INT J TUBERC LUNG DIS 13(11):1320–1330

STATE OF THE ART SERIES
Drug-resistant tuberculosis, *Edited by* C-Y. Chiang
NUMBER 1 IN THE SERIES

Mechanisms of drug resistance in *Mycobacterium tuberculosis*

Y. Zhang,* W. W. Yew†

***Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA; †Tuberculosis and Chest Unit, Grantham Hospital, Hong Kong, China**

SUMMARY

The increasing emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis (TB) in the era of human immunodeficiency virus (HIV) infection presents a major threat to effective control of TB. Drug resistance in *Mycobacterium tuberculosis* arises from spontaneous chromosomal mutations at low frequency. Clinical drug-resistant TB largely occurs as a result of man-made selection during disease treatment of these genetic alterations through erratic drug supply, suboptimal physician prescription and poor patient adherence. Molecular mechanisms of drug resistance have been elucidated for the major first- and second-line drugs rifampicin, isoniazid, pyrazinamide, ethambutol, the aminoglycosides and the fluoroquinolones. The relationship between drug resistance in *M. tuberculosis* strains and their virulence/transmissibility needs to be further investigated. Understanding the mechanisms of drug resistance in *M. tuberculosis* would enable the development of rapid molecular diagnostic tools and furnish possible insights into new drug development for the treatment of TB.

KEY WORDS: tuberculosis; drug resistance; mechanisms

THE CURRENTLY PREVAILING SCENARIOS of drug-resistant tuberculosis (TB)[1] are particularly alarming, and pose a significant threat to the control of the disease globally. There is also much concern that the TB situation will become worse with the growing human immunodeficiency virus (HIV) pandemic worldwide, as the viral infection can weaken the host immune system and predispose to endogenous reactivation and exogenous re-infection with TB. Drug-resistant TB and HIV infection, a lethal combination, presents a serious challenge for effective TB control.

In the most recent report on the Anti-tuberculosis Drug Resistance Surveillance Global Project published by the World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease (The Union),[2] the proportion of multidrug resistance (MDR), denoting resistance to at least rifampicin (RMP) and isoniazid (INH), in new cases ranged from 0% to 22.3%. The highest proportion of MDR-TB reported was 60% among previously treated cases. It has been estimated that 489 139 cases of MDR-TB emerged in 2006, and the global proportion of such resistance among all cases amounted to 4.8%. The proportion of extensively drug-resistant TB (XDR-TB), defined as MDR-TB with additional bacillary resistance to fluoroquinolones (FQs) and at least one second-line injectable drug, among MDR-TB cases in different settings, ranged from 0% to 30% globally. Around 40 000 cases of XDR-TB are estimated to emerge globally each year.[2]

Understanding the mechanisms of mycobacterial resistance to the anti-tuberculosis drugs not only enables the development of more rapid molecular diagnostic tests and furnishes implications for designing new anti-tuberculosis drugs,[3] it also helps to implement measures to prevent the development of such resistance. In this article, we will provide an update on drug resistance mechanisms in *Mycobacterium tuberculosis,* and highlight issues pertaining to the control of drug-resistant TB, as well as areas that need further study. Rapid diagnosis of MDR-TB has been reviewed recently[4,5] and will not be covered here.

BASIC CONCEPTS IN THE DEVELOPMENT OF DRUG-RESISTANT TUBERCULOSIS

Drug-resistant TB is not a recent phenomenon. *M. tuberculosis* strains that were resistant to streptomycin (SM) appeared soon after the introduction of the

Correspondence to: Ying Zhang, Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, 615 N Wolfe Street, Baltimore, MD 21205, USA. Tel: (+1) 410 614 2975. Fax: (+1) 410 955 0105. e-mail: yzhang@jhsph.edu

[A version in French of this article is available from the Editorial Office in Paris and from the Union website www.theunion.org]

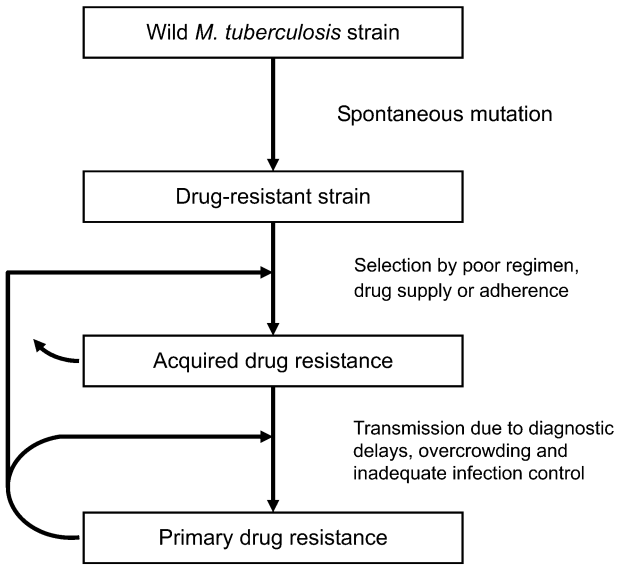

Figure Concepts in the development of drug-resistant TB.

drug for treatment of TB in 1944. Genetic resistance to an anti-tuberculosis drug is due to spontaneous chromosomal mutations at a frequency of 10^{-6} to 10^{-8} mycobacterial replications. Mobile genetic elements such as plasmids and transposons, which are known to mediate drug resistance in various bacterial species, do not do so in *M. tuberculosis*. Because such mutations resulting in drug resistance are unlinked, the probability of developing bacillary resistance to three drugs used simultaneously becomes 10^{-18} to 10^{-20}. In theory, the chance of drug resistance is thus virtually non-existent when three effective drugs are used in combination for TB treatment.

Amplification of the afore-mentioned genetic mutation through human error results in clinically drug-resistant TB. These include 'monotherapy' due to irregular drug supply, inappropriate doctor prescription and, most importantly, poor patient adherence to treatment.[6] Subsequent transmission of resistant *M. tuberculosis* strains from the index patient to others aggravates the problem (Figure). The MDR/XDR phenotype is caused by sequential accumulation of mutations in different genes involved in individual drug resistance (Table).

Although the definitions of 'acquired' and 'primary' drug resistance are conceptually relatively clear, in reality they are often subject to misclassification when previous treatment cannot be readily ascertained. The term 'initial' drug resistance is thus often preferred to 'primary' drug resistance to include 'unknown' or 'undisclosed' acquired drug resistance. The matter is currently further simplified by categorizing drug resistance in new cases and previously treated cases of TB.[2] The latter refers to cases with treatment lasting for at least 1 month.

CLINICAL RELEVANCE OF ANTI-TUBERCULOSIS DRUG RESISTANCE

INH resistance is the most common form of anti-tuberculosis drug resistance encountered, whether in isolation or in combination with other drugs.[2] INH-monoresistant TB is relatively easy to treat. Standard

Table Mechanisms of drug resistance in *M. tuberculosis*

Drug (year of discovery)	MIC µg/ml	Gene(s) involved in resistance	Gene function	Role	Mechanism of action	Mutation frequency %
Isoniazid (1952)	0.02–0.2	*kat*G *inh*A	Catalase-peroxidase Enoyl ACP reductase	Pro-drug conversion Drug target	Inhibition of mycolic acid biosynthesis and other multiple effects	50–95 8–43
Rifampicin (1966)	0.05–1	*rpo*B	β subunit of RNA polymerase	Drug target	Inhibition of RNA synthesis	95
Pyrazinamide (1952)	16–50 (pH 5.5)	*pnc*A	Nicotinamidase/pyrazinamidase	Pro-drug conversion	Depletion of membrane energy	72–97
Ethambutol (1961)	1–5	*emb*B	Arabinosyl transferase	Drug target	Inhibition of arabinogalactan synthesis	47–65
Streptomycin (1944)	2–8	*rps*L *rrs* *gid*B	S12 ribosomal protein 16S rRNA rRNA methyltransferase (G527 in 530 loop)	Drug target Drug target Drug target	Inhibition of protein synthesis	52–59 8–21 ?
Amikacin/kanamycin (1957)	2–4	*rrs*	16S rRNA 16S rRNA	Drug target	Inhibition of protein synthesis	76
Capreomycin (1960)		*tly*A	2′-O-methyltransferase			
Quinolones (1963)	0.5–2.5	*gyr*A *gyr*B	DNA gyrase subunit A DNA gyrase subunit B	Drug target	Inhibition of DNA gyrase	75–94
Ethionamide (1956)	2.5–10	*eta*A/*eth*A	Flavin monooxygenase	Prodrug conversion	Inhibition of mycolic acid synthesis	37
		*inh*A		Drug target		56
PAS (1946)	1–8	*thy*A	Thymidylate synthase	Drug activation?	Inhibition of folic acid and iron metabolism?	36

