49**:3192–7.
49. Maus CE, Pilkaytis BB, Shinnick TM. Mutation of *tlyA* confers capreomycin resistance in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2005;**49**:571–7.
50. Rustomjee R, Lienhardt C, Kanyok T, et al. A phase II study of the sterilizing activities of ofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. *Int J Tuberc Lung Dis* 2008;**12**:128–38.
51. Ginsburg AS, Grosset JH, Bishai WR. Fluoroquinolones, tuberculosis, and resistance. *Lancet Infect Dis* 2003;**3**: 432–42.
52. Pitaksajjakul P, Wongwit W, Punprasit W, Eampokalap B, Peacock S, Ramasoota P. Mutations in the *gyrA* and *gyrB* genes of fluoroquinolone-resistant *Mycobacterium tuberculosis* from TB patients in Thailand. *Southeast Asian J Trop Med Public Health* 2005;**36**:228–37.
53. Wang JY, Lee LN, Lai HC, et al. Fluoroquinolones resistance in *Mycobacterium tuberculosis* isolates: associated genetic mutations and relationship to antimicrobial exposure. *J Antimicrob Chemother* 2007;**59**:860–5.
54. Morlock GP, Metchock B, Sikes D, Crawford JT, Cooksey RC. *ethA*, *inhA*, and *katG* loci of ethionamide-resistant clinical *Mycobacterium tuberculosis* isolates. *Antimicrob Agents Chemother* 2003;**47**:3799–805.
55. Brodie D, Schluger NW. The diagnosis of tuberculosis. *Clin Chest Med* 2005;**26**:247–71.

56. Palomino JC. Nonconventional and new methods in the diagnosis of tuberculosis: feasibility and applicability in the field. *Eur Respir J* 2005;**26**:339—50.
57. Cheng VCC, Yew WW, Yuen KY. Molecular diagnostics in tuberculosis. *Eur J Clin Microbiol Infect Dis* 2005;**24**:711—20.
58. Mokaddas E, Ahmad S. Development and evaluation of a multiplex PCR for rapid detection and differentiation of *Mycobacterium tuberculosis* complex members from non-tuberculous mycobacteria. *Jap J Infect Dis* 2007;**60**:140—4.
59. Tortoli E, Mariottini A, Mazarelli G. Evaluation of INNO-LiPA MYCOBACTERIA v2: improved reverse hybridization multiple DNA probe assay for mycobacterial identification. *J Clin Microbiol* 2003;**41**:4418—20.
60. Russo C, Tortoli E, Manichella D. Evaluation of the new Genotype *Mycobacterium* assay for identification of mycobacterial species. *J Clin Microbiol* 2006;**44**:334—9.
61. Mokaddas E, Ahmad S. Species spectrum of nontuberculous mycobacteria causing infections in Kuwait. *Curr Microbiol* 2008;**56**:413—7.
62. Tortoli E, Benedetti M, Fontanelli A, Simonetti T. Evaluation of automated BACTEC MGIT 960 system for testing susceptibility of *Mycobacterium tuberculosis* to four major antituberculous drugs: comparison with the radiometric BACTEC 460TB method and the agar plate method of proportion. *J Clin Microbiol* 2002;**40**:607—10.
63. Scarparo C, Ricordi P, Ruggiaro G, Piccoli P. Evaluation of the fully automated BACTEC MGIT 960 system for testing susceptibility of *Mycobacterium tuberculosis* to pyrazinamide, streptomycin, isoniazid, rifampin, and ethambutol and comparison with the radiometric BACTEC 460TB method. *J Clin Microbiol* 2004;**42**:1109—14.
64. Piersimoni C, Olivieri A, Benacchio L, Scarparo C. Current perspective on drug susceptibility testing of *Mycobacterium tuberculosis* complex: the automated nonradiometric systems. *J Clin Microbiol* 2006;**44**:20—8.
65. Rusch-Gerdes S, Pfyffer GE, Casal M, Chadwick M, Siddiqi S. Multicenter laboratory validation of the BACTEC MGIT 960 technique for testing susceptibilities of *Mycobacterium tuberculosis* to classical second-line and newer antimicrobials. *J Clin Microbiol* 2006;**44**:688—92.
