Mechanisms of Action and Resistance of the Antimycobacterial Agents

25

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1 Introduction

Historically, mycobacterial infections have been associated with significant morbidity and mortality worldwide. In particular, *Mycobacterium tuberculosis* is a highly successful human pathogen, causing ~8.7 million cases of active tuberculosis (TB) and ~1.4 million deaths annually [1]. The organism is unique in its ability to establish persistent infection, requiring prolonged treatment with antimicrobials in order to achieve clinical cure. In general, the goals of antituberculosis therapy include rapid reduction of the massive numbers of actively multiplying bacilli in the diseased host, prevention of acquired drug resistance, and sterilization of infected host tissues to prevent clinical relapse. In order to achieve these goals, currently accepted guidelines recommend administration of multiple active drugs for a minimum duration of 6 months [2]. In areas where drug resistance is prevalent and resources permit, *M. tuberculosis* clinical isolates should be routinely tested for susceptibility to first-line antituberculosis agents in order to optimally guide therapy. The emergence of multidrug-resistant TB (MDR-TB) [3], defined as resistance to the first-line drugs isoniazid and rifampicin, extensively drug-resistant TB (XDR-TB), defined as MDR-TB with additional resistance to fluoroquinolones and at least one of the injectable second-line drugs (capreomycin, kanamycin, and amikacin), and totally drug-resistant tuberculosis (TDR-TB), loosely used for TB strains resistant to a wider range of drugs than those classified as XDR-TB, poses formidable challenges to global TB control efforts [4–6]. The global incidence of MDR-TB is estimated to be ~500,000 cases annually, of which 5–7 % represent XDR-TB.

Prior to the advent of highly active antiretroviral therapy (HAART), disseminated infection with *M. avium* complex was the most common bacterial opportunistic infection in adults infected with HIV-1 in the developed world, occurring annually in 10–20 % of individuals with AIDS [7, 8]. The availability of HAART, as well as the use of effective prophylaxis with azithromycin or clarithromycin, has reduced the annual incidence of disseminated *M. avium* complex infection among individuals with advanced HIV disease to less than 1 % per year [9]. Nevertheless, *M. avium* complex continues to cause disseminated disease in persons with HIV and advanced immunosuppression not receiving or unable to tolerate HAART. In addition, *M. avium* complex is an important cause of pulmonary infection, particularly in HIV-negative persons with underlying lung disease or other immunosuppression [10]. Infections with other mycobacteria, including *M. kansasii*, *M. genavense*, *M. hemophilum*, *M. fortuitum*, *M. xenopi*, *M. chelonae*, have been reported with increasing frequency, particularly in the setting of HIV infection [11–13].

This chapter will review the mechanisms of action and resistance of the antimycobacterial agents (Table 25.1), with an emphasis on the four first-line antituberculosis drugs isoniazid, rifampin, pyrazinamide, and ethambutol. The mechanisms of action of other drugs used to treat mycobacterial infections, including the fluoroquinolones, aminoglycosides, and the macrolides, will be reviewed elsewhere in this book and this chapter will focus on specific mutations associated with resistance to these agents in *M. tuberculosis* and *M. avium* complex. This chapter includes mechanistic studies carried out in *M. smegmatis*, which, because of its relatedness to *M. tuberculosis*, its fast-growing nature and lack of pathogenicity, and its relative genetic tractability, is widely used as a model system to study mycobacterial physiology. The phenomenon of *M. tuberculosis* phenotypic drug tolerance will not be addressed in this section, and discussion will be limited to genotypic mechanisms of drug resistance.

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Table 25.1 Mechanisms of action and resistance of the antimycobacterial agents

Drug/drug class	Cellular process inhibited	Drug target	Resistance mutations in clinical isolates	Frequency	Comments
Isoniazid (INH)	Mycolic acid synthesis	InhA	*katG* (S315T)	50–80 %	INH is a pro-drug requiring activation by the *M. tuberculosis* catalase-peroxidase KatG [26]
			inhA	15–34 %	
Rifampin	mRNA synthesis	RNA polymerase β subunit	*rpoB* (codons 507–533)	>90 %	>90 % of rifampin-resistant isolates are also resistant to INH
Pyrazinamide (PZA)	Depletion of membrane energy	Unknown	*pncA*	70–90 %	PZA is a pro-drug requiring activation by *M. tuberculosis* pyrazinamidase, which is encoded by *pncA* [153]
Ethambutol	Arabinogalactan synthesis	EmbB	*embB*	50–70 %	*embB* mutations may not be sufficient to confer resistance to EMB [202]
Streptomycin	Translation	30 S ribosomal subunit	*rpsL* (codons 43 and 88)	~50 %	Cross-resistance may not be observed with kanamycin or amikacin
			rrs	~20 %	
Amikacin/ Kanamycin	Translation	30 S ribosomal subunit	*rrs* (codon 1400)		Cross-resistance is observed with capreomycin, but not with streptomycin
Fluoroquinolones	DNA synthesis and transcription	DNA gyrase	*gyrA*	42–85 %	Cross-resistance is generally observed among the fluoroquinolones
			gyrB		
Macrolides	Translation	50 S ribosomal subunit	23S rRNA gene (Domain V loop)		Mechanisms of action and resistance listed are for *M. avium* complex; *M. tuber-culosis* is inherently resistant to the macrolides
Ethionamide	Mycolic acid synthesis	InhA	*ethA*		Ethionamide is a pro-drug requiring activation by the monooxygenase EthA [290, 318]
			inhA		
Capreomycin	Translation	16S rRNA	*rrs*		Cross-resistance is observed with kanamycin/amikacin
Cycloserine	Peptidoglycan synthesis	AlrA	*alrA*		Mechanisms of resistance have been shown in *M. smegmatis*, but not in *M. tuberculosis*
		Ddl			
Paraaminosalicylic acid (PAS)	Folic acid biosynthesis	Unknown	*thyA*		The mechanisms of action and resistance for PAS remain poorly characterized
	Iron uptake?				

2 Isoniazid

Isoniazid (isonicotinic acid hydrazide [INH]) has been the most commonly used drug in the armamentarium against *M. tuberculosis* since recognition of its clinical activity by Robitzek and Selikoff in 1952 [14]. Consisting of a pyridine ring and a hydrazide group (Fig. 25.1), INH is a nicotinamide analog structurally related to the antituberculosis drugs ethionamide and pyrazinamide [15]. Because of its significant bactericidal activity, it has become a critical component of first-line antituberculous chemotherapy. However, in the last two decades, resistance to INH has been reported with increasing frequency, ranging from 3 % to as high as one-quarter of all *M. tuberculosis* isolates from previously untreated individuals [16–20], with the highest rates of resistance reported from southeast Asia and the Russian Federation [19, 21].

2.1 Mechanism of Action

Despite the widespread use of INH for more than half a century, its mechanism of action has only recently begun to be elucidated. The drug appears to penetrate host cells readily [22, 23], and diffuses across the *M. tuberculosis* membrane [24, 25]. INH is a pro-drug, requiring oxidative activation by the *M. tuberculosis katG*-encoded catalase-peroxidase enzyme [26]. The resulting isonicotinoyl radical reacts non-enzymatically with oxidized NAD^+ to generate several different 4-isonicotinoyl-NAD adducts [27]. Although the active metabolites of INH have been reported to inhibit multiple essential cellular pathways, including synthesis of nucleic acids [28] and phospholipids [29], and NAD metabolism [30, 31], the primary pathway inhibited by the drug appears to be the synthesis of mycolic acids [32–34], manifesting as a loss of acid-fast staining of the organisms following INH treatment [35].