MIC = minimum inhibitory concentration; ACP = acyl carrier protein; PAS = para-aminosalicylic acid.

short-course chemotherapy (SCC) can achieve good success (around 98% cure and less than 5% relapse) when all four drugs—INH, RMP, pyrazinamide (PZA) and ethambutol (EMB)/SM—are used throughout the 6 months of treatment.[7] When the four drugs are reduced to RMP and INH after 2 months, the relapse rate after 6 months of treatment rises to 10%.[8] As there may be a small chance of resistance amplification with involvement of RMP,[9] some authorities recommend alternative regimens such as RMP+ EMB, or RMP+EMB+PZA, for more prolonged durations.[10,11]

RMP-resistant TB carries a much more ominous prognosis, as the outcome of standard SCC for such disease is poor in terms of both disease status on cessation of 6-month treatment and relapse.[12] Recommendation has been made to treat such disease with INH, PZA and EMB for 18–24 months.[13] Some authorities feel that the duration of treatment can be shortened to 12 months by the addition of an FQ to this three-drug regimen.[14] Furthermore, RMP monoresistance in *M. tuberculosis* is rare, except perhaps in HIV-infected patients,[13,15] and RMP resistance thus generally serves as a surrogate marker for dual resistance to RMP and INH, i.e., MDR-TB.[16] This proxy appears especially valid for previously treated patients. SCC can cure less than 60% of MDR-TB cases,[17] with a high recurrence rate of about 28% among those with apparent success.[18] It is thus quite clear today that alternative specific chemotherapy using second-line drugs is required for the management of this formidable condition.[19]

Increased risk for the development of bacillary resistance to EMB and PZA likely occurs when a WHO Category I (4-drug) treatment regimen and a Category II (5-drug) retreatment regimen are repeatedly administered despite observed treatment failure with the standard short-course regimen for TB.[9,20,21] The diagnosis of resistance to PZA and/or EMB is of prognostic value in MDR-TB; as such, resistance in addition to dual resistance to INH and RMP generally portends an even more adverse prognosis,[22] particularly when patients receive only 'standard' second-line anti-tuberculosis drug regimens, which often comprise largely those two drugs plus an FQ and aminoglycoside/capreomycin (CPM) for MDR-TB treatment.[9] A high prevalence of bacillary resistance to PZA and/or EMB would also hamper the efficacy of standard 6-month SCC, as PZA plays a very unique role in sterilizing TB lesions to curtail disease relapse.[23]

FQs are generally regarded to have a pivotal position in the treatment of MDR-TB.[24–26] In vitro resistance to FQs has been shown to predict a poor outcome in the treatment of MDR-TB.[24,27,28] Most FQ resistance in *M. tuberculosis* is associated with the injudicious use of this class of drugs in the management of TB, particularly MDR-TB.[29,30] FQ-resistant MDR-TB likely results from the use of suboptimal second-line drug regimens comprising inadequate number and/or dosage of agents accompanying the key drug FQ, as previously mentioned.[9] The alternative pathway in the development of FQ-resistant TB might be related to the excess use of this class of antimicrobials in the treatment of lower respiratory tract infections,[31,32] as well as other types of community-acquired infections. In settings where FQs are widely used for treating respiratory infections, the prevalence of FQ-resistant TB could be substantial.[33] This phenomenon can be a cause for concern in different parts of the world.[34,35]

As the aminoglycosides or CPM have potent anti-tuberculosis activity, the loss of these second-line injectables in addition, through suboptimal use in the management of MDR-TB, would result in XDR-TB, with a much worse prognosis than MDR-TB.[36] Currently, the cure rate of XDR-TB is generally much less than 50% with the chemotherapy available.[37]

MECHANISMS OF RESISTANCE TO FIRST- AND SECOND-LINE AGENTS

A great deal of progress has been made in our understanding of the molecular basis of drug action and resistance in *M. tuberculosis* (Table). Below we will provide an update on this topic.

Isoniazid

INH is the most widely used first-line anti-tuberculosis drug. Since its discovery in 1952, INH has been the cornerstone of all effective regimens for the treatment of TB disease and latent infection. *M. tuberculosis* is highly susceptible to INH (minimum inhibitory concentration [MIC] 0.02–0.2 μg/ml). INH is only active against growing tubercle bacilli, and is not active against non-replicating bacilli or under anaerobic conditions.

INH is a prodrug that is activated by the catalase-peroxidase enzyme (KatG) encoded by the *kat*G gene[38] to generate a range of highly reactive species which then attack multiple targets in *M. tuberculosis*.[39] The reactive species produced by KatG-mediated INH activation include both reactive oxygen species such as superoxide, peroxide and hydroxyl radical,[40] nitric oxide[41] and reactive organic species such as isonicotinic-acyl radical or anion,[42,43] and certain electrophilic species.[44] The primary target of INH inhibition is thought to be the InhA enzyme (enoyl-acyl carrier protein reductase), involved in elongation of fatty acids in mycolic acid synthesis.[45] The active species (isonicotinic-acyl radical or anion) derived from KatG-mediated INH activation reacts with NAD(H) (nicotinamide adenine dinucleotide) to form an INH-NAD adduct, and then attacks InhA.[42,43] A recent study showed that INH-NAD(P) adducts react with other protein targets besides InhA, such as DfrA (an NADPH-dependent dihydrofolate reductase involved in DNA synthesis).[46] Further studies are needed to assess the role of these putative targets in INH action and resistance.

Resistance to INH occurs more frequently than for most anti-tuberculosis drugs, at a frequency of 1 in 10^{5-6} bacilli in vitro.[47] INH-resistant clinical isolates of *M. tuberculosis* often lose catalase and peroxidase enzyme[48] encoded by *kat*G, especially in high-level resistant strains (MIC > 5 μg/ml).[47] Low-level resistant strains (MIC < 1 μg/ml) often still possess catalase activity.[47] Mutation in *kat*G is the main mechanism of INH resistance (Table).[38,49] KatG S315T mutation is the most common mutation in INH-resistant strains, accounting for 50–95% of INH-resistant clinical isolates.[39,49] Resistance to INH can also occur by mutations in the promoter region of *mab*A/*inh*A operon, causing overexpression of InhA, or by mutations at the InhA active site, lowering the InhA affinity to the INH-NAD adduct.[42,45] Mutations in *inh*A or its promoter region are usually associated with low-level resistance (MICs = 0.2 − 1 μg/ml) and are less frequent than *kat*G mutations (Table).[39,49] INH-resistant *M. tuberculosis* harboring *inh*A mutations could have additional mutations in *kat*G, conferring higher levels of INH resistance.[50] Mutations in *inh*A not only cause INH resistance, they also confer cross-resistance to the structurally related drug, ethionamide (ETH).[45] In KatG-negative INH-resistant strains, mutations in the promoter region of *ahp*C, encoding an alkylhydroperoxide reductase, leading to increased expression of the enzyme, have been observed as a compensation for the lack of catalase-peroxidase in such strains.[51,52] Overexpression of AphC did not appear to confer significant INH resistance.[53] About 10–25% of low-level INH-resistant strains do not have mutations in *kat*G or *inh*A,[49] and may be due to new mechanism(s) of resistance. Mutations in *msh*A, encoding an enzyme involved in mycothiol biosynthesis, have recently been shown to confer INH and ETH resistance in *M. tuberculosis* strains in vitro,[54] but its role in clinical resistance remains to be demonstrated.