66. Moore DAJ, Evans CAW, Gilman RH, et al. Microscopic-observation drug-susceptibility assay for the diagnosis of TB. *N Engl J Med* 2006;**355**:1539—50.
67. Shiferaw G, Woldeamanuel Y, Gebeyehu M, Girmachew F, Demessie D, Lemma E. Evaluation of microscopic observation drug susceptibility assay for the detection of multidrug-resistant *Mycobacterium tuberculosis*. *J Clin Microbiol* 2007; **45**:1093—7.
68. Ejigu GS, Woldeamanuel Y, Shah NS, Gebeyehu M, Silassie A, Lemma E. Microscopic-observation drug susceptibility assay provides rapid and reliable identification of MDR-TB. *Int J Tuberc Lung Dis* 2008;**12**:332—7.
69. Palomino JC, Martin A, Portaels F. Rapid drug resistance detection in *Mycobacterium tuberculosis*. a review of colourimetric methods. *Clin Microbiol Infect* 2007;**13**:754—62.
70. Abate G, Aseffa A, Selassie A, et al. Direct colorimetric assay for rapid detection of rifampin-resistant *Mycobacterium tuberculosis*. *J Clin Microbiol* 2004;**42**:871—3.
71. Martin A, Portaels F, Palomino JC. Colorimetric redox-indicator methods for the rapid detection of multidrug resistance in *Mycobacterium tuberculosis*: a systematic review and meta-analysis. *J Antimicrob Chemother* 2007; **59**:175—83.
72. Affolabi D, Odoun M, Martin A, Palomino JC, Anagonou S, Portaels F. Evaluation of direct detection of *Mycobacterium tuberculosis* rifampin resistance by a nitrate reductase assay applied to sputum samples in Cotonou, Benin. *J Clin Microbiol* 2007;**45**:2123—5.
73. Hazbon MH, Guarin N, Ferro BE, et al. Photographic and luminometric detection of luciferase reporter phages for susceptibility testing of *Mycobacterium tuberculosis* isolates. *J Clin Microbiol* 2003;**41**:4865—9.
74. Banaiee N, January V, Barthus C, et al. Evaluation of semi-automated reporter phage assay for susceptibility testing of *Mycobacterium tuberculosis* isolates in South Africa. *Tuberculosis* 2008;**88**:64—8.
75. Traore H, van Deun A, Shamputa IC, Rigouts L, Portaels F. Direct detection of *Mycobacterium tuberculosis* complex DNA and rifampin resistance in clinical specimens from tuberculosis patients by line probe assay. *J Clin Microbiol* 2006;**44**: 4384—8.
76. Hillemann D, Gerdes RS, Richter E. Evaluation of Genotype MTBDR *plus* assay for rifampin and isoniazid susceptibility testing of *Mycobacterium tuberculosis* strains and clinical specimens. *J Clin Microbiol* 2007;**45**:2635—40.
77. Feasibility of the GenoType MTBDR *sl* assay for fluoroquinolone, amikacin-capreomycin and ethambutol resistance testing of *Mycobacterium tuberculosis* strains and clinical specimens. *J Clin Microbiol* 2009;**47**:1767—72.
78. Mokrousov I, Bhanu NV, Suffys PN, Kadival GV, Yap SF, Cho SN, et al. Multicenter evaluation of reverse line blot assay for detection of drug resistance in *Mycobacterium tuberculosis* clinical isolates. *J Microbiol Methods* 2004;**57**:323—35.
79. Sougakoff W, Rodrigues M, Truffot-Pernot C, et al. Use of a high-density DNA probe array for detecting mutations involved in rifampicin resistance in *Mycobacterium tuberculosis*. *Clin Microbiol Infect* 2004;**10**:289—94.
80. Bergval IL, Vijzelaar RNCP, Dalla Costa ER, et al. Development of multiplex assay for rapid characterization of *Mycobacterium tuberculosis*. *J Clin Microbiol* 2008;**46**:689—99.