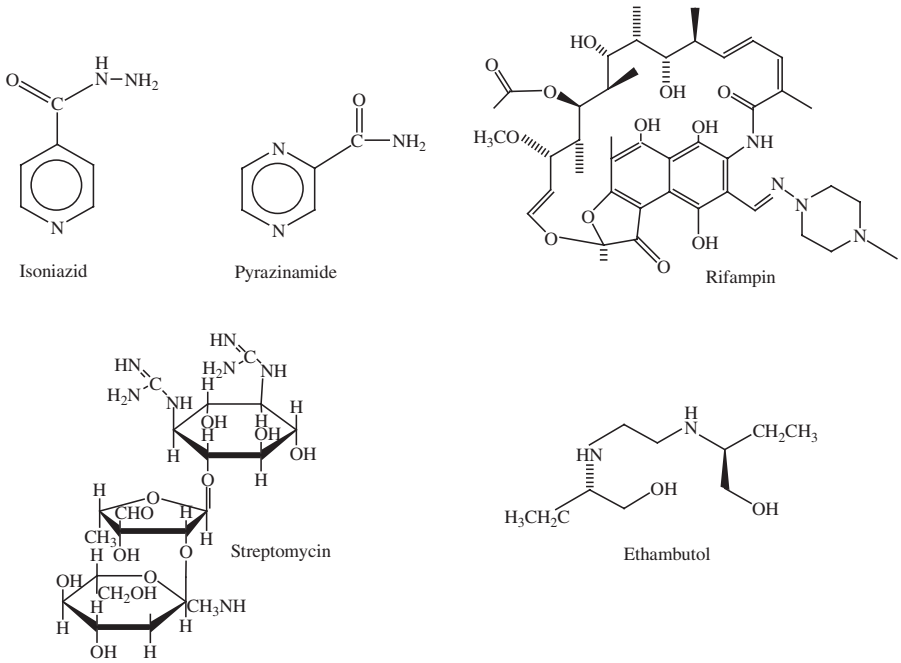

Fig. 25.1 Chemical structures of frontline TB drugs

Mycolic acids are high-molecular-weight α-alkyl, β-hydroxy fatty acids, which are unique outer cell wall components of mycobacteria and other Actinomycetales [36]. Mycolic acids are covalently attached to arabinogalactan and, together with other lipids of the outer leaflet, constitute a very hydrophobic barrier [37] responsible for resistance to certain drugs [38, 39]. Disruption of this hydrophobic barrier is believed to result in a loss of cellular integrity [40]. INH interrupts mycolic acid synthesis by binding tightly to the NADH-dependent enoyl acyl carrier protein (ACP) reductase InhA [41], a component of the fatty acid synthase II system of mycobacteria, which is essential for fatty acid elongation [42]. Genetic, biochemical, and structural data provide compelling evidence that InhA is the primary target for INH in the mycolic acid synthesis pathway. When transferred on a multicopy plasmid, the wild-type *inhA* gene of *M. tuberculosis* or *M. smegmatis* confers INH resistance to *M. smegmatis* and *M. bovis* BCG [41], as well as to *M. tuberculosis* [43]. A missense mutation within the mycobacterial *inhA* gene leading to the amino acid substitution S94A confers INH resistance to *M. smegmatis* [41] and *M. bovis* [44]. In addition, the same single point mutation in *inhA* (S94A) was sufficient to cause fivefold increased resistance to INH and inhibition of mycolic acid biosynthesis in *M. tuberculosis* [45]. Interestingly, overexpression of or mutation within *inhA* also confers resistance to the structurally related second-line antituberculosis drug ethionamide in *M. tuberculosis*, *M. smegmatis*, and *M. bovis*, suggesting that *inhA* encodes the target of both INH and ethionamide in these mycobacteria [41]. In addition, enoyl reductases, and specifically mycobacterial InhA, have been shown to be targets for the widely used topical disinfectant triclosan, and particular *M. smegmatis* mutants in *inhA* are cross-resistant to INH and triclosan [46]. However, although it affects InhA function, INH does not directly interact with InhA. Biochemical and structural studies have shown that InhA catalyzes the NADH-specific reduction of 2-trans-enoyl-ACP, and that the INH-resistant phenotype of S94A mutant InhA is related to reduced NADH binding [42, 47]. X-ray crystallographic and mass spectrometry data revealed that the activated form of INH covalently attaches to the nicotinamide ring of NAD bound within the active site of InhA, causing NADH to dissociate from InhA [47, 48].

Although inhibition of DNA synthesis by INH had been observed long ago [28], only relatively recently was a mechanism of action for this phenomenon proposed. Argyrou and colleagues cloned and overexpressed the *M. tuberculosis* gene encoding dihydrofolate reductase (DHFR), *dfrA*, in *M. smegmatis* and demonstrated a twofold increase in MIC [49]. *M. tuberculosis* DHFR was shown to selectively bind and co-crystallize with an active INH metabolite which is distinct from that which binds InhA [48]. However, this work requires further biochemical and genetic confirmation. Mutations in *dfrA* have yet to be reported among INH-resistant clinical isolates of *M. tuberculosis*.

Despite the identification of specific cellular targets in the last 15 years, the precise mechanism by which INH kills *M. tuberculosis* remains elusive. Interestingly, depletion of mycolic acids does not necessarily result in loss of viability in other mycobacteria in vitro [50, 51]. However, inhibition of mycolic acid synthesis may more severely compromise the intracellular survival of *M. tuberculosis* in vivo. It remains

to be shown that inhibition of mycolic acid synthesis is both necessary and sufficient for the highly potent in vivo bactericidal activity of INH against *M. tuberculosis*.

2.2 Mechanisms of Drug Resistance

Spontaneous INH resistance may be observed at a rate of 10^{-5} to 10^{-6} per bacterium per generation in *M. tuberculosis* cultures grown in vitro [52]. Because INH is the most commonly used antituberculosis drug, resistance to INH occurs more frequently among clinical isolates than to any other agent [53]. INH resistance varies geographically [54], and may be as high as 20–30 % in some parts of the world [19, 21]. Mutations are most commonly detected in the *katG* gene, occurring in 50–80 % of INH-resistant clinical isolates, or in the *inhA* gene, accounting for 15–34 % of INH resistance [53]. Depending on the mutation, the degree of INH resistance may vary from low (0.2 μg/mL) to high (100 μg/mL) [55]. Interestingly, a report from South Africa noted a higher frequency of mutations in the *inh*A region among patients with XDR-TB than among those with MDR-TB. In this study, mutations in the *inh*A promoter region accounted for ~50 to 60 % of cases of MDR-TB and 85–90 % of XDR-TB [56].

2.3 katG

INH resistance among clinical isolates of *M. tuberculosis* has long been associated with loss of catalase and peroxidase enzyme activities [57]. In general, there is a strong inverse correlation between degree of INH resistance and catalase activity [58]. Zhang and colleagues first demonstrated that deficiency of *katG*, which encodes the *M. tuberculosis* catalase-peroxidase enzyme, accounts for the observed resistance to INH in drug-resistant clinical isolates of *M. tuberculosis* [26, 59]. Mutations in KatG reduce the ability of the enzyme to activate the pro-drug INH, thus leading to resistance. The *M. tuberculosis katG* gene is situated in a highly variable and unstable region of the genome, perhaps because of the presence of repetitive DNA sequences [60], thereby potentially predisposing to a high frequency of *katG* mutations. Point mutations in *katG* are more common than deletions in INH-resistant clinical isolates, and a single point mutation resulting in substitution of threonine for serine at residue 315 (S315T) accounts for the majority of INH-resistant clinical isolates [61–63]. The S315T mutation is associated with a 50 % reduction in catalase and peroxidase activity and high-level INH resistance (MIC = 5–10 μg/mL) [58, 64]. The availability of the crystal structure for *M. tuberculosis* KatG [65] has provided greater insight into the process of INH activation and may permit a more accurate interpretation of structural and functional effects of mutations implicated in causing INH resistance in clinical isolates. Down-regulation of *katG* expression has also been shown recently to be associated with resistance to INH [66]. Three novel mutations in the *furA-katG* intergenic region were identified in 4 % of 108 INH-resistant strains studied; none of these was present in 51 INH-susceptible strains. Reconstructing these mutations in the *furA-katG* intergenic region of isogenic strains decreased the expression of *katG* and conferred resistance to INH.

2.4 inhA

INH resistance may arise either from mutations in *inhA*, resulting in reduced affinity of the enzyme for NADH without affecting its enoyl reductase activity [67], or in the promoter region of the *mabAinhA* operon [63], resulting in overexpression of the wild-type enzyme. Mutations in the *mabA* promoter region appear to be more frequent, but overexpression of MabA alone does not confer INH resistance in mycobacteria [68]. Unlike mutations in *katG*, which can confer low-level or high-level INH resistance, depending on the extent to which catalase-peroxidase enzyme activity is affected, mutations in *inhA* or in the promoter region of its operon usually confer low-level resistance (MIC = 0.2–1 μg/mL) [69]. Similarly, mutations in the intergenic region *oxyR-ahpC* can reduce the level of expression of *inhA* and have been associated with resistance to INH. A study by Dalla Costa et al. [70] found mutations in the intergenic region *oxyR-ahpC* in 8.9 % of 224 INH-resistant strains studied, confirming its less frequent involvement as a cause of resistance to INH. However, the precise role of these genes in INH resistance has not been completely elucidated.

2.5 Other Genes

The role of mutations in *kasA*, which encodes a β-ketoacyl ACP synthase of the type II fatty acid synthase system, with respect to INH resistance is controversial. Initial reports identified an association between clinical INH resistance and four independent mutations in *kasA* [71], but subsequent studies reported the presence of three of these mutations in INH-sensitive *M. tuberculosis* strains [72, 73]. In addition, although one group reported a fivefold increase in the MIC of INH following *kasA* overexpression in *M. tuberculosis* [74], another group found that overexpression of *kasA* conferred resistance to thiolactomycin, a known KasA inhibitor, but no increased resistance to INH in *M. smegmatis*, *M. bovis* BCG, and *M. tuberculosis* [43]. Using radioactive INH, Mdluli and colleagues reported KasA to be covalently associated with INH and ACP in *M. tuberculosis* [71], but Kremer and colleagues used anti-KasA antibodies to show that INH

treatment in mycobacteria does not result in significant KasA sequestering, and also demonstrated that activated INH does not inhibit KasA activity in an in vitro assay [75]. Although the preponderance of evidence suggests that InhA, and not KasA, is the primary target of INH in the mycolic acid synthesis pathway, the role of *kasA* mutations in INH resistance of clinical *M. tuberculosis* isolates requires further investigation.

Mutations in *ndh*, encoding a NADH dehydrogenase, were first shown in *M. smegmatis* to confer resistance to INH and ethionamide, as well as to exhibit other phenotypes, including thermosensitive lethality and auxotrophy [76]. Subsequently, *ndh* mutations were detected in almost 10 % of INH-resistant *M. tuberculosis* clinical isolates, which did not contain mutations in *katG*, *inhA*, or *kasA* [72]. Defective NADH dehydrogenase, which normally oxidizes NADH and transfers electrons to quinones of the respiratory chain, could lead to an increased ratio of NADH/NAD, which may interfere with KatG-mediated peroxidation of the drug, or displace the INH/NAD adduct from the InhA active site [76].