Rifampicin

RMP is an important first-line drug for the treatment of TB. RMP is bactericidal for *M. tuberculosis,* with MICs ranging from 0.05 to 1 μg/ml on solid or liquid media, but the MIC is higher in egg media (MIC = 2.5–10 μg/ml). Strains with MICs < 1 μg/ml in liquid or agar medium or MICs < 40 μg/ml in Löwenstein-Jensen (LJ) medium are considered RMP-susceptible. RMP is active against both growing and stationary phase bacilli with low metabolic activity. The latter activity is related to its high sterilizing activity in vivo, correlating with its ability to shorten TB treatment from 12–18 months to 9 months.[55]

RMP interferes with RNA synthesis by binding to the β subunit of the RNA polymerase. The RNA polymerase is an oligomer consisting of a core enzyme formed by four chains $\alpha 2\beta\beta'$ in association with the σ subunit to specifically initiate transcription from promoters. The RMP-binding site is located upstream of the catalytic center and physically blocks the elongation of the RNA chain. In *M. tuberculosis*, resistance to RMP occurs at a frequency of 10^{-7} to 10^{-8}. As in other bacteria, mutations in a defined region of the 81 base pair (bp) region of the *rpo*B are found in about 96% of RMP-resistant *M. tuberculosis* isolates.[56] Mutations at positions 531, 526 and 516 are among the most frequent mutations in RMP-resistant strains. Mutations in *rpo*B generally result in high-level resistance (MIC > 32 μg/ml) and cross-resistance to all rifamycins. However, specific mutations in codons 511, 516, 518 and 522 are associated with lower-level resistance to RMP and rifapentine, but retain susceptibility to rifabutin and rifalazil.[57,58]

An intriguing and potentially worrying finding is the observation of RMP-dependent strains of *M. tuberculosis* in clinical settings.[59,60] These strains had poor growth on egg-based media but grew better in the presence of RMP. RMP-dependent strains in some ways exhibit similarity to L-form bacteria. Strictly speaking, these strains are not RMP-dependent, as they could still show very poor growth in the absence of RMP. This is unlike the strictly SM-dependent strains that only grow in the presence of SM. RMP-dependent strains have not been widely reported, probably because current diagnostic practice uses only drug-free media. These strains have common and additional *rpo*B mutations (B Zhu, M Zhong and Y Zhang, unpublished observations). The circumstances under which the RMP-dependent strains arise remain unclear, but they often occur as MDR-TB and seem to develop upon repeated treatment with rifamycins in retreatment patients. Continued use of rifamycins for the treatment of patients harboring RMP-dependent strains can worsen the disease (M Zhong and Y Zhang, unpublished observations). It will be of interest to determine the mechanism of RMP dependence and assess the role of such strains in treatment failures.

Pyrazinamide

PZA is an important first-line drug used along with INH and RMP. PZA plays a unique role in shortening TB treatment from the previous 9–12 months to 6 months because it kills a population of persister bacilli in acidic pH environment in the lesions that is not killed by other drugs.[55]

PZA is an unconventional and paradoxical antituberculosis drug that has high sterilizing activity in vivo,[23] but no activity against tubercle bacilli at normal culture conditions near neutral pH.[61] PZA is only active against *M. tuberculosis* at acid pH (e.g., 5.5).[62] Even at acid pH (5.5), PZA activity is quite poor, with MICs in the range of 6.25–50 μg/ml.[23] PZA activity is enhanced under low oxygen or anaerobic conditions[63] and by agents that compromise the membrane energy status, such as weak acids,[64] and energy inhibitors such as DCCD (dicyclohexylcarbodiimide), azide and rotenone.[65]

PZA is a prodrug that requires conversion to its active form, pyrazinoic acid (POA), by the pyrazinamidase/nicotinamidase enzyme encoded by the *pnc*A gene of *M. tuberculosis*.[66] The POA produced intracellularly reaches the cell surface through passive diffusion and a defective efflux.[67] The extracellular acid pH facilitates the formation of uncharged protonated POA, which then permeates through the membrane and causes accumulation of POA and disrupts membrane potential in *M. tuberculosis*.[65] The protonated POA brings protons into the cell and could eventually cause cytoplasmic acidification and de-energize the membrane by collapsing the proton motive force, which affects membrane transport.[65] The target of PZA is related to membrane energy metabolism,[65] although the specific target remains to be identified. Fas-I was proposed as a target for PZA,[68] but its validity is questioned.[69] More details on PZA can be found in the review by Zhang and Mitchison.[23]

PZA-resistant *M. tuberculosis* strains lose pyrazinamidase/nicotinamidase activity.[70] We have cloned the *M. tuberculosis pnc*A[66] and shown that defective pyrazinamidase activity due to *pnc*A mutations is the major cause of PZA resistance.[66,71,72] Various other studies have subsequently confirmed our findings.[20,73–80]

The *pnc*A mutations are highly diverse and scattered along the gene, which is unique to PZA resistance. Despite the highly diverse and scattered distribution of *pnc*A mutations, there is some degree of clustering at three regions of the PncA, 3–17, 61–85 and 132–142.[71,75] Most PZA-resistant *M. tuberculosis* strains (72–97%) have mutations in *pnc*A;[20,71–78] however, some resistant strains do not have *pnc*A mutations. One type of such strains is pyrazinamidase-negative, with a high level of resistance,[72,75,76] which may be due to mutations in an undefined *pnc*A regulatory gene. Another type of such strains has low-level resistance (MICs = 200–300 μg/ml, with a resistance cut-off of 100 μg/ml PZA) and positive pyrazinamidase activity without *pnc*A mutations; their mechanism of resistance remains to be determined. The lower percentage of PZA-resistant strains with *pncA* mutations (e.g., 72%)[73] reported in some studies could be caused by false resistance due to well-known problems with PZA susceptibility.[23]

PZA is active only against *M. tuberculosis* complex organisms (*M. tuberculosis, M. africanum* and *M. microti*), but not *M. bovis,* due to a characteristic mutation in its *pnc*A gene.[66] Strains of *M. bovis*, including bacille Calmette-Guérin (BCG), are naturally resistant to PZA and lack pyrazinamidase; these features are commonly used to distinguish *M. bovis* from *M. tuberculosis*. The natural PZA resistance in *M. bovis* and BCG is due to a single point mutation of 'C' to 'G' at nucleotide position 169 of the *pnc*A gene compared with the *M. tuberculosis pnc*A sequence, causing amino acid substitution at position 57 of the PncA sequence.[66] However, the correlation between pyrazinamidase activity and PZA susceptibility is not true for other naturally PZA-resistant mycobacterial species whose intrinsic PZA resistance is most likely due to their highly active POA efflux mechanism.[67]

Ethambutol

EMB [(*S,S′*)-2,2′(ethylenediimino)di-1-butanol] is a first-line drug that is used in combination with INH, RMP and PZA to prevent the emergence of drug resistance. The MICs of EMB for *M. tuberculosis* are in the range of 0.5–2 μg/ml. EMB is a bacteriostatic agent that is active for growing bacilli and has no effect on non-replicating bacilli. EMB interferes with the biosynthesis of cell wall arabinogalactan.[81] It inhibits the polymerization of cell-wall arabinan of arabinogalactan and of lipoarabinomannan, and induces the accumulation of D-arabinofuranosyl-P-decaprenol, an intermediate in arabinan biosynthesis.[82,83] Arabinosyl transferase, encoded by *emb*B, an enzyme involved in the synthesis of arabinogalactan, has been proposed as the target of EMB in *M. tuberculosis*[84] and *M. avium*.[85] In *M. tuberculosis, emb*B is organized into an operon with *emb*C and *emb*A in the order *emb*CAB. *emb*C, *emb*B and *emb*A share over 65% amino acid identity with each other and are predicted to encode transmembrane proteins.[84]

Strains resistant to EMB have MICs > 7.5 μg/ml.[86] Mutation to EMB resistance occurs at a frequency of 10^{-5}. Mutations in the *emb*CAB operon, in particular *emb*B, and occasionally *emb*C, are responsible for resistance to EMB.[84] The *emb*B codon 306 mutation is most frequent in clinical isolates resistant to EMB, accounting for as high as 68% resistant strains.[87,88] Some inconsistent reports have suggested that the EmbB306 mutation is not involved in EMB resistance,[89–92] but is instead associated with the development of other drug resistance, including MDR-TB.[90,91,93] This discrepancy was recently resolved by carefully assessing the role of individual mutations causing different amino acid substitutions in EMB resistance using site-directed mutagenesis and allelic exchange in *M. tuberculosis*.[94] It was found that mutations leading to certain amino acid changes are indeed causing EMB resistance while other amino acid substitutions have little effect on EMB resistance.[94] However, about 35% of EMB-resistant strains (MIC $<$ 10 μg/ml) do not have *emb*B mutations,[95] suggesting that there may be other mechanisms of EMB resistance. Further studies are needed to identify potential new mechanisms of EMB resistance.