81. Cockerill III FR, Uhl JR, Temesgen Z, et al. Rapid identification of a point mutation of the *Mycobacterium tuberculosis* catalase—peroxidase (*katG*) gene associated with isoniazid resistance. *J Infect Dis* 1995;**171**:240—5.
82. Ahmad S, Mokaddas E, Jaber AA. Rapid detection of ethambutol-resistant *Mycobacterium tuberculosis* strains by PCR-RFLP targeting *embB* codons 306 and 497 and *iniA* codon 501 mutations. *Mol Cell Probes* 2004;**18**:299—306.
83. Ahmad S, Mokaddas E. Contribution of AGC to ACC and other mutations at codon 315 of the *katG* gene in isoniazid-resistant *Mycobacterium tuberculosis* isolates from the Middle East. *Int J Antimicrob Agents* 2004;**23**:473—9.
84. Marttila HJ, Makinen J, Marjamaki M, Soini H. Prospective evaluation of pyrosequencing for the rapid detection of isoniazid and rifampin resistance in clinical *Mycobacterium tuberculosis* isolates. *Eur J Clin Microbiol Infect Dis* 2009;**28**: 33—8.
85. Ruiz M Torres MJ, Llanos AC, Arroyo A, Palomares JC, Aznar J. Direct detection of rifampin- and isoniazid-resistant *Mycobacterium tuberculosis* in auramine-rhodamine-positive sputum specimens by real-time PCR. *J Clin Microbiol* 2004;**42**: 1585—9.
86. Espasa M, Gonzales-Martin J, Alcaide F, et al. Direct detection in clinical samples of multiple gene mutations causing resistance of *Mycobacterium tuberculosis* to isoniazid and rifampicin using fluorogenic probes. *J Antimicrob Chemother* 2005; **55**:860—5.
87. Viveiros M, Leandro C, Rodrigues L, et al. Direct application of the INNO-LiPA Rif. TB line-probe assay for rapid identification of *Mycobacterium tuberculosis* complex strains and detection of resistance in 360 smear-positive respiratory specimens from an area of high incidence of multidrug-resistant tuberculosis. *J Clin Microbiol* 2005;**43**:4880—4.
88. Vijdea R, Stegger M, Sosnovskaja A, Andersen AB, Thomsen VO, Bang D. Multidrug-resistant tuberculosis: rapid detection of resistance to rifampin and high or low levels of

isoniazid in clinical specimens and isolates. *Eur J Clin Microbiol Infect Dis* 2008;**27**:1079–86.
89. Evans J, Stead MC, Nicol MP, Segal H. Rapid genotypic assays to identify drug-resistant *Mycobacterium tuberculosis* in South Africa. *J Antimicrob Chemother* 2009;**63**:11–6.
90. Giannoni F, Iona E, Sementilli F, Brunori L, Pardini M, Migliori GB, et al. Evaluation of a new line probe assay for rapid identification of *gyrA* mutations in Mycobacterium tuberculosis. *J Clin Microbiol* 2005;**49**:2928–33.
91. Kim SY, Park YJ, Song E, et al. Evaluation of the CombiChip Mycobacteria Drug-Resistance detection DNA chip for identifying mutations associated with resistance to isoniazid and rifampin in Mycobacterium tuberculosis. *Diagn Microbiol Infect Dis* 2006;**54**:203–10.
92. Donald PR, Diacon AH. The early bactericidal activity of antituberculosis drugs: a literature review. *Tuberculosis* 2008;**88**: S75–83.
93. Fortun J, Martin-Davila P, Navas E, et al. Linezolid for the treatment of multidrug-resistant tuberculosis. *J Antimicrob Chemother* 2005;**56**:180–5.
94. Dietze R, Hadad DJ, McGee B, et al. Early and extended early bactericidal activity of linezolid in pulmonary tuberculosis. *Am J Respir Crit Care Med* 2008;**178**:1180–5.
95. Espinal MA, Kim SJ, Suarez PG, et al. Standard short-course chemotherapy for drug-resistant tuberculosis. *JAMA* 2000; **283**:2537–45.