Mutations in the promoter region of *ahpC*, leading to overexpression of an alkylhydroperoxide reductase, have been observed in INH-resistant *M. tuberculosis* [77]. Although rarely found in some INH-resistant strains with apparently intact KatG [78], the *ahpC* mutation is usually found in KatG-negative INH-resistant *M. tuberculosis*, presumably as a compensatory mechanism for the loss of catalase-peroxidase activity in such strains [79–81]. AhpC does not appear to play a direct role in INH resistance, since *ahpC* overexpression in a wild-type reference strain of *M. tuberculosis* does not appreciably increase the MIC of INH, but mutations in the *ahpC* promoter region may serve as a useful marker for detection of INH resistance [78].

The *M. tuberculosis iniA* gene (Rv0342), part of a three-gene operon (Rv0341, Rv0342, Rv0343) induced in the presence of INH, appears to contribute to the development of tolerance to both INH and ethambutol, perhaps functioning through an MDR-pump-like mechanism, although IniA does not appear to directly transport INH from the cell [82]. INH also induces several other genes, including an operon cluster of five genes that code type II fatty acid synthase enzymes and *fbpC*, which encodes trehalose dimycolyl transferase. Other genes also induced are *efpA*, *fadE23*, *fadE24*, and *ahpC*, which mediate processes linked to the toxic activity of the drug and efflux mechanisms [83].

Despite the identification of several genetic mutations associated with resistance to INH, as many as a quarter of all clinical INH-resistant isolates do not have mutations in any of the above genes, suggesting alternative mechanisms of INH resistance. Tessema et al. reported 8 % of phenotypically defined isoniazid-resistant strains had no mutations in codon 315 of the *katG* gene and in the regulatory region of the *inhA* gene, demonstrating that other mechanisms or mutations in other codons of the katG gene may be responsible for the development of INH resistance in *M. tuberculosis* strains [84].

3 Rifampin and Other Rifamycins

The rifamycins were first isolated in 1957 from *Amycolatopsis* (formerly *Streptomyces*) *mediterranei* as part of an antibiotic screening program in Italy [85]. Their discovery and widespread use has revolutionized antituberculosis therapy, allowing for the reduction of the duration of treatment from 18 months to 9 months [86]. Although the early bactericidal activity of the rifamycins is inferior to that of INH [87–89], the former are the most potent sterilizing agents available in TB chemotherapy, continuing to kill persistent tubercle bacilli throughout the duration of therapy [90, 91]. Rifampin is a broad-spectrum antibiotic and the most widely used rifamycin to treat tuberculosis. Rifabutin, another rifamycin with reduced induction of the hepatic cytochrome P-450 enzyme system, was originally shown to be effective for the prophylaxis [92] and treatment [93] of *M. avium-intracellulare* complex infection in persons with advanced HIV disease. Rifapentine is a rifamycin with favorable pharmacokinetic properties [94, 95], including substantially greater maximum serum concentration and extended half-life, which permits highly intermittent therapy for HIV-negative patients who do not have cavitation on chest radiograph and who are sputum culture-negative after 2 months of therapy [96].

3.1 Mechanism of Action

The rifamycins are characterized by a unique chemical structure consisting of an aromatic nucleus linked on both sides by an aliphatic bridge (see figure) [97]. Although structural changes at positions C-21, C-23, C-8, or C-1 may significantly reduce microbiological activity, modifications at C-3 do not alter antituberculous activity. Rifampin is a 3-formyl derivative of rifamycin S; rifabutin is a spiropiperidyl derivative of rifamycin S; and rifapentine is a cyclopentyl-substituted rifampin [97].

The rifamycins are highly protein-bound in plasma, but easily diffuse across the *M. tuberculosis* cell membrane due to their lipophilic nature [69]. The bactericidal activity of the rifamycins has been attributed to their ability to inhibit mRNA synthesis by binding with high affinity to bacterial DNA-dependent RNA polymerase [98]. The core structure of RNA polymerase, comprising the subunits $\alpha_2\beta\beta'\omega$, is evolutionarily conserved among prokaryotes [99], explaining the antimicrobial activity of the rifamycins against a broad range of bacteria. X-ray crystallographic data examining the interaction of rifampin and RNA polymerase from *Thermus*

aquaticus revealed that rifampin exerts its effect by binding in a pocket between two structural domains of the RNA polymerase β subunit and directly blocking the path of the elongating RNA transcript at the 5′ end beyond the second or third nucleotide [100].

Although the molecular target of rifampin has been well characterized, the precise mechanism by which this interaction leads to mycobacterial killing remains unclear. Interestingly, transcriptional inhibition of the toxin-antitoxin *mazEF* module by rifampin was shown to trigger programmed cell death in *Escherichia coli* by reducing cellular levels of the labile antitoxic protein MazE, allowing the unrestrained lethal action of the long-lived toxic protein MazF [101]. Although *M. tuberculosis* contains homologous toxin-antitoxin gene modules [102, 103], it appears that these modules may play a role in *M. tuberculosis* growth arrest and persistence under adverse conditions, rather than in programmed cell death, as originally suggested [103].

3.2 Mechanism of Resistance

Although resistance to INH alone is common in *M. tuberculosis*, resistance to rifampin alone is rare, and more than 90 % of rifampin-resistant isolates are also resistant to INH. Therefore, rifampin resistance has been used as a surrogate marker for multidrug-resistant tuberculosis [104]. Resistance to rifampin develops in a single step at a frequency of 10^{-7} to 10^{-8} organisms in *M. tuberculosis* [105].

As in *E. coli* [106–108], resistance to rifampin in *M. tuberculosis* arises from mutations in *rpoB*, which encodes the β-subunit of RNA polymerase [109]. Over 90 % of rifampin-resistant clinical isolates contain point mutations clustered in an 81-base pair region between codons 507 and 533 of the *rpoB* gene [110, 111]. Although at least 35 distinct *rpoB* mutant allelic variants have been described [110], amino acid substitutions at one of two positions (Ser_{531} and His_{526}) account for the great majority of mutations conferring clinical resistance to rifampin [109, 111–113]. Consistent with the clinical data, selection of spontaneous rifampin resistance in vitro in the *M. tuberculosis* laboratory reference strain H37Rv yields *rpoB* mutations only at Ser_{531} and His_{526}, with the Ser_{531}Leu mutation predominating [114]. Strains with the point mutations CAC → TAC (His → Tyr) at codon 526 and TCG → TTG (Ser → Leu) at codon 531 account for 30 % and 25 %, respectively, of rifampin-resistant clinical isolates in the USA [112], while the same mutations represent 12 % and 47 %, respectively, of predominantly foreign rifampin-resistant isolates [109], suggesting that there may be geographic variation in the frequency of occurrence of particular *rpoB* mutations [110, 115]. Unlike mutations in codons 531 and 526, which confer high-level resistance to rifampin (MIC > 32 μg/mL) and cross-resistance to all rifamycins [69], mutations in codons 511, 516, and 522 are associated with low- or high-level resistance to rifampin and rifapentine (MIC 2–32 μg/mL), but preservation of susceptibility to rifabutin and the new rifamycin rifalazil [116–118]. In particular, MDR strains with the *rpoB* point mutation Asp516Val were almost always identified as rifabutin-susceptible [119]. Rare mutations in *M. tuberculosis* also have been reported in the 5′ region of the *rpoB* gene, and one such mutation at V176F confers intermediate- to high-level resistance to rifampin [120–123].

Several fast-growing strains of mycobacteria, including *M. smegmatis*, *M. chelonae*, *M. flavescens*, and *M. vaccae*, are able to inactivate rifampin by ribosylation, leading to inherent resistance to this antibiotic [124, 125]. However, this mechanism of rifampin resistance has not been described in *M. tuberculosis*. Nevertheless, a small percentage of rifampin-resistant isolates (<5 %) do not contain any mutations in the *rpoB* gene, suggesting additional molecular mechanisms of rifampin resistance in *M. tuberculosis*, such as altered rifampin permeability or mutations in other RNA polymerase subunits [110].

An important finding related to resistance to rifampicin is that almost all rifampicin-resistant strains also show resistance to other drugs, particularly to isoniazid. For this reason, rifampicin resistance detection has been proposed as a surrogate molecular marker for MDR [126].

4 Pyrazinamide

The use of pyrazinamide (PZA) in combination with rifampin in modern antituberculosis regimens has permitted shortening the duration of therapy from the previous 9–12 months to the current 6 months [127]. PZA is one of the key components of primary drug therapy against TB, especially when MDR has been diagnosed [128, 129]. Although its bactericidal activity is inferior to that of isoniazid and rifampin [130], the reduction of relapse rates associated with the addition of PZA in 6-month regimens is attributed to the drug's unique ability to target semi-dormant populations of bacilli residing within an acidic environment [131]. Consistent with this hypothesis, the drug was shown to be more active against old non-growing tubercle bacilli than against young, actively replicating organisms [132]. Interestingly, despite its established activity in vivo [133–136], PZA is inactive against *M. tuberculosis* grown under normal conditions in vitro [137], and requires acidification of the medium pH to demonstrate antituberculosis activity [138].

4.1 Mechanism of Action

PZA is an amide derivative of pyrazine-2-carboxylic acid and a nicotinamide analog (see figure) [139]. Despite recognition of its antituberculosis activity more than half a century

ago [133], the mechanism of action of PZA remains poorly understood. Because of the strict requirement for an acidic microenvironment, it was originally hypothesized that the site of action of PZA was in the macrophage phagolysosome [140], where intracellular *M. tuberculosis* resides. However, the interior pH of these organelles may be neutral or only slightly acidic [141, 142], well above the range where PZA is active [143]. In addition, although older studies suggested otherwise [140, 144], more recent studies have demonstrated that PZA has neither bacteriostatic nor bactericidal activity against intracellular *M. tuberculosis* in human monocyte-derived macrophages [145]. An alternative hypothesis is that PZA acts against bacilli residing in acidified compartments of the lung that are present during the early inflammatory stages of infection [131], which is consistent with the clinical observation that the potent sterilizing activity of PZA is limited to the first 2 months of therapy [146–148]. Anaerobic and microaerophilic conditions in vitro have been shown to enhance the activity of PZA against *M. tuberculosis*, suggesting an alternative explanation for the higher sterilizing activity of PZA against in vivo organisms residing within oxygen-deprived granulomas as compared to bacilli grown under in vitro conditions with ambient oxygen tension [149].