Aminoglycosides (streptomycin, kanamycin/ amikacin/capreomycin)

SM is an aminoglycoside antibiotic that is active against a variety of bacterial species, including *M. tuberculosis*. SM kills actively growing tubercle bacilli with MICs of 2–4 μg/ml,[86] but it is inactive against

non-growing or intracellular bacilli.[55] SM inhibits protein synthesis by binding to the 30S subunit of bacterial ribosome, causing misreading of the mRNA message during translation.[96] The site of action of SM is the 30S subunit of the ribosome at the ribosomal protein S12 and the 16S rRNA. Resistance to SM is caused by mutations in the S12 protein encoded by *rps*L gene and 16S rRNA encoded by *rrs* gene.[97] Mutations in *rps*L and *rrs* are the major mechanism of SM resistance,[97–99] accounting for respectively about 50% and 20% of SM-resistant strains.[97–99] The most common mutation in *rps*L is a substitution in codon 43 from lysine to arginine,[97–99] causing high-level resistance to SM. Mutation in codon 88 is also common.[97–99] Mutations of the *rrs* gene occur in the loops of the 16S rRNA and are clustered in two regions around nucleotides 530 and 915.[97–99] SM-dependent SM-resistant *M. tuberculosis* strains appear to be caused by a 'C' insertion in the 530 loop.[100] However, about 20–30% of SM-resistant strains with a low level of resistance (MIC < 32 μg/ml) do not have mutations in *rps*L or *rrs*,[101] which indicates other mechanism(s) of resistance. Recently, a mutation in *gid*B, encoding a conserved 7-methylguanosine (m(7)G) methyltransferase specific for 16S rRNA, has been found to cause low-level SM resistance in 33% of resistant *M. tuberculosis* isolates.[102] A subsequent study showed that while L16R change is a polymorphism not involved in SM resistance, other mutations in *gid*B appear to be involved in low-level SM resistance.[103] In addition, some low-level SM resistance seems to be caused by increased efflux as efflux pump inhibitors caused increased sensitivity to SM, although the exact mechanism remains to be identified.[103]

Kanamycin (KM) and its derivative amikacin (AMK) are also inhibitors of protein synthesis through modification of ribosomal structures at the 16S rRNA.[104,105] Mutations at 16S rRNA (*rrs*) position 1400 are associated with high-level resistance to KM and AMK.[104,105] CPM is a polypeptide antibiotic. A gene called *tly*A encoding rRNA methyltransferase was shown to be involved in resistance to CPM.[106] The rRNA methyltransferase modifies nucleotide C1409 in helix 44 of 16S rRNA and nucleotide C1920 in helix 69 of 23S rRNA.[107] Variable cross-resistance may be observed between KM, AMK, CPM or viomycin (VM).[47] Mutants resistant to CPM and VM could have *tly*A, C1402T, or G1484T *rrs* mutations, while mutants resistant to CPM but not VM could have an A1401G *rrs* mutation.[108] Mutants with an A1401G mutation could cause resistance to KM and CPM but not VM.[108] Mutants resistant to CPM, KM and VM could have either a C1402T or a G1484T mutation in the *rrs* gene.[108] Multiple mutations may occur in the *rrs* gene in one strain, conferring cross-resistance among these agents.[108] SM-resistant strains are usually still susceptible to KM and AMK.

Fluoroquinolones

DNA topoisomerases are a diverse set of essential enzymes responsible for maintaining chromosomes in an appropriate topological state. In the cell, topoisomerases regulate DNA supercoiling and unlink tangled nucleic acid strands to meet replicative and transcriptional needs.[109] In most bacterial species, FQs inhibit DNA gyrase (topoisomerase II) and topoisomerase IV, resulting in microbial death. DNA gyrase is a tetrameric A2B2 protein. The A subunit carries the breakage-reunion active site, whereas the B subunit promotes adenosine triphosphate hydrolysis. *M. tuberculosis* has respectively *gyr*A and *gyr*B encoding the A and B subunits.[110] A conserved region, the quinolone-resistance-determining region (QRDR) of *gyr*A (320 bp) and *gyr*B (375 bp), has been found to be a most important area involved in the exhibition of FQ resistance in *M. tuberculosis*.[110] Mutations within the QRDR of *gyr*A have been identified in clinical and laboratory-selected isolates of *M. tuberculosis*, largely clustered at codons 90, 91, 94,[110–114] with Asp94 being relatively frequent.[112,115] Other major isolates involved also include codons 74, 83, 87.[111,115,116] Mutation at codon 95 is considered a polymorphism not involved in quinolone resistance.[117] A less common involvement is codon 88.[118] For clinical isolates, *gyr*B mutations appear to be of much rarer occurrence.[113,114,119] Generally, two mutations in *gyr*A or concomitant mutations in *gyr*A plus *gyr*B are required for the development of higher levels of resistance.[110,120]

It has also been shown that the frequency of mutations conferring FQ resistance in *M. tuberculosis*, and the distribution of resistance alleles selected, possibly depend on the FQ concentration.[121] Selection at low FQ concentration produced many low-level resistance mutants. None contained mutations in QRDR of *gyr*A, the principal drug target. However, as selection pressure increased, a variety of *gyr*A alterations became prevalent. High concentrations of FQ reduced the variety to a few types, and eventually a concentration was revealed at which no mutant was recovered, which is called mutant prevention concentration.[121]

It is of immense interest to note that the percentage of FQ-resistant *M. tuberculosis* clinical isolates with discernible *gyr* mutations was found to vary markedly among different studies: $<50\%$ in some of them,[112,114,119] with an extreme of 2%,[114] and $\geqslant 50\%$ in many others[113,115,116,122,123] with an extreme approaching 100%.[116,123] Possible explanations of the observed variance might include differences in the molecular detection methodology used,[110,123] especially in relation to the extent of coverage of the genome—whether QRDR or beyond in *gyr*A, as well as for *gyr*B; the definition of FQ resistance in *M. tuberculosis* (MICs of ofloxacin $\geqslant 2$ μg/ml vs. $\geqslant 4$ μg/ml) (some low-level quinolone-resistant strains may be falsely resistant);[111,112] and perhaps other underlying

mechanisms responsible for the mycobacterial resistance to FQs, such as decreased cell-wall permeability to drug, drug efflux pump, drug sequestration, or perhaps even drug inactivation.[109] Recently, a new mechanism of quinolone resistance mediated by MfpA was identified.[124] MfpA is a member of the pentapeptide repeat family of proteins from *M. tuberculosis*, whose expression causes resistance to FQ drugs. MfpA binds to DNA gyrase and inhibits its activity in the form of a DNA mimicry, which explains its inhibitory effect on DNA gyrase and quinolone resistance.[124] The *M. tuberculosis Rv2686c-Rv2687c-Rv2688c* operon, encoding an ATP-binding cassette transporter, has been shown to confer resistance to ciprofloxacin and, to a lesser extent, norfloxacin, moxifloxacin and sparfloxacin in *M. smegmatis*.[125] The resistance level was found to decrease in the presence of efflux pump inhibitors such as reserpine and verapamil. However, it remains to be determined if clinical strains elaborate MfpA or the *Rv2686c-Rv2687c-Rv2688c* operon to develop clinical resistance to quinolones.

The alternative mechanisms accounting for *M. tuberculosis* resistance to FQs are likely associated with lower levels of resistance, unlike that due to *gyr* mutations.[112] However, when these alternative mechanisms co-exist with *gyr* mutations, the displayed resistance can be anticipated to be considerable. Furthermore, it has been suggested that, regarding *M. tuberculosis* resistance to FQs, the underlying genetic mutations can show substantial disparity among different geographic regions.[114]

Ethionamide/prothionamide and thioamides

ETH (2-ethylisonicotinamide) is a derivative of isonicotinic acid, and is bactericidal only against *M. tuberculosis, M. avium-intracellulare* and *M. leprae*. The MICs of ETH for *M. tuberculosis* are 0.5–2 μg/ml in liquid medium, 2.5–10 μg/ml in 7H11 agar, and 5–20 μg/ml in LJ medium. Like INH, ETH is also a prodrug that is activated by EtaA/EthA (a monooxygenase)[126,127] and inhibits the same target as INH, the InhA of the mycolic acid synthesis pathway.[45] Prothionamide (PTH, 2-ethyl-4-pyridinecarbothioamide) shares structure and activity almost identical to that of ETH. EtaA or EthA is a flavin adenosine dinucleotide (FAD) containing enzyme that oxidizes ETH to the corresponding S-oxide, which is further oxidized to 2-ethyl-4-amidopyridine, presumably via the unstable oxidized sulfinic acid intermediate.[128] EtaA also activates thiacetazone, thiocarlide, thiobenzamide, and perhaps other thioamide drugs,[128] which explains the cross-resistance between ETH and thiacetazone, thiocarlide and other thioamides and thioureas.[129] Mutations in the drug-activating enzyme EtaA/EthA[126,127] cause resistance to ETH and other thioamides. In addition, mutations in the target InhA confer resistance to both ETH and INH.

EPILOGUE

To meet the challenge of MDR-TB and XDR-TB worldwide, huge monetary resource instillation and extensive human resource development are required for the prevention and management of these formidable drug resistance scenarios. Among the response priorities, rapid detection of anti-tuberculosis drug resistance, use of appropriate regimens for treatment, and new drug development are of paramount importance. Recent advances in high throughput DNA sequencing will allow whole genome sequencing of unique drug-resistant strains to be performed at a much more rapid speed and significantly less cost, which will facilitate the identification of new and unknown mechanisms of drug resistance and ultimately lead to more efficient detection of drug resistance. Improved understanding of the mechanisms of drug resistance in *M. tuberculosis* would be conducive to the accelerated development of these new strategies for control of drug-resistant TB. However, strengthening of the current TB control programs globally should continue to be maintained. Adequate monitoring of drug resistance, especially MDR/XDR-TB in new patients and its transmission, molecular characterization of the drug-resistant strains, and analysis of patients' immune status and genetic susceptibility are also needed to address the problem of the fitness, virulence and transmissibility of drug-resistant *M. tuberculosis* strains.

Acknowledgement

YZ was supported by National Institutes of Health grant AI44063 and the Changjiang Scholar Program.

References

1 Zignol M, Hosseini M S, Wright A, et al. Global incidence of multidrug-resistant tuberculosis. J Infect Dis 2006; 194: 479–485.
2 World Health Organization. Anti-tuberculosis drug resistance in the world. Report no. 4. WHO/HTM/TB/2008.394. Geneva, Switzerland: WHO, 2008. http://www.who.int/tb/publications/2008/drs_report4_26feb08.pdf Accessed July 2009.
3 Zhang Y. The magic bullets and tuberculosis drug targets. Ann Rev Pharmacol Toxicol 2005; 45: 529–564.
4 Ling D I, Zwerling A A, Pai M. GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis. Eur Respir J 2008; 32: 1165–1174.
5 Chang K, Yew W, Zhang Y. A systematic review of rapid drug susceptibility tests for multidrug-resistant tuberculosis using rifampicin-resistance as a surrogate. Expert Opin Med Diagn 2009; 3: 99–122.
6 Vareldzis B P, Grosset J, de Kantor I, et al. Drug-resistant tuberculosis: laboratory issues. World Health Organization recommendations. Tubercle Lung Dis 1994; 75: 1–7.
7 Hong Kong Chest Service/British Medical Research Council. Five-year follow-up of a controlled trial of five 6-month regimens of chemotherapy for pulmonary tuberculosis. Am Rev Respir Dis 1987; 136: 1339–1342.
8 Hong Kong Chest Service/British Medical Research Council. Controlled trial of 2, 4 and 6 months of pyrazinamide in

6 month, three-times-weekly regimens for smear-positive pulmonary tuberculosis, including an assessment of a combined preparation of isoniazid, rifampin and pyrazinamide. Am Rev Respir Dis 1991; 143: 700–706.
9 Caminero J A. Likelihood of generating MDR-TB and XDR-TB under adequate National Tuberculosis Control Programme implementation. Int J Tuberc Lung Dis 2008; 12: 869–877.
10 Davidson P T. Drug resistance and the selection of therapy for tuberculosis. Am Rev Respir Dis 1987; 136: 255–257.
11 Joint Tuberculosis Committee of the British Thoracic Society. Chemotherapy and management of tuberculosis in the United Kingdom: recommendations 1998. Thorax 1998; 53: 536–548.
12 Mitchison D A, Nunn A J. Influence of initial drug resistance on the response to short-course chemotherapy of pulmonary tuberculosis. Am Rev Respir Dis 1986; 133: 423–430.
13 Barnes P F, Bloch A B, Davidson P T, et al. Tuberculosis in patients with human immunodeficiency virus infection. N Engl J Med 1991; 324: 1644–1650.
14 Centers for Disease Control and Prevention. Treatment of tuberculosis, American Thoracic Society, CDC, and Infectious Diseases Society of America. MMWR 2003; 52 (RR-11): 1–77.
15 Vernon A, Burman W, Benator D, et al. Acquired rifamycin monoresistance in patients with HIV-related tuberculosis treated with once-weekly rifapentine and isoniazid. Tuberculosis Trials Consortium. Lancet 1999; 353: 1843–1847.
16 Traore H, Fissette K, Bastian I, et al. Detection of rifampicin resistance in *Mycobacterium tuberculosis* isolates from diverse countries by a commercial line probe assay as an initial indicator of multidrug resistance. Int J Tuberc Lung Dis 2000; 4: 481–484.
17 Espinal M A, Kim S J, Suarez P G, et al. Standard short-course chemotherapy for drug-resistant tuberculosis: treatment outcomes in 6 countries. JAMA 2000; 283: 2537–2545.
18 Becerra M C, Freeman J, Bayona J, et al. Using treatment failure under effective directly observed short-course chemotherapy programs to identify patients with multidrug-resistant tuberculosis. Int J Tuberc Lung Dis 2000; 4: 108–114.
19 World Health Organization. Guidelines for the programmatic management of drug-resistant tuberculosis. WHO/HTM/TB/2006.361. Geneva, Switzerland: WHO, 2006.
20 Louw G E, Warren R M, Donald P R, et al. Frequency and implications of pyrazinamide resistance in managing previously treated tuberculosis patients. Int J Tuberc Lung Dis 2006; 10: 802–807.
21 Hanif M, Malik S, Dhingra V K. Acquired drug resistance pattern in tuberculosis cases at the State Tuberculosis Centre, Delhi, India. Int J Tuberc Lung Dis 2009; 13: 74–78.
22 Migliori G B, Besozzi G, Girardi E, et al. Clinical and operational value of the extensively drug-resistant tuberculosis definition. Eur Respir J 2007; 30: 623–626.
23 Zhang Y, Mitchison D. The curious characteristics of pyrazinamide: a review. Int J Tuberc Lung Dis 2003; 7: 6–21.
24 Yew W W, Chan C K, Chau C H, et al. Outcomes of patients with multidrug-resistant pulmonary tuberculosis treated with ofloxacin/levofloxacin-containing regimens. Chest 2000; 117: 744–751.
25 Chan E D, Laurel V, Strand M J, et al. Treatment and outcome analysis of 205 patients with multidrug-resistant tuberculosis. Am J Respir Crit Care Med 2004; 169: 1103–1109.
26 Chiang C Y, Enarson D A, Yu M C, et al. Outcome of pulmonary multidrug-resistant tuberculosis: a 6-yr follow-up study. Eur Respir J 2006; 28: 980–985.
27 Park S K, Lee W C, Lee D H, et al. Self-administered, standardised regimens for multidrug-resistant tuberculosis in South Korea. Int J Tuberc Lung Dis 2004; 8: 361–368.
28 Leimane V, Riekstina V, Holtz T H, et al. Clinical outcome of individualised treatment of multidrug-resistant tuberculosis in Latvia: a retrospective cohort study. Lancet 2005; 365: 318–326.
29 Huang T S, Kunin C M, Shin-Jung Lee S, et al. Trends in fluoroquinolone resistance of *Mycobacterium tuberculosis* complex in a Taiwanese medical centre: 1995–2003. J Antimicrob Chemother 2005; 56: 1058–1062.
30 Park I N, Hong S B, Oh Y M, et al. Impact of short-term exposure to fluoroquinolones on ofloxacin resistance in HIV-negative patients with tuberculosis. Int J Tuberc Lung Dis 2007; 11: 319–324.
31 Ginsburg A S, Hooper N, Parrish N, et al. Fluoroquinolone resistance in patients with newly diagnosed tuberculosis. Clin Infect Dis 2003; 37: 1448–1452.
32 Wang J Y, Hsueh P R, Jan I S, et al. Empirical treatment with a fluoroquinolone delays the treatment for tuberculosis and is associated with a poor prognosis in endemic areas. Thorax 2006; 61: 903–908.
33 Sterling T R. The WHO/IUATLD diagnostic algorithm for tuberculosis and empiric fluoroquinolone use: potential pitfalls. Int J Tuberc Lung Dis 2004; 8: 1396–1400.
34 Grimaldo E R, Tupasi T E, Rivera A B, et al. Increased resistance to ciprofloxacin and ofloxacin in multidrug-resistant *Mycobacterium tuberculosis* isolates from patients seen at a tertiary hospital in the Philippines. Int J Tuberc Lung Dis 2001; 5: 546–550.
35 Agrawal D, Udwadia Z F, Rodriguez C, et al. Increasing incidence of fluoroquinolone-resistant *Mycobacterium tuberculosis* in Mumbai, India. Int J Tuberc Lung Dis 2009; 13: 79–83.
36 Migliori G B, Lange C, Centis R, et al. Resistance to second-line injectables and treatment outcomes in multidrug-resistant and extensively drug-resistant tuberculosis cases. Eur Respir J 2008; 31: 1155–1159.
37 Kim D H, Kim H J, Park S K, et al. Treatment outcomes and long-term survival in patients with extensively drug-resistant tuberculosis. Am J Respir Crit Care Med 2008; 178: 1075–1082.
38 Zhang Y, Heym B, Allen B, et al. The catalase-peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. Nature 1992; 358: 591–593.
39 Zhang Y, Telenti A. Genetics of drug resistance in *Mycobacterium tuberculosis*. In: Hatfull G, Jacobs W R, eds. Molecular genetics of mycobacteria. Washington DC, USA: ASM Press, 2000: pp 235–254.
40 Shoeb H A, Bowman B U Jr, Ottolenghi A C, et al. Peroxidase-mediated oxidation of isoniazid. Antimicrob Agents Chemother 1985; 27: 399–403.
41 Timmins G S, Master S, Rusnak F, et al. Nitric oxide generated from isoniazid activation by KatG: source of nitric oxide and activity against *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2004; 48: 3006–3009.
42 Rozwarski D A, Grant G A, Barton D H, et al. Modification of the NADH of the isoniazid target (InhA) from *Mycobacterium tuberculosis*. Science 1998; 279: 98–102.
43 Rawat R, Whitty A, Tonge P J. The isoniazid-NAD adduct is a slow, tight-binding inhibitor of InhA, the *Mycobacterium tuberculosis* enoyl reductase: adduct affinity and drug resistance. Proc Natl Acad Sci USA 2003; 100: 13881–13886.
44 Johnsson K, King D S, Schultz P G. Studies on the mechanism of action of isoniazid and ethionamide in the chemotherapy of tuberculosis. J Am Chem Soc 1995; 117: 5009–5010.
45 Banerjee A, Dubnau E, Quemard A, et al. *inh*A, a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis*. Science 1994; 263: 227–230.
46 Argyrou A, Jin L, Siconilfi-Baez L, et al. Proteome-wide profiling of isoniazid targets in *Mycobacterium tuberculosis*. Biochemistry 2006; 45: 13947–13953.
47 Winder F. Mode of action of the antimycobacterial agents and associated aspects of the molecular biology of mycobacteria. In: Ratledge C, Stanford J, eds. The biology of mycobacteria. Vol I. New York, NY, USA: Academic Press, 1982: pp 354–438.
48 Middlebrook G. Isoniazid resistance and catalase activity of tubercle bacilli. Am Rev Tuberc 1954; 69: 471–472.

49 Hazbon M H, Brimacombe M, Bobadilla del Valle M, et al. Population genetics study of isoniazid resistance mutations and evolution of multidrug-resistant *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2006; 50: 2640–2649.

50 Heym B, Alzari P M, Honore N, et al. Missense mutations in the catalase-peroxidase gene, *kat*G, are associated with isoniazid resistance in *Mycobacterium tuberculosis*. Mol Microbiol 1995; 15: 235–245.

51 Deretic V, Philipp W, Dhandayuthapani S, et al. *Mycobacterium tuberculosis* is a natural mutant with an inactivated oxidative-stress regulatory gene: implications for sensitivity to isoniazid. Mol Microbiol 1995; 17: 889–900.

52 Sherman D R, Sabo P J, Hickey M J, et al. Disparate responses to oxidative stress in saprophytic and pathogenic mycobacteria. Proc Natl Acad Sci USA 1995; 92: 6625–6629.

53 Heym B, Stavropoulos E, Honore N, et al. Effects of overexpression of the alkyl hydroperoxide reductase AhpC on the virulence and isoniazid resistance of *Mycobacterium tuberculosis*. Infect Immun 1997; 65: 1395–1401.

54 Vilcheze C, Av-Gay Y, Attarian R, et al. Mycothiol biosynthesis is essential for ethionamide susceptibility in *Mycobacterium tuberculosis*. Mol Microbiol 2008; 69: 1316–1329.

55 Mitchison D A. The action of anti-tuberculosis drugs in short course chemotherapy. Tubercle 1985; 66: 219–225.

56 Telenti A, Imboden P, Marchesi F, et al. Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*. Lancet 1993; 341: 647–650.

57 Bodmer T, Zurcher G, Imboden P, et al. Mutation position and type of substitution in the beta-subunit of the RNA polymerase influence in-vitro activity of rifamycins in rifampicin-resistant *Mycobacterium tuberculosis*. J Antimicrob Chemother 1995; 35: 345–348.

58 Williams D L, Spring L, Collins L, et al. Contribution of *rpo*B mutations to development of rifamycin cross-resistance in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 1998; 42: 1853–1857.

59 Nakamura M, Harano Y, Koga T. [Isolation of a strain of *M. tuberculosis* which is considered to be rifampicin-dependent, from a patient with long-lasted smear positive and culture difficult (SPCD) mycobacteria]. Kekkaku 1990; 65: 569–574. [Japanese]

60 Zhong M, Wang Y, Sun C, et al. [Growth of rifampin-dependent *Mycobacterium tuberculosis* in conditions without rifampin]. Zhonghua Jie He He Hu Xi Za Zhi 2002; 25: 588–590. [Chinese]

61 Tarshis M S, Weed W A Jr. Lack of significant in vitro sensitivity of *Mycobacterium tuberculosis* to pyrazinamide on three different solid media. Am Rev Tuberc 1953; 67: 391–395.

62 McDermott W, Tompsett R. Activation of pyrazinamide and nicotinamide in acidic environment in vitro. Am Rev Tuberc 1954; 70: 748–754.

63 Wade M M, Zhang Y. Anaerobic incubation conditions enhance pyrazinamide activity against *Mycobacterium tuberculosis*. J Med Microbiol 2004; 53: 769–773.

64 Wade M M, Zhang Y. Effects of weak acids, UV and proton motive force inhibitors on pyrazinamide activity against *Mycobacterium tuberculosis* in vitro. J Antimicrob Chemother 2006; 58: 936–941.

65 Zhang Y, Wade M M, Scorpio A, et al. Mode of action of pyrazinamide: disruption of *Mycobacterium tuberculosis* membrane transport and energetics by pyrazinoic acid. J Antimicrob Chemother 2003; 52: 790–795.

66 Scorpio A, Zhang Y. Mutations in *pnc*A, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. Nat Med 1996; 2: 662–667.

67 Zhang Y, Scorpio A, Nikaido H, et al. Role of acid pH and deficient efflux of pyrazinoic acid in unique susceptibility of *Mycobacterium tuberculosis* to pyrazinamide. J Bacteriol 1999; 181: 2044–2049.

68 Zimhony O, Cox J S, Welch J T, et al. Pyrazinamide inhibits the eukaryotic-like fatty acid synthetase I (FASI) of *Mycobacterium tuberculosis*. Nat Med 2000; 6: 1043–1047.

69 Boshoff H I, Mizrahi V, Barry C E III. Effects of pyrazinamide on fatty acid synthesis by whole mycobacterial cells and purified fatty acid synthase I. J Bacteriol 2002; 184: 2167–2172.

70 Konno K, Feldmann F M, McDermott W. Pyrazinamide susceptibility and amidase activity of tubercle bacilli. Am Rev Respir Dis 1967; 95: 461–469.

71 Scorpio A, Lindholm-Levy P, Heifets L, et al. Characterization of *pnc*A mutations in pyrazinamide-resistant *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 1997; 41: 540–543.

72 Cheng S J, Thibert L, Sanchez T, et al. *pnc*A mutations as a major mechanism of pyrazinamide resistance in *Mycobacterium tuberculosis*: spread of a monoresistant strain in Quebec, Canada. Antimicrob Agents Chemother 2000; 44: 528–532.

73 Sreevatsan S, Pan X, Zhang Y, et al. Mutations associated with pyrazinamide resistance in *pnc*A of *Mycobacterium tuberculosis* complex organisms. Antimicrob Agents Chemother 1997; 41: 636–640.

74 Hirano K, Takahashi M, Kazumi Y, et al. Mutation in *pnc*A is a major mechanism of pyrazinamide resistance in *Mycobacterium tuberculosis*. Tubercle Lung Dis 1997; 78: 117–122.

75 Lemaitre N, Sougakoff W, Truffot-Pernot C, et al. Characterization of new mutations in pyrazinamide-resistant strains of *Mycobacterium tuberculosis* and identification of conserved regions important for the catalytic activity of the pyrazinamidase PncA. Antimicrob Agents Chemother 1999; 43: 1761–1763.

76 Marttila H J, Marjamaki M, Vyshnevskaya E, et al. *pnc*A mutations in pyrazinamide-resistant *Mycobacterium tuberculosis* isolates from northwestern Russia. Antimicrob Agents Chemother 1999; 43: 1764–1766.

77 Morlock G P, Crawford J T, Butler W R, et al. Phenotypic characterization of *pnc*A mutants of *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2000; 44: 2291–2295.

78 Portugal I, Barreiro L, Moniz-Pereira J, et al. *pnc*A mutations in pyrazinamide-resistant *Mycobacterium tuberculosis* isolates in Portugal. Antimicrob Agents Chemother 2004; 48: 2736–2738.

79 Jureen P, Werngren J, Toro J C, et al. Pyrazinamide resistance and *pnc*A gene mutations in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2008; 52: 1852–1854.

80 Mestdagh M, Fonteyne P A, Realini L, et al. Relationship between pyrazinamide resistance, loss of pyrazinamidase activity, and mutations in the *pnc*A locus in multidrug-resistant clinical isolates of *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 1999; 43: 2317–2319.

81 Takayama K, Kilburn J. Inhibition of synthesis of arabinogalactan by ethambutol in *Mycobacterium smegmatis*. Antimicrob Agents Chemother 1989; 33: 1493–1499.

82 Mikusov K, Slayden R, Besra G, et al. Biogenesis of the mycobacterial cell wall and the site of action of ethambutol. Antimicrob Agents Chemother 1995; 39: 2484–2489.

83 Wolucka B, McNeil M, de Hoffmann E, et al. Recognition of the lipid intermediate for arabinogalactan/arabinomannan biosynthesis and its relation to the mode of action of ethambutol on mycobacteria. J Biol Chem 1994; 269: 23328–23335.

84 Telenti A, Philipp W J, Sreevatsan S, et al. The *emb* operon, a unique gene cluster of *Mycobacterium tuberculosis* involved in resistance to ethambutol. Nature Med 1997; 3: 567–570.

85 Belanger A, Besra G, Ford M, et al. The embAB genes of *Mycobacterium avium* encode an arabinosyl transferase involved in cell wall arabinan biosynthesis that is the target for the antimycobacterial drug ethambutol. Proc Natl Acad Sci USA 1996; 93: 11919–11924.

86 Heifets L, Desmond E. Clinical mycobacteriology laboratory. In: Cole S, Eisenach K, McMurray D, Jacobs W Jr, eds. Tuberculosis and the tubercle bacillus. Washington DC, USA: ASM Press, 2005: pp 49–70.

87 Ramaswamy S V, Amin A G, Goksel S, et al. Molecular genetic analysis of nucleotide polymorphisms associated with ethambutol resistance in human isolates of *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2000; 44: 326–336.
88 Sreevatsan S, Stockbauer K, Pan X, et al. Ethambutol resistance in *Mycobacterium tuberculosis*: critical role of embB mutations. Antimicrob Agents Chemother 1997; 41: 1677–1681.
89 Mokrousov I, Otten T, Vyshnevskiy B, et al. Detection of *embB306* mutations in ethambutol-susceptible clinical isolates of *Mycobacterium tuberculosis* from northwestern Russia: implications for genotypic resistance testing. J Clin Microbiol 2002; 40: 3810–3813.
90 Hazbon M H, Bobadilla del Valle M, Guerrero M I, et al. Role of *embB* codon 306 mutations in *Mycobacterium tuberculosis* revisited: a novel association with broad drug resistance and IS*6110* clustering rather than ethambutol resistance. Antimicrob Agents Chemother 2005; 49: 3794–3802.
91 Shi R, Zhang J, Otomo K, et al. Lack of correlation between *embB* mutation and ethambutol MIC in *Mycobacterium tuberculosis* clinical isolates from China. Antimicrob Agents Chemother 2007; 51: 4515–4517.
92 Perdigao J, Macedo R, Ribeiro A, et al. Genetic characterisation of the ethambutol resistance-determining region in *Mycobacterium tuberculosis*: prevalence and significance of embB306 mutations. Int J Antimicrob Agents 2009; 33: 334–338.
93 Parsons L M, Salfinger M, Clobridge A, et al. Phenotypic and molecular characterization of *Mycobacterium tuberculosis* isolates resistant to both isoniazid and ethambutol. Antimicrob Agents Chemother 2005; 49: 2218–2225.
94 Safi H, Sayers B, Hazbon M H, et al. Transfer of *embB* codon 306 mutations into clinical *Mycobacterium tuberculosis* strains alters susceptibility to ethambutol, isoniazid, and rifampin. Antimicrob Agents Chemother 2008; 52: 2027–2034.
95 Alcaide F, Pfyffer G E, Telenti A. Role of *emb*B in natural and acquired resistance to ethambutol in mycobacteria. Antimicrob Agents Chemother 1997; 41: 2270–2273.
96 Davies J, Gorini L, Davis B. Misreading of RNA codewords induced by aminoglycoside antibiotics. Mol Pharmacol 1965; 1: 93–106.
97 Finken M, Kirschner P, Meier A, et al. Molecular basis of streptomycin resistance in *Mycobacterium tuberculosis*: alterations of the ribosomal protein S12 gene and point mutations within a functional 16S ribosomal RNA pseudoknot. Mol Microbiol 1993; 9: 1239–1246.
98 Honore N, Cole S T. Streptomycin resistance in mycobacteria. Antimicrob Agents Chemother 1994; 38: 238–242.
99 Nair J, Rouse D A, Bai G H, et al. The *rps*L gene and streptomycin resistance in single and multiple drug-resistant strains of *Mycobacterium tuberculosis*. Mol Microbiol 1993; 10: 521–527.
100 Honore N, Marchal G, Cole S T. Novel mutation in 16S rRNA associated with streptomycin dependence in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 1995; 39: 769–770.
101 Cooksey R C, Morlock G P, McQueen A, et al. Characterization of streptomycin resistance mechanisms among *Mycobacterium tuberculosis* isolates from patients in New York City. Antimicrob Agents Chemother 1996; 40: 1186–1188.
102 Okamoto S, Tamaru A, Nakajima C, et al. Loss of a conserved 7-methylguanosine modification in 16S rRNA confers low-level streptomycin resistance in bacteria. Mol Microbiol 2007; 63: 1096–1106.
103 Spies F S, da Silva P E, Ribeiro M O, et al. Identification of mutations related to streptomycin resistance in clinical isolates of *Mycobacterium tuberculosis* and possible involvement of efflux mechanism. Antimicrob Agents Chemother 2008; 52: 2947–2949.
104 Alangaden G, Kreiswirth B, Aouad A, et al. Mechanism of resistance to amikacin and kanamycin in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 1998; 42: 1295–1297.
105 Suzuki Y, Katsukawa C, Tamaru A, et al. Detection of kanamycin-resistant *Mycobacterium tuberculosis* by identifying mutations in the 16S rRNA gene. J Clin Microbiol 1998; 36: 1220–1225.
106 Maus C E, Plikaytis B B, Shinnick T M. Mutation of *tly*A confers capreomycin resistance in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2005; 49: 571–577.
107 Johansen S, Maus C, Plikaytis B, et al. Capreomycin binds across the ribosomal subunit interface using tlyA-encoded 2′-O-methylations in 16S and 23S rRNAs. Mol Cell 2006; 23: 173–182.
108 Maus C E, Plikaytis B B, Shinnick T M. Molecular analysis of cross-resistance to capreomycin, kanamycin, amikacin, and viomycin in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2005; 49: 3192–3197.
109 Drlica K, Malik M. Fluoroquinolones: action and resistance. Curr Top Med Chem 2003; 3: 249–282.
110 Takiff H, Salazar L, Guerrero C, et al. Cloning and nucleotide sequence of *Mycobacterium tuberculosis* gyrA and gyrB genes and detection of quinolone resistance mutations. Antimicrob Agents Chemother 1994; 38: 773–780.
111 Alangaden G J, Manavathu E K, Vakulenko S B, et al. Characterization of fluoroquinolone-resistant mutant strains of *Mycobacterium tuberculosis* selected in the laboratory and isolated from patients. Antimicrob Agents Chemother 1995; 39: 1700–1703.
112 Cheng A F, Yew W W, Chan E W, et al. Multiplex PCR amplimer conformation analysis for rapid detection of *gyrA* mutations in fluoroquinolone-resistant *Mycobacterium tuberculosis* clinical isolates. Antimicrob Agents Chemother 2004; 48: 596–601.
113 Pitaksajjakul P, Wongwit W, Punprasit W, et al. 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 Suppl 4: 228–237.
114 Lee A S, Tang L L, Lim I H, et al. Characterization of pyrazinamide and ofloxacin resistance among drug resistant *Mycobacterium tuberculosis* isolates from Singapore. Int J Infect Dis 2002; 6: 48–51.
115 Sun Z, Zhang J, Zhang X, et al. Comparison of *gyr*A gene mutations between laboratory-selected ofloxacin-resistant *Mycobacterium tuberculosis* strains and clinical isolates. Int J Antimicrob Agents 2008; 31: 115–121.
116 Sulochana S, Narayanan S, Paramasivan C N, et al. Analysis of fluoroquinolone resistance in clinical isolates of *Mycobacterium tuberculosis* from India. J Chemother 2007; 19: 166–171.
117 Sreevatsan S, Pan X, Stockbauer K E, et al. Restricted structural gene polymorphism in the *Mycobacterium tuberculosis* complex indicates evolutionarily recent global dissemination. Proc Natl Acad Sci USA 1997; 94: 9869–9874.
118 Matrat S, Veziris N, Mayer C, et al. Functional analysis of DNA gyrase mutant enzymes carrying mutations at position 88 in the A subunit found in clinical strains of *Mycobacterium tuberculosis* resistant to fluoroquinolones. Antimicrob Agents Chemother 2006; 50: 4170–4173.
119 Wang J Y, Lee L N, Lai H C, et al. Fluoroquinolone resistance in *Mycobacterium tuberculosis* isolates: associated genetic mutations and relationship to antimicrobial exposure. J Antimicrob Chemother 2007; 59: 860–865.
120 Kocagoz T, Hackbarth C, Unsal I, et al. Gyrase mutations in laboratory selected, fluoroquinolone-resistant mutants of *Mycobacterium tuberculosis* H37Ra. Antimicrob Agents Chemother 1996; 40: 1768–1774.

121 Zhou J, Dong Y, Zhao X, et al. Selection of antibiotic-resistant bacterial mutants: allelic diversity among fluoroquinolone-resistant mutations. J Infect Dis 2000; 182: 517–525.
122 van Doorn H R, An D D, de Jong M D, et al. Fluoroquinolone resistance detection in *Mycobacterium tuberculosis* with locked nucleic acid probe real-time PCR. Int J Tuberc Lung Dis 2008; 12: 736–742.
123 Antonova O V, Gryadunov D A, Lapa S A, et al. Detection of mutations in *Mycobacterium tuberculosis* genome determining resistance to fluoroquinolones by hybridization on biological microchips. Bull Exp Biol Med 2008; 145: 108–113.
124 Hegde S S, Vetting M W, Roderick S L, et al. A fluoroquinolone resistance protein from *Mycobacterium tuberculosis* that mimics DNA. Science 2005; 308: 1480–1483.
125 Pasca M R, Guglierame P, Arcesi F, et al. Rv2686c-Rv2687c-Rv2688c, an ABC fluoroquinolone efflux pump in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2004; 48: 3175–3178.
126 DeBarber A, Mdluli K, Bosman M, et al. Ethionamide activation and sensitivity in multidrug-resistant *Mycobacterium tuberculosis*. Proc Natl Acad Sci USA 2000; 97: 9677–9682.
127 Baulard A, Betts J, Engohang-Ndong J, et al. Activation of the pro-drug ethionamide is regulated in mycobacteria. J Biol Chem 2000; 275: 28326–28331.
128 Vannelli T, Dykman A, Ortiz de Montellano P. The antituberculosis drug ethionamide is activated by a flavoprotein monooxygenase. J Biol Chem 2002; 277: 12824–12829.
129 Trnka L. Thiosemicarbazones. In: Bartmann K, ed. Antituberculosis drugs. Berlin, Germany: Spring-Verlag, 1988: pp 92–103.

RÉSUMÉ

La survenue croissante des tuberculoses à germes multirésistants (TB-MDR) et ultrarésistants (TB-XDR) a l'ère de l'infection par le virus de l'immunodéficience humaine (VIH) représente une menace majeure pour la lutte efficiente contre la TB. La résistance aux médicaments chez *Mycobacterium tuberculosis* provient de mutations chromosomiques spontanées survenant à une faible fréquence. La TB clinique à germes résistants aux médicaments apparaît par suite d'une sélection d'origine humaine des modifications génétiques au cours du traitement de la maladie due à la fourniture erratique des médicaments, à une prescription médicale suboptimale et à une adhésion médiocre des patients. Les mécanismes moléculaires de la résistance aux médicaments ont été élucidés pour les principaux médicaments de première et de deuxième ligne que sont la rifampicine, l'isoniazide, le pyrazinamide, l'éthambutol, les aminoglycosides et les fluoroquinolones. Les relations entre la résistance aux médicaments dans les souches de *M. tuberculosis* et leur virulence ou leur contagiosité doivent être étudiées de façon plus approfondie. Une meilleure connaissance des mécanismes de la résistance de *M. tuberculosis* aux médicaments permettrait l'élaboration d'outils rapides de diagnostic moléculaire et permettrait d'entrevoir des possibilités de développement de nouveaux médicaments pour le traitement de la TB.

RESUMEN

La aparición cada vez más frecuente de casos de tuberculosis (TB) multidrogorresistente (MDR) y extensivamente drogorresistente (XDR) en la era de la infección por el virus de la inmunodeficiencia humana (VIH) constituye una amenaza considerable al control eficaz de la TB. La resistencia de *Mycobacterium tuberculosis* a los medicamentos aparece por mutaciones cromosómicas espontáneas de baja frecuencia. La TB clínicamente drogorresistente se presenta en gran medida como consecuencia de una selección de las cepas con alteraciones genéticas determinada por el hombre durante el tratamiento de la enfermedad ; las causas de esta selección son la inconstancia en el suministro de los medicamentos, la prescripción incorrecta por parte de los médicos y el incumplimiento terapéutico de los pacientes. Ya se han elucidado los mecanismos moleculares de resistencia a los principales medicamentos antituberculosos de primera y segunda línea como la rifampicina, la isoniazida, la pirazinamida, el etambutol, los aminoglucósidos y las fluoroquinolonas. Sin embargo, la relación entre la drogorresistencia de algunas cepas de *M. tuberculosis* y su virulencia y transmisibilidad precisa nuevas investigaciones. La comprensión de los mecanismos de resistencia de *M. tuberculosis* a los medicamentos permitirá la elaboración de herramientas moleculares de diagnóstico rápido y ofrecerá posibles hipótesis de trabajo en el desarrollo de nuevos medicamentos antituberculosos.