96. Mak A, Thomas A, Sel Granado M, Zalekis R, Mouzafarova N, Menzies D. Influence of multidrug-resistance on tuberculosis treatment outcomes with standardized regimens. *Am J Respir Crit Care Med* 2008;**178**:306–12.
97. Lew W, Pai M, Oxdale O, Martin D, Menzies D. Initial drug resistance and tuberculosis treatment outcomes: systematic review and meta-analysis. *Ann Intern Med* 2008;**149**:123–34.
98. Fischl MA, Daikos GL, Uttamchandani RB, et al. Clinical presentation and outcome of patients with HIV infection and tuberculosis caused by multiple-drug-resistant bacilli. *Ann Intern Med* 1992;**117**:184–90.
99. Park MM, Davis AL, Schluger NW, Cohen H, Rom WN. Outcome of MDR-TB patients, 1983–1993: prolonged survival with appropriate therapy. *Am J Respir Crit Care Med* 1996;**153**: 317–24.
100. Turett GS, Telzak EE, Torian LV, et al. Improved outcomes for patients with multidrug-resistant tuberculosis. *Clin Infect Dis* 1995;**21**:1238–44.
101. Mukherjee JS, Rich ML, Socci AR, et al. Programmes and principles in treatment of multidrug-resistant tuberculosis. *Lancet* 2004;**363**:474–81.
102. Furin J. The clinical management of drug-resistant tuberculosis. *Curr Opin Pulm Med* 2007;**13**:212–7.
103. Mitnick CD, Appleton SC, Shin SS. Epidemiology and treatment of multidrug-resistant tuberculosis. *Semin Respir Crit Care Med* 2008;**29**:499–524.
104. Harries AD, Boxshall M, Phiri S, Kwanjana J. Providing HIV care for tuberculosis patients in sub-Saharan Africa. *Int J Tuberc Lung Dis* 2006;**10**:1306–11.
105. Rich ML, Socci AR, Mitnick CD, et al. Representative drug susceptibility patterns for guiding design of retreatment regimens for MDR-TB. *Int J Tuberc Lung Dis* 2006;**10**:290–6.
106. Perkins MD, Smith LM. Facing the crises: improving the diagnosis of tuberculosis in the HIV era. *J Infect Dis* 2007; **196**(Suppl. 1):S15–27.
107. Leimane V, Riekstina V, Holtz TH, et al. Clinical outcome of individualised treatment of multidrug-resistant tuberculosis in Latvia: a retrospective cohort study. *Lancet* 2005;**365**:318–26.
108. Saravia JC, Appleton SC, Rich ML, Sarria M, Bayona J, Becerra MC. Retreatment management strategies when first-line tuberculosis therapy fails. *Int J Tuberc Lung Dis* 2005;**9**: 421–9.
109. Shin SS, Yagui M, Ascencios L, et al. Scale-up of multidrug-resistant tuberculosis laboratory services, Peru. *Emerg Infect Dis* 2008;**15**:701–8.
110. Holtz TH, Sternberg M, Kammerer S, et al. Time to sputum culture conversion in multidrug-resistant tuberculosis: predictors and relationship to treatment outcome. *Ann Intern Med* 2006;**144**:650–9.
111. Han LL, Sloutsky A, Canales R, et al. Acquisition of drug resistance in multidrug-resistant *Mycobacterium tuberculosis* during directly observed empiric treatment with standardized regimens. *Int J Tuberc Lung Dis* 2005;**9**:818–21.
112. Dominguez-Castellano A, Muniain MA, Muniain MA, et al. Factors associated with time to sputum smear conversion in active pulmonary tuberculosis. *Int J Tuberc Lung Dis* 2003;**7**: 432–8.
113. Shin SS, Pasechnikov AD, Gelmanova IY, et al. Treatment outcomes in an integrated civilian and prison MDR-TB treatment program in Russia. *Int J Tuberc Lung Dis* 2006;**10**:402–8.