PZA enters *M. tuberculosis* through passive diffusion and via an ATP-dependent transport system [150]. The drug accumulates intracellularly because of an inefficient efflux system unique to *M. tuberculosis* [151]. Similar to INH, PZA is a pro-drug, which requires activation to its active form, pyrazinoic acid (POA), by the enzyme pyrazinamidase (PncA) [152, 153]. PncA is expressed constitutively in the cytoplasm of *M. tuberculosis* [154]. After conversion of PZA into POA, the drug exerts its cidal effect on tubercle bacilli by destabilizing the membrane potential and affecting membrane transport function [155]. The uptake and accumulation of POA in *M. tuberculosis* is enhanced when the extracellular pH is acidic [151]. The inhibitory effects of POA accumulation initially were attributed to direct inhibition of the mycobacterial fatty acid synthase I (FAS-I) enzyme [156], which is responsible for de novo synthesis of C_{16} fatty acids from acetyl-CoA primers and their elongation to $C_{24–26}$ fatty acyl-CoA derivatives [157, 158]. However, subsequent studies showed that, although the PZA analog 5-chloropyrazinamide irreversibly inhibits fatty acid synthesis through inhibition of FAS-I, POA does not directly inhibit purified mycobacterial FAS-I, suggesting that the enzyme is not the immediate target of PZA [159]. It has been proposed that the antituberculosis activity of PZA is not attributable to inhibition of a specific cellular target, but rather may reflect disruption of the proton motive force required for essential membrane transport functions by POA at acidic pH [160]. These findings could explain the enhanced susceptibility to PZA of old, non-replicating bacilli, which have a relatively low membrane potential [151] and reduced ability to maintain membrane energetics [161], as compared to young, actively replicating organisms [160]. Alternatively, the accumulation of POA or other weak organic acids has been hypothesized to lower the intracellular pH sufficiently to inactivate FAS-I or other vital enzymes required for cellular metabolism [159].

Recently, the ribosomal protein S1 (RpsA), a vital protein involved in protein translation and the ribosome-sparing process of trans-translation, was identified as a target of POA [162]. RpsA overexpression in *M. tuberculosis* conferred increased PZA resistance, and POA was shown to bind RpsA, thereby inhibiting trans-translation. Since the latter process is essential for freeing scarce ribosomes in nonreplicating organisms, inhibition of RpsA by POA may explain the sterilizing activity of PZA against persistent bacilli.

4.2 Mechanisms of Resistance

It has been known for some time that PZA resistance in *M. tuberculosis* is associated with loss of PZase activity [152]. More recently, pyrazinamide resistance has been attributed to mutations in *pncA*, the gene encoding PZase [153]. Consistent with these findings, integration of wild-type *pncA* into a pyrazinamide-resistant *pncA* mutant of *M. tuberculosis* is sufficient to restore susceptibility to PZA [163]. *M. bovis*, another member of the *M. tuberculosis* complex, is inherently resistant to PZA, most frequently because of a point mutation at codon 169 of the *pncA* gene, which renders the enzyme nonfunctional [164]. In contrast, studies of PZA-resistant clinical isolates of *M. tuberculosis* revealed that 72–97 % of these strains may contain various missense mutations, insertions, deletions, or termination mutations throughout the *pncA* gene or its promoter [165–167]. Recent data indicate that three mutations (D8G, S104R, and C138Y) in PncA confer excessive hydrogen bonding between PZA-binding residues and their neighboring residues, creating a rigid binding cavity, which in turn abolishes conversion of PZA into POA [168].

Resistance to PZA is also mediated by mutations in *rpsA* [162], which encodes a protein required for trans-translation in nonreplicating bacilli. A rare type of PZA-resistant isolate was found containing a deleted alanine at the C terminus of RpsA, preventing binding of the protein to tmRNA [162, 169, 170].

A small percentage of isolates with high-level PZA resistance contain no mutation in *pncA* or its promoter, suggesting other potential mechanisms of resistance to the drug [165], including perhaps deficient uptake [150], enhanced efflux, or altered *pncA* regulation. Alternatively, these findings may reflect the intrinsic problems associated with PZA susceptibility testing, since PZA resistance may be reported erroneously when the culture medium contains excessive bovine serum albumin, or a high inoculum of *M. tuberculosis*,

as both of these conditions may raise the pH of the medium and falsely elevate the MIC of the drug [132]. In fact, reliable methods for susceptibility testing of PZA have only recently been developed, using media with slightly higher pH (6.0–6.2) and higher concentrations of PZA (ranging from 300 μg/mL to as high as 1200 μg/mL, depending on the culture broth) [171].

5 Ethambutol

Ethambutol (EMB; dextro-2,2′-(ethylenediimino)-di-1-butanol), a synthetic compound structurally similar to D-arabinose (see figure) [172], was initially reported to have antituberculosis activity in 1961 [173]. In addition to its role as a first-line agent against *M. tuberculosis*, EMB is an important component of combination therapy against *M. avium* complex [9], and the drug exhibits activity against other mycobacteria, including *M. kansasii*, *M. xenopi*, and *M. marinum* [174]. EMB kills only actively multiplying bacilli [175], although its early bactericidal activity is not as potent as that of INH [176, 177]. EMB has poor sterilizing activity, as its addition to a regimen of INH, rifampin, and streptomycin does not improve culture conversion rates after 2 months of therapy [178], and its substitution for PZA increases clinical relapse rates [179]. Because of its modest contribution to the standard regimen of INH, rifampin, and PZA, the principal role of EMB is in the empiric treatment of individuals who are deemed at increased risk for harboring INH-resistant or multidrug-resistant *M. tuberculosis*, until drug susceptibility results become available.

5.1 Mechanism of Action

The mechanism of action of EMB remains incompletely understood. EMB has been reported to inhibit numerous mycobacterial cellular pathways, including RNA metabolism [175, 180], transfer of mycolic acids into the cell wall [181], phospholipid synthesis [182, 183], and spermidine biosynthesis [184]. However, the primary pathway affected by EMB appears to be that of arabinogalactan biosynthesis [185], through inhibition of cell wall arabinan polymerization [186].

Initial studies showed that treatment of *M. smegmatis* with EMB results in rapid bacterial disaggregation and morphological changes, consistent with alterations in cell wall composition [187]. A potential explanation for this phenomenon was provided by the observations that EMB inhibits transfer of mycolic acids to the cell wall in *M. smegmatis* [181], leading to rapid accumulation of trehalose monomycolate, trehalose dimycolate, and free mycolic acids in the culture medium [188]. Subsequently, EMB was shown to inhibit arabinogalactan synthesis, since MIC levels of the drug immediately inhibited the transfer of label from D-[^{14}C] glucose into the D-arabinose residue of arabinogalactan in EMB-susceptible *M. smegmatis*, but not in a drug-resistant strain [185]. In addition to inhibiting the synthesis of the arabinan component of the mycobacterial cell wall core polymer arabinogalactan, EMB inhibits biosynthesis of the arabinan of lipoarabinomannan, a lipoglycan noncovalently associated with the cell envelope [189, 190]. The observations that the latter effect is delayed relative to the former [186], and that EMB treatment results in rapid accumulation of β-D-arabinofuranosyl-1-monophosphoryldecaprenol (decaprenol phosphoarabinose) [191], an intermediate in arabinan biosynthesis, suggested that the primary site of EMB action is not on de novo synthesis of D-arabinose or on its activation, but rather in the final polymerization steps [186].

Using target overexpression by a plasmid vector as a selection tool, Belanger et al. demonstrated that the translationally coupled *embA* and *embB* genes of *M. avium* are both necessary and sufficient to render a naturally susceptible *M. smegmatis* strain resistant to EMB [192]. Subsequently, the *embCAB* gene cluster encoding the homologous arabinosyl transferase enzymes EmbA, EmbB, and EmbC was cloned, sequenced, and characterized in *M. tuberculosis* [193]. Although it has been proposed that these genes constitute an operon, there is evidence to suggest that the *embB* gene can be expressed from a unique promoter [194], the location of which remains unknown. The Emb proteins are thought to be integral membrane proteins with 12 transmembrane domains and a large carboxyl-terminal globular region of approximately 375 amino acids [193, 195]. Genetic and biochemical studies have shown that the EmbA and EmbB proteins are involved in the formation of the proper terminal hexaarabinofuranoside motif during arabinogalactan synthesis [196], while EmbC is involved in lipoarabinomannan synthesis [197]. Since the majority of EMB-resistant clinical isolates contain mutations in *embB* (see below) [193, 195, 198], the EmbB protein has been proposed as the main target of EMB, although X-ray crystallographic data supporting this interaction are lacking.

5.2 Mechanisms of Resistance

In *M. smegmatis*, high-level resistance to EMB appears to require multiple steps, including overexpression of the Emb proteins, as well as mutations in the conserved region of EmbB or further increases in protein expression levels [193]. Resistance to EMB in *M. tuberculosis* is usually associated with point mutations in the *embCAB* operon, commonly involving amino acid substitutions at codon Met306 of the *embB* gene [193, 195, 198]. EmbB mutations have been identified in 30–70 % of EMB-resistant isolates of *M. tuberculosis* [193, 198–200]. Mutations in the *embB* gene were

reported to be associated with high-level EMB resistance [201], with the mutations Met306Leu or Met306Val yielding a higher MIC (40 μg/mL) than the Met306Ile substitution (20 μg/mL) [198]. However, a study of 183 epidemiologically unlinked *M. tuberculosis* isolates collected in St. Petersburg, Russia, detected the presence of *embB* mutations at codon 306 in 48 % of EMB-resistant isolates, but also in 31 % of EMB-susceptible isolates, suggesting that *embB* mutations may not be sufficient to confer resistance to EMB, or the presence of a compensatory mutation that reverses the EMB resistance phenotype of *embB* mutants [202]. Interestingly, the discrepancy in phenotypic and genotypic EMB resistance tests was restricted to strains already resistant to other antituberculosis drugs; specifically, *embB* mutations at codon 306 were noted in 40 of the 69 (60 %) of EMB-susceptible strains resistant to isoniazid, rifampin, and streptomycin but none of the 43 pan-susceptible strains [202]. Huang et al. identified several novel mutations in *embB*, including at codon 319 and codon 497 [203]. Interestingly, Lacoma et al. found that two EMB-susceptible strains harbored a mutation at codon 306 [204].

Nucleotide polymorphisms in the *embC-embA* intergenic region have been reported in association with resistance-associated amino acid replacements in EmbA or EmbB, suggesting that these intergenic mutations represent secondary or compensatory changes [195]. Other potential mutations involved in EMB resistance include a Gln379Arg replacement in *M. tuberculosis embR*, a homologue of the synonymous gene encoding a putative transcriptional activator of *embAB* in *M. avium* [192], as well as mutations in *rmlD* and *rmlA2*, which encode proteins involved in rhamnose modification [195]. In addition, mutations associated with EMB resistance have been described in *Rv0340* [195], a gene transcribed in the same orientation and upstream of the *iniBAC* operon, which is significantly upregulated following exposure to EMB in vitro [205]. As many as one-quarter of all EMB-resistant *M. tuberculosis* isolates do not harbor mutations in any of the genes described above, suggesting alternative mechanisms of EMB resistance [206].

6 Aminoglycosides

The discovery of streptomycin (see figure) in the early 1940s represented the first breakthrough in the chemotherapy of tuberculosis [207]. Patients treated with streptomycin and bed rest improved initially compared to those assigned to bed rest alone, but streptomycin monotherapy led inevitably to relapses with streptomycin-resistant *M. tuberculosis* [208]. Although relapse rates are comparable when streptomycin is substituted for ethambutol as the fourth drug in addition to INH, rifampin, and PZA, the poor oral absorption of streptomycin, which necessitates parenteral administration, as well as the toxicity profile of the aminoglycosides have favored the use of ethambutol in first-line antituberculosis therapy [209]. Other aminoglycosides with significant antimycobacterial activity include kanamycin and amikacin [210]. The detailed mechanisms of action of the aminoglycosides will be addressed elsewhere, and this section will cover mechanisms of aminoglycoside resistance identified specifically in *M. tuberculosis*.

As in other bacteria, the mode of action of the aminoglycosides against mycobacterial species is through their binding to the 30S ribosomal subunit, which affects polypeptide synthesis and ultimately results in inhibition of translation [211]. In clinically relevant bacteria, resistance to the aminoglycosides most often results from modification of the aminoglycoside molecule. Although genes encoding aminoglycoside-modifying enzymes have been identified in the chromosome of slow-growing mycobacteria [212, 213], and disruption of aminoglycoside 2′-*N*-acetyltransferase genes has been correlated with increased aminoglycoside susceptibility in *M. smegmatis* [214], this mechanism of resistance has not been described for *M. tuberculosis* [105]. Instead, resistance to streptomycin and the other aminoglycosides in *M. tuberculosis* usually develops by mutation of the ribosome target binding sites. Interestingly, although cross-resistance is observed between amikacin and kanamycin [215], these drugs are not cross-resistant with streptomycin [216], suggesting distinct mechanisms of resistance. Amikacin is a derivative of kanamycin, and the two drugs are structurally related, each containing a 2-deoxystreptamine moiety, while streptomycin is structurally distinct, containing a streptidine moiety. High-level resistance to amikacin and kanamycin with preserved susceptibility to streptomycin has been reported in *M. abscessus* and *M. chelonae* [217], and in *M. tuberculosis* [218] in association with a point mutation at position 1400 (corresponding to position 1408 in *E. coli*) of the *rrs* gene, which encodes 16S rRNA [216, 217]. On the other hand, streptomycin resistance in mycobacteria is most commonly associated with mutations in the *rpsL* gene, which encodes the ribosomal protein S12 [219–224]. Specifically, a missense mutation resulting in a substitution of an arginine for a lysine at codon 43, as well as point mutations in codon 88 account for the majority of *rpsL* mutations in *M. tuberculosis* [224]. As in *E. coli*, streptomycin resistance in *M. tuberculosis* also commonly arises from *rrs* mutations, which are usually clustered in the regions surrounding nucleotides 530 or 912 [219, 220, 225]. Unlike most other bacteria, which have multiple copies of the *rrs* gene, *M. tuberculosis* and other slow-growing mycobacteria have a single copy of the gene, making it an easily selected mutation site. Thus, alterations in the drug target arising from reduced association of the 16S rRNA with the S12 ribosomal protein lead to an inability of aminoglycosides to disrupt translation of mycobacterial mRNA, thereby resulting

in antibiotic resistance. Mutations in *rpsL* and *rrs*, which occur in about 50 % and 20 %, respectively, of streptomycin-resistant *M. tuberculosis* clinical isolates, are usually associated with intermediate-(MIC 64–512 μg/mL) or high-level resistance (MIC > 1000 μg/mL) [105]. The mechanisms responsible for streptomycin resistance in other *M. tuberculosis* isolates, particularly those with low-level resistance (MIC 4–32 μg/mL), are unknown, but may involve changes in cell envelope permeability and diminished drug uptake [219, 221].

Reeves et al. recently identified aminoglycoside cross-resistance in *M. tuberculosis* due to mutations in the 5′ untranslated region of *whiB7*. These mutations led to an increase in the number of *whiB7* transcripts and increased expression of both *eis* (Rv2416c) and *tap* (Rv1258c) [226]. An association has been reported between *M. tuberculosis* clinical isolates harboring a variety of mutations in the *gidB* gene (Rv3919c) and low-level streptomycin resistance [227–229]. These data show that a mutation at either the *whiB7* or *gidB* locus leads to the acquisition of high-level streptomycin resistance at an elevated frequency, which may partly explain why streptomycin resistance can develop so quickly in the host [226, 229]. However, recent results suggest that for *gidB* this may remain problematic both because the number of mutations required to accurately assess *gidB* status is large and also because the impact of specific mutations in *gidB* on the resistance level of the isolate remains unclear [230]. A recent systematic review described additional mutations in the *rrs*, *tlyA*, *eis* promoter, and *gidB* genes appear to be associated with resistance to the injectable agents amikacin, kanamycin, and/or capreomycin [231]. Mutations in the gene *tlyA* encoding a 2′-*O*-methyltransferase of 16S rRNA and 23S rRNA have been implicated in resistance to capreomycin and viomycin [232]. Based on our understanding of aminoglycoside cross-resistance, the best order in which to introduce an injectable agent with the hope of preventing cross-resistance to other injectables would be streptomycin first, then capreomycin, then kanamycin, and finally amikacin [233].

7 Fluoroquinolones

The fluoroquinolones demonstrate excellent activity against several mycobacterial species, including *M. tuberculosis*, *M. kansasii*, and *M. fortuitum*, but not against others, such as *M. avium*, *M. marinum*, *M. chelonae*, and *M. abscessus* [234]. In particular, drugs of the fluoroquinolone class are highly active against *M. tuberculosis* both *in vitro* [235, 236] and in animal models [237–239]. In descending order of activity, fluoroquinolones active against *M. tuberculosis* include moxifloxacin, sparfloxacin, levofloxacin, ofloxacin, and ciprofloxacin [240]. The 8-methoxy-fluoroquinolone moxifloxacin has bactericidal activity similar to that of INH against *M. tuberculosis* both in vitro and in the murine model of TB [239, 241, 242], as well as early bactericidal activity comparable to INH in patients with pulmonary TB [243–245]. Unlike gatifloxacin, which appears to lack sterilizing activity against stationary-phase cultures of *M. tuberculosis* [246], moxifloxacin, when substituted for INH, is able to shorten the duration of therapy needed to effect stable cure in murine TB [247, 248], suggesting that the drug has significant sterilizing activity. Until recently, the fluoroquinolones have been recommended primarily as second-line agents in the treatment of multidrug-resistant tuberculosis [2]. However, the use of a fluoroquinolone as the only active agent in a failing regimen for treatment of multidrug-resistant *M. tuberculosis* constitutes the most frequent cause of fluoroquinolone resistance [240]. Resistance to fluoroquinolones also may arise extremely rapidly following use of these drugs for other infections [249, 250]. Despite the widespread use of fluoroquinolones to treat a variety of bacterial infections, fluoroquinolone resistance is detected in fewer than 2 % of *M. tuberculosis* isolates in the United States and Canada [251]. Because of its potent bactericidal and sterilizing activities, moxifloxacin is currently under investigation as a first-line agent in the treatment of tuberculosis. The mechanism of action and detailed mechanisms of resistance to this class of drugs will be discussed in another chapter, and this section will highlight specific mutations identified in fluoroquinolone-resistant *M. tuberculosis*.

Fluoroquinolones exert their powerful antibacterial activity by trapping gyrase and topoisomerase IV on DNA as ternary complexes and blocking the movement of replication forks and transcription complexes [252]. Unlike most other bacterial species, *M. tuberculosis* lacks topoisomerase IV but does contain the genes *gyrA* and *gyrB*, which encode the A and B subunits, respectively, of DNA gyrase [212]. Consequently, fluoroquinolone resistance in *M. tuberculosis* is most commonly associated with mutations in the quinolone resistance-determining region (QRDR) of *gyrA* and *gyrB*, conserved regions involved in the interaction between the drug and DNA gyrase [240]. Spontaneous fluoroquinolone resistance develops in laboratory strains of *M. tuberculosis* at frequencies of 2×10^{-6} to 1×10^{-8} [253]. The most frequent mutations associated with high-level fluoroquinolone resistance involve substitutions at codons 88, 90, 91, and 94 of the *gyrA* gene [254–256].

The degree of resistance to fluoroquinolones depends on the specific amino acid substitution in the QRDR, and on the number of resistance mutations present. Therefore, while single mutations in *gyrA* may confer low-level resistance (MIC > 2 μg/mL) [257], high-level resistance to fluoroquinolones usually requires a stepwise process of at least two

mutations in *gyrA* or the combination of mutations in *gyrA* and *gyrB* [255, 257]. Mutations in the QRDR of *gyrA* do not occur following exposure of *M. tuberculosis* to low concentrations of fluoroquinolones in vitro, and the selection pressure for mutants in *gyrA* increases when *M. tuberculosis* is exposed to high concentrations of fluoroquinolones in vitro [258]. However, mutations in the QRDR region of *gyrA* are identified in only 42–85 % of fluoroquinolone-resistant clinical isolates, suggesting alternative mechanisms of resistance.

Mutations in the QRDR of the *gyrB* gene in the absence of *gyrA* mutations have been identified in some laboratory isolates [257, 258], but are rare in fluoroquinolone-resistant *M. tuberculosis* clinical isolates [259]. Pantel et al. reported four GyrB substitutions in fluoroquinolone-resistant *M. tuberculosis* clinical strains (D500A, N538T, T539P, and E540V) suggesting that the GyrB QRDR may extend from positions 500–540 [260]. Functional genetic analysis and structural modeling of GyrB suggest that N538D, E540V, and R485C/T539N conferred resistance to four different fluoroquinolones in at least one genetic background. The GyrB D500H and D500N mutations conferred resistance only to levofloxacin and ofloxacin while N538K and E540D consistently conferred resistance to moxifloxacin only. These findings indicate that certain mutations in *gyrB* may be sufficient to confer fluoroquinolone resistance, but the level and pattern of resistance varies among different mutations [261].

The *lfrA* gene, which encodes a multidrug efflux pump, has been shown to confer low-level resistance to fluoroquinolones when expressed on multicopy plasmids in *M. smegmatis* [262, 263]. Furthermore, expression of MfpA, a member of the pentapeptide repeat family of bacterial proteins [264], which includes McbG in *E. coli* and Qnr in *K. pneumoniae*, confers low-level resistance (four- to eightfold increase in the MIC) in *M. smegmatis* to ciprofloxacin and sparfloxacin [265]. Fluoroquinolone resistance related to MfpA has been attributed to DNA mimicry, as MfpA can directly bind to and inhibit DNA gyrase, thus preventing the formation of the DNA gyrase-DNA complex required for fluoroquinolone binding [266]. High-level resistance of *M. smegmatis* to ciprofloxacin (MIC = 64 2 μg/mL) also has been associated with overexpression and chromosomal amplification of the *pstB* gene, which encodes a putative ATPase subunit of the phosphate-specific transport (Pst) system, and disruption of this gene in *M. smegmatis* results in a twofold increase in sensitivity to fluoroquinolones relative to the isogenic wild-type strain [267]. Although homologues of *lfrA*, *mfpA*, and *pstB* appear to be present in *M. tuberculosis* [212], mutations or amplifications of these genes have not been identified in fluoroquinolone-resistant clinical isolates.

8 Macrolides

Clinical outcomes of patients with AIDS and disseminated *M. avium* complex have improved substantially since the introduction of the extended-spectrum macrolides, which are now considered the cornerstone of any potent regimen [268–270]. However, combination therapy with at least one other antimycobacterial agent, usually ethambutol, is necessary to prevent the emergence of macrolide resistance [271–273]. Although clarithromycin and azithromycin are both effective against disseminated *M. avium* complex infection, several studies directly comparing these two drugs when used in combination with ethambutol suggest trends toward more rapid clearance of bacteremia with clarithromycin [271, 272]. The mechanism of action of the macrolide antibiotics will be covered elsewhere in this book, and this section will focus on known macrolide resistance mutations occurring in *M. avium* complex.

The macrolides exert their antibacterial effect by binding to the bacterial 50S ribosomal subunit and inhibiting RNA-dependent protein synthesis [274]. However, these drugs have limited activity against wild-type *M. tuberculosis* [275]. This intrinsic resistance is believed to be associated with expression of the *erm* gene [276, 277], which is induced upon exposure of *M. tuberculosis* to clarithromycin [278]. Interestingly, disruption of the *pks12* gene, which encodes a polyketide synthase required for synthesis of the major cell wall lipid dimycocerosyl phthiocerol, results in increased susceptibility of *M. tuberculosis* to clarithromycin relative to its parent strain, but no change in susceptibility to ciprofloxacin or penicillin [279].

In *M. avium*, spontaneous resistance to clarithromycin has been estimated to occur at a rate of 10^{-8} to 10^{-9} organisms [280, 281]. Clarithromycin resistance in *M. avium* isolated from patients with pulmonary disease has been associated with point mutations in the generally conserved loop of domain V of 23S rRNA [282], corresponding to position 2058 in *E. coli* 23S rRNA, which confer resistance to erythromycin and the macrolides-lincomide-streptogramin B antibiotics [283]. Similarly, clarithromycin-resistant *M. avium* isolates obtained from patients with AIDS and disseminated *M. avium* infection contained point mutations in the domain V sequences of 23S rRNA at position 2274 [284]. Mutations in the *M. avium* 23S rRNA gene are associated with high-level resistance (MIC ≥ 128 μg/mL) [285]. As in *M. avium*, clarithromycin resistance in *M. chelonae* and *M. abscessus* has been associated with point mutations in the 23S rRNA peptidyltransferase region at positions 2058 or 2059 in strains with a single chromosomal copy of the rRNA operon [286, 287]. However, a few clarithromycin-resistant *M. avium* isolates, particularly with low-level resistance, have been described in which no mutation can be identified in the peptidyltransferase region of the 23S rRNA [281, 288], suggesting alternative mechanisms of drug resistance.

8.1 Cross-Resistance of Antimycobacterial Agents

In general, there is low cross-resistance among most antituberculosis drugs. When present, the degree of cross-resistance depends on the particular mutations and mechanism of drug resistance. Although the most commonly observed INH resistance mutations, i.e., those involving *katG*, do not generate cross-resistance to other agents, mutations in *inhA* itself or in its promoter region confer resistance to the second-line antituberculosis drug ethionamide [41, 43, 289]. Mutations in *ethA*, which confer ethionamide resistance, also yield cross-resistance to thiacetazone and thiocarlide [290].

Mutations in the *rpoB* gene of *M. tuberculosis*, particularly in codons Ser531 and His526, have been associated with high-level resistance (MIC > 32 μg/mL) to rifampin and cross-resistance to all the rifamycins. On the other hand, the *rpoB* mutations L511P, D516Y, D516V, or S522L, which are associated with low- to high-level resistance to rifampin and rifapentine, do not significantly alter susceptibility to rifabutin (MIC 0.5 μg/mL) or rifalazil (MIC 0.01–0.04 μg/mL) [118, 291]. In one study of 25 rifampin-resistant *M. tuberculosis* isolates (MIC > 2 μg/mL), three of these isolates (12 %) retained susceptibility to rifabutin [292]. Another study of 112 *M. tuberculosis* clinical isolates detected 73 % cross-resistance between rifabutin and rifampin [293], suggesting that rifabutin may have a role in the therapy of multidrug-resistant tuberculosis in cases where the isolate retains susceptibility to rifabutin.

Cross-resistance among the aminoglycosides is variable. Thus, cross-resistance is usually seen between the 2-deoxystreptamine aminoglycosides amikacin and kanamycin [218], but not between these two drugs and the streptidine aminoglycoside streptomycin [216]. In addition, cross-resistance may be observed between kanamycin and capreomycin or viomycin [294, 295]. Although cross-resistance has not been reported between fluoroquinolones and other classes of antituberculosis agents, mutations associated with individual fluoroquinolone resistance appear to confer cross-resistance to the entire class of drugs [240]. Similarly, resistance to clarithromycin or azithromycin in *M. avium* complex is usually associated with class-wide resistance to the macrolides [275, 284].

8.2 Mechanism of Spread of Resistance

Although drug resistance may be spread by plasmids or transposons among many bacterial species, including the fast-growing *M. fortuitum* [262], these mobile genetic elements are not known to cause drug resistance in *M. tuberculosis* [105]. As described above, drug resistance in *M. tuberculosis* is caused by mutations in specific chromosomal genes. In general, genetic resistance of *M. tuberculosis* to specific antimycobacterial drugs does not alter the fitness or virulence of the organism [105], suggesting that drug-resistant isolates may spread to previously uninfected individuals and cause disease equivalent to that caused by drug-susceptible isolates. One notable exception to this rule is in the case of certain INH-resistant *M. tuberculosis* isolates with reduced catalase activity, which demonstrate decreased virulence in the guinea pig model of tuberculosis [57]. Reduced catalase activity in these isolates correlates well with increased INH resistance, as well as decreased virulence [105]. Molecular genetic studies have shown that integration of a functional *katG* gene into the genome of INH-resistant, catalase-defective *M. bovis* restores INH susceptibility, as well as virulence in the guinea pig model [44]. Consistent with these findings, KatG-deficient *M. tuberculosis* is attenuated relative to a wild-type strain during infection of immunocompetent mice and mouse-derived macrophages, as a result of exposure to the peroxides generated by the phagocyte NADPH oxidase [296]. Although *M. tuberculosis* clinical isolates containing the S315T mutation appear to retain full virulence and transmissibility in humans [297], it is unknown if other *katG* mutants, with more greatly reduced catalase activity, are less transmissible or virulent in humans. On the other hand, restoration of virulence may be associated with promoter-up mutations in the *ahpC* gene, which may compensate for loss of catalase activity resulting from mutations in *katG* [79]. Full transmissibility and virulence are expected among *M. tuberculosis* strains in which INH resistance is mediated by mutations in genes other than *katG*, such as *inhA* or *ndh* [105].

The efficient spread of drug-resistant isolates certainly may occur from person to person, as evidenced by the ecologically successful strain W. This strain, which is resistant to as many as 11 antimycobacterial drugs, caused a multidrug-resistant outbreak of tuberculosis in New York City and spread across the United States [298]. However, the emergence of drug resistance in a particular individual is most often not due to primary infection with a drug-resistant isolate, but rather a result of human error. Thus, a prior history of tuberculosis and antituberculosis therapy has been implicated strongly in the causation of multidrug-resistant *M. tuberculosis* [299]. Factors associated with acquisition of drug resistance include incomplete and inadequate treatment, such as the use of a single drug to treat tuberculosis, the addition of a single drug to a failing regimen and the failure to identify preexisting resistance, as well as inadequate treatment adherence on the part of the patient [300]. Mathematical models predict that the future of the MDR and XDR-TB epidemic will depend to a large extent on the transmission efficiency or relative fitness of drug-resistant *M. tuberculosis* compared to drug-susceptible strains [301].

8.3 Alternative Agents

M. tuberculosis strains that are resistant to either isoniazid or rifampin may be treated effectively with other first-line drugs. However, strains that are resistant to both drugs, termed "multidrug-resistant" strains, require the use of "second-line drugs," which are generally less effective and more toxic [299]. These drugs include ethionamide, capreomycin, cycloserine, and paraaminosalicylic acid. Promising new antituberculosis drugs [302, 303], such as the nitroimidazoles (PA-824, OPC-67683) [304, 305], a diarylquinoline (TMC207/bedaquiline) [306], an ethylene diamine (SQ-109) [307], oxazolidinones (Linezolid, PNU-100480/sutezolid) [308, 309], benzothiazinones [310], clofazimine, and thioridazine [311–313], are currently being tested in preclinical or clinical trials [314]. Known mechanisms of action and resistance for each of these drugs will be discussed briefly in this section.

Ethionamide, a synthetic compound structurally related to INH, was shown to have antituberculosis activity in the late 1950s [315]. Although less potent than INH, ethionamide also inhibits mycolic acid synthesis [41, 316]. Ethionamide is a pro-drug requiring activation by the monooxygenase EthA [290, 317, 318], which itself is negatively regulated by the transcriptional repressor EthR. [317] Similarly to INH, ethionamide inhibits mycolic acid synthesis by binding the ACP reductase InhA [41]. Using a cell-based activation method, Wang et al. recently showed that the thioamide drugs ethionamide and prothionamide form covalent adducts with NAD, which are tight-binding inhibitors of *M. tuberculosis* and *M. leprae* InhA [319]. Approximately three-quarters of *M. tuberculosis* isolates with high-level ethionamide resistance (MIC > 50 μg/mL) have mutations in *ethA* or *inhA* [289]. Recently, other potential mechanisms of resistance have been identified, as *M. tuberculosis mshA* deletion mutants were found to be defective in mycothiol biosynthesis and resistant to ethionamide, likely due to defective activation of the drug [320].

Although often grouped together with the aminoglycosides because of similar activity and toxicities, capreomycin is a macrocyclic polypeptide antibiotic isolated from *Streptomyces capreolus* [210]. Like streptomycin and kanamycin, capreomycin inhibits protein synthesis through modification of ribosomal structures at the 16S rRNA [69]. Recent studies using site-directed mutagenesis have identified the binding site of capreomycin on 16S rRNA helix 44 [321]. In *M. smegmatis*, mutations in *vicA* and *vicB*, which encode components of the 50S and 30S ribosomal subunits, confer resistance to capreomycin and viomycin [294, 295]. In *M. tuberculosis*, mutations in the *rrs* gene encoding 16S rRNA have been associated with resistance to capreomycin as well as kanamycin [218, 322]. The *rrs* mutation A1401G causes high-level amikacin/kanamycin and low-level capreomycin resistance. C1402T is associated with capreomycin (and viomycin) resistance and low-level kanamycin resistance. G1484T has been linked to high-level amikacin/kanamycin and capreomycin/viomycin resistance [323–325]. Various single-nucleotide polymorphisms (SNPs) in the *tlyA* gene have been also associated with capreomycin resistance [326].

Cycloserine interrupts peptidoglycan synthesis by inhibiting the enzymes D-alanine racemase (AlrA) and D-alanine:alanine ligase (Ddl) [327, 328]. Overexpression of *M. tuberculosis* AlrA and Ddl on a multicopy vector results in resistance to D-cycloserine in *M. smegmatis* and *M. bovis* BCG [327, 328], and *M. smegmatis alrA* mutants lacking D-alanine racemase activity display hypersusceptibility to D-cycloserine [329]. In *E. coli*, cycloserine resistance has been attributed to mutations in *cycA*, which encodes a permease responsible for uptake of the drug [330], but such a mechanism of resistance has not been described for mycobacteria. In addition, mutations in a gene homologous to that encoding *E. coli* penicillin binding protein 4 (PBP4) were shown to confer resistance to D-cycloserine, as well as to vancomycin in *M. smegmatis* [331]. However, the mechanism of cycloserine resistance in *M. tuberculosis* remains unknown.

Paraaminosalicylic acid (PAS) was introduced in 1945 [332, 333]. Although its activity was inferior to that of streptomycin when used alone, the combination of PAS with streptomycin significantly reduced the emergence of streptomycin-resistant organisms [334]. The mechanisms of action and resistance to PAS have not been well characterized, but it has been suggested that the drug may inhibit folic acid biosynthesis and uptake of iron [69]. Recently, PAS-resistant transposon mutants of *M. bovis* BCG were found to harbor insertions in the *thyA* gene, which encodes the enzyme thimidylate synthesis in the folate biosynthesis pathway [335]. In addition, mutations in the *thyA* gene resulting in diminished enzymatic activity were identified in PAS-resistant *M. tuberculosis* clinical isolates, suggesting that PAS may act as a folate antagonist and that *thyA* mutations may mediate clinical PAS resistance [335]. However, only slightly more than a third of the evaluated PAS-resistant strains had mutations in *thyA*, suggesting the existence of additional mechanisms of PAS resistance. Thr202Ala has been reported as the most common mutation associated with PAS resistance, although this mutation has also been identified in several PAS-susceptible isolates [336].

PA-824, a small molecule nitroimidazopyran related to metronidazole, was recently shown to have bactericidal activity against replicating and static *M. tuberculosis* cultures in vitro, as well as in murine and guinea pig models of tuberculosis [337]. In the mouse model, PA-824 has bactericidal activity comparable to that of INH [338, 339]. However, unlike INH, but like metronidazole, the drug also has potent activity against nonreplicating bacilli exposed to microaerophilic conditions [337, 338]. In addition, PA-824 is highly

active against multidrug-resistant clinical isolates of *M. tuberculosis* (MIC < 1 μg/mL), suggesting no cross-resistance with current antituberculosis drugs [338]. Like metronidazole, PA-824 is a pro-drug which requires bioreductive activation of an aromatic nitro group in order to exert an antitubercular effect [337]. Although the precise mechanism by which PA-824 exerts its lethal effect is unknown, the drug appears to inhibit the oxidation of hydroxymycolates to ketomycolates, a terminal step in mycolic acid synthesis [337]. Similar to INH, resistance to PA-824 is most commonly mediated by mutations which lead to loss of pro-drug activation. Mutations in *fgd1* and *fbiC* result in the loss of a specific glucose-6-phosphate dehydrogenase and its deazaflavin cofactor F_{420}, respectively, which together provide electrons for the reductive activation of PA-824 [340]. In addition, resistance to PA-824 has been associated with mutations in *Rv3547*, a gene encoding a conserved hypothetical protein which appears to be involved in PA-824 activation [340, 341]. Among laboratory strains, the frequency of resistance to PA-824 is slightly less than that to INH, approximately 9.0×10^{-7} [337].

As in the case of PA-824, mutations in the Rv3547 gene have been identified in strains resistant to OPC-67683, indicating defective drug activation [342]. Resistance to TMC207 is mediated by mutations in the *atpE* gene encoding the transmembrane and oligomeric C subunit of ATP synthase, typically at positions 63 or 66 [343]. However, more recent studies have shown that a majority of in vitro-generated mutants resistant to TMC207 lacked mutations in *atpE*, indicating alternative mechanisms of drug resistance [344].

Whether upregulation of *ahpC* expression, observed in strains resistant to INH, EMB, and SQ109, plays a role in resistance to SQ109 or merely reflects a compensatory metabolic mechanism remains to be determined [345].

While resistance to linezolid in *M. tuberculosis* clinical isolates is rarely reported, in vitro-selected mutants with high-level resistance to linezolid (MIC = 16–32 mg/L) have been found to contain mutations at G2061T and G2576T in the 23S rRNA gene [346]. On the other hand, mutants with lower level linezolid resistance (MIC = 4–8 mg/L) lack mutations in the 23S rRNA gene, implicating other possible mechanisms of resistance, such as the possible involvement of efflux pumps or other non-ribosomal alterations, as has been shown in *M. smegmatis* mutants [347, 348]. Sutezolid (PNU-100480) [349] is undergoing Phase I studies and resistance mechanisms are expected to be similar to those of linezolid.

Although spontaneous benzothiazinone-resistant laboratory mutants were found to have a Ser or Gly substitution at codon Cys387 of *dprE1*, resistance to benzothiazinones has not been reported in clinical *M. tuberculosis* isolates [350].

Several existing drugs used for other medical conditions have been "repurposed" for the treatment of tuberculosis. A recent study showed that the antitubercular activity of clofazimine, including against MDR-TB, is due to the generation of reactive oxygen species formed as a consequence of Ndh-mediated reduction of clofazimine [351]. Xu et al. reported that clofazimine mutants resistant to 0.48 and 1.92 μg/mL were not observed using the indirect method in mice during 90 days of treatment [352]. The mechanism of antitubercular activity of thioridazine is likely multifactorial [353, 354], as the drug appears to act on enzymes involved in fatty acid metabolism and membrane proteins, particularly efflux pumps, in addition to inhibiting type II NADH:menaquinone oxidoreductase as a phenothiazine [355]. Mechanisms of *M. tuberculosis* resistance to the phenothiazines remain to be elucidated.

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References

1. World Health Organization. WHO: Global Tuberculosis Report 2012. WHO/HTM/TB/2012.6.
2. Blumberg HM, Burman WJ, Chaisson RE, Daley CL, Etkind SC, Friedman LN, Fujiwara P, Grzemska M, Hopewell PC, Iseman MD, Jasmer RM, Koppaka V, Menzies RI, O'Brien RJ, Reves RR, Reichman LB, Simone PM, Starke JR, Vernon AA. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis. Am J Respir Crit Care Med. 2003;167:603–62.
3. Lanzas F, Karakousis PC, Sacchettini JC, Ioerger TR. Multidrug-resistant tuberculosis in panama is driven by clonal expansion of a multidrug-resistant Mycobacterium tuberculosis strain related to the KZN extensively drug-resistant M. tuberculosis strain from South Africa. J Clin Microbiol. 2013;51:3277–85.
4. Royce S, Falzon D, van Weezenbeek C, Dara M, Hyder K, Hopewell P, Richardson MD, Zignol M. Multidrug resistance in new tuberculosis patients: burden and implications [Short communication]. Int J Tuberc Lung Dis. 2013;17:511–3.
5. Udwadia ZF. MDR, XDR, TDR tuberculosis: ominous progression. Thorax. 2012;67:286–8.
6. Zar HJ, Udwadia ZF. Advances in tuberculosis 2011–2012. Thorax. 2013;68:283–7.
7. Chaisson RE, Moore RD, Richman DD, Keruly J, Creagh T, The Zidovudine Epidemiology Study Group. Incidence and natural history of Mycobacterium avium-complex infections in patients with advanced human immunodeficiency virus disease treated with zidovudine. Am Rev Respir Dis. 1992;146:285–9.
8. Nightingale SD, Byrd LT, Southern PM, Jockusch JD, Cal SX, Wynne BA. Incidence of Mycobacterium avium-intracellulare complex bacteremia in human immunodeficiency virus-positive patients. J Infect Dis. 1992;165:1082–5.
9. Karakousis PC, Moore RD, Chaisson RE. Mycobacterium avium complex in patients with HIV infection in the era of highly active antiretroviral therapy. Lancet Infect Dis. 2004;4:557–65.
10. Field SK, Fisher D, Cowie RL. Mycobacterium avium complex pulmonary disease in patients without HIV infection. Chest. 2004;126:566–81.
11. Canueto-Quintero J, Caballero-Granado FJ, Herrero-Romero M, Dominguez-Castellano A, Martin-Rico P, Verdu EV, Santamaria DS, Cerquera RC, Torres-Tortosa M. Epidemiological, clinical,

and prognostic differences between the diseases caused by Mycobacterium kansasii and Mycobacterium tuberculosis in patients infected with human immunodeficiency virus: a multicenter study. Clin Infect Dis. 2003;37:584–90.
12. Bottger EC, Teske A, Kirschner P, Bost S, Chang HR, Beer V, Hirschel B. Disseminated "Mycobacterium genavense" infection in patients with AIDS. Lancet. 1992;340:76–80.
13. Bessesen MT, Shlay J, Stone-Venohr B, Cohn DL, Reves RR. Disseminated Mycobacterium genavense infection: clinical and microbiological features and response to therapy. Aids. 1993;7:1357–61.
14. Robitzek EH, Selikoff IJ. Hydrazine derivatives of isonicotinic acid (rimifon marsilid) in the treatment of active progressive caseous-pneumonic tuberculosis; a preliminary report. Am Rev Tuberc. 1952;65:402–28.
15. Slayden RA, Barry III CE. The genetics and biochemistry of isoniazid resistance in mycobacterium tuberculosis. Microbes Infect. 2000;2:659–69.
16. Al-Tawfiq JA, Al-Muraikhy AA, Abed MS. Susceptibility pattern and epidemiology of Mycobacterium tuberculosis in a Saudi Arabian hospital: a 15-year study from 1989 to 2003. Chest. 2005;128:3229–32.
17. Anuradha B, Aparna S, Hari Sai Priya V, Vijaya Lakshmi V, Akbar Y, Suman Latha G, Murthy KJ. Prevalence of drug resistance under the DOTS strategy in Hyderabad, South India, 2001–2003. Int J Tuberc Lung Dis. 2006;10:58–62.
18. Perez del Molino Bernal ML, Tunez V, Cruz-Ferro E, Fernandez-Villar A, Vazquez-Gallardo R, Diaz-Cabanela D, Anibarro L. Study of Mycobacterium tuberculosis drug resistance in the region of Galicia, Spain. Int J Tuberc Lung Dis. 2005;9:1230–5.
19. Quy HT, Buu TN, Cobelens FG, Lan NT, Lambregts CS, Borgdorff MW. Drug resistance among smear-positive tuberculosis patients in Ho Chi Minh City, Vietnam. Int J Tuberc Lung Dis. 2006;10:160–6.
20. Swaminathan S, Paramasivan CN, Ponnuraja C, Iliayas S, Rajasekaran S, Narayanan PR. Anti-tuberculosis drug resistance in patients with HIV and tuberculosis in South India. Int J Tuberc Lung Dis. 2005;9:896–900.
21. Balabanova Y, Drobniewski F, Fedorin I, Zakharova S, Nikolayevskyy V, Atun R, Coker R. The directly observed therapy short-course (DOTS) strategy in Samara Oblast, Russian Federation. Respir Res. 2006;7:44.
22. Mackaness GB, Smith N. The action of isoniazid (isonicotinic acid hydrazide) on intracellular tubercle bacilli. Am Rev Tuberc. 1952;66:125–33.
23. Suter E. Multiplication of tubercle bacilli within phagocytes cultivated in vitro, and effect of streptomycin and isonicotinic acid hydrazide. Am Rev Tuberc. 1952;65:775–6.
24. Tsukamura M, Tsukamura S, Nakano E. The uptake of isoniazid by mycobacteria and its relation to isoniazid susceptibility. Am Rev Respir Dis. 1963;87:269–75.
25. Bardou F, Raynaud C, Ramos C, Laneelle MA, Laneelle G. Mechanism of isoniazid uptake in Mycobacterium tuberculosis. Microbiology. 1998;144(Pt 9):2539–44.
26. Zhang Y, Heym B, Allen B, Young D, Cole S. The catalase-peroxidase gene and isoniazid resistance of Mycobacterium tuberculosis. Nature. 1992;358:591–3.
27. Nguyen M, Claparols C, Bernadou J, Meunier B. A fast and efficient metal-mediated oxidation of isoniazid and identification of isoniazid-NAD(H) adducts. Chembiochem. 2001;2:877–83.
28. Gangadharam PR, Harold FM, Schaefer WB. Selective inhibition of nucleic acid synthesis in Mycobacterium tuberculosis by isoniazid. Nature. 1963;198:712–4.
29. Brennan PJ, Rooney SA, Winder FG. The lipids of Mycobacterium tuberculosis BCG: fractionation, composition, turnover and the effects of isoniazid. Ir J Med Sci. 1970;3:371–90.
30. Zatman LJ, Kaplan NO, Colowick SP, Ciotti MM. Effect of isonicotinic acid hydrazide on diphosphopyridine nucleotidases. J Biol Chem. 1954;209:453–66.
31. Bekierkunst A. Nicotinamide-adenine dinucleotide in tubercle bacilli exposed to isoniazid. Science. 1966;152:525–6.
32. Winder FG, Collins PB. Inhibition by isoniazid of synthesis