114. Riekstina V, Leimane V, Holtz TH, Leimans J, Wells CD. Treatment outcome cohort analysis in an integrated DOTS and DOTS-Plus TB program in Latvia. *Int J Tuberc Lung Dis* 2007; **11**:585–7.
115. Van Deun A, Salim MA, Das AP, Bastian I, Portaels F. Results of a standardised regimen for multidrug-resistant tuberculosis in Bangladesh. *Int J Tuberc Lung Dis* 2004;**8**:560–7.
116. Nathanson E, Lambregts-van Weezenbeek C, Rich ML, et al. Multidrug-resistant tuberculosis management in resource-limited settings. *Emerg Infect Dis* 2006;**12**:1389–97.
117. Tupasi TE, Gupta R, Quelapio MI, et al. Feasibility and cost-effectiveness of treating multidrug-resistant tuberculosis: a cohort study in the Philippines. *PLoS Med* 2006;**3**:e352.
118. World Health Organization. *Guidelines for the programmatic management of drug-resistant tuberculosis*. Geneva, Switzerland: WHO; 2006.
119. Mitchison DA. Antimicrobial therapy of tuberculosis: justification for currently recommended treatment regimens. *Semin Respir Crit Care Med* 2004;**25**:307–15.
120. Connolly LE, Edelstein PH, Ramakrishnan L. Why is long-term therapy required to cure tuberculosis. *PLoS Med* 2007;**4**: e120.
121. Salomon JA, Lloyd-Smith JO, Getz WM, et al. Prospects for advancing tuberculosis control through novel therapies. *PLoS Med* 2006;**3**:e273.
122. Ginsberg AM. Emerging drugs for active tuberculosis. *Semin Respir Crit Care Med* 2008;**29**:552–9.
123. Stover CK, Warrener P, VanDevanter DR, et al. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. *Nature* 2000;**405**:962–6.
124. Nuermberger E, Tyagi S, Tasneen T, et al. Powerful bactericidal and sterilizing activity of a regimen containing PA-824, moxifloxacin and pyrazinamide in a murine model of tuberculosis. *Antimicrob Agents Chemother* 2008;**52**:1522–4.
125. Matsumoto M, Hashizume H, Tomishige T, et al. OPC-67683, a nitro-dihydro-imidazooxazole derivative with promising action against tuberculosis in vitro and in mice. *PLoS Med* 2006;**3**:e466.
126. Manjunatha UH, Boshoff H, Dowd CS, et al. Identification of a nitroimidazo-oxazine-specific protein involved in PA-824 resistance in *Mycobacterium tuberculosis*. *Proc Natl Acad Sci USA* 2006;**103**:431–6.
127. Andries K, Verhasselt P, Guillemont J, et al. A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science* 2005;**307**:223–7.
128. Lounis N, Veziris N, Chauffour A, Truffot-Pernot C, Andries K, Jarlier V. Combinations of R207910 with drugs used to treat multidrug-resistant tuberculosis have the potential to shorten treatment duration. *Antimicrob Agents Chemother* 2006;**50**: 3543–7.

129. Protopopova M, Hanrahan C, Nikonenko B, et al. Identification of a new antitubercular drug candidate, SQ109, from a combinatorial library of 1,2-ethylenediamines. *J Antimicrob Chemother* 2005;**56**:968–74.
130. Nikonenko BV, Protopopova MN, Samala R, Einck L, Nacy CA. Drug therapy of experimental TB: improved outcome by combining SQ109, a new diamine antibiotic with existing TB drugs. *Antimicrob Agents Chemother* 2007;**51**:1563–5.
131. Villar R, Vicente E, Solano B, et al. *In vitro* and *in vivo* anti-mycobacterial acitivities of ketone and amide derivatives of quinoxaline 1,4-di-N-oxide. *J Antimicrob Chemother* 2008;**62**: 547–54.
132. Lilienkampf A, Mao J, Wan B, Wang Y, Franzblau SG, Kozikowski AP. Structure-activity relationships for a series of quinoline-based compounds against replicating and non-replicating. *J Med Chem* 2009;**52**:2109–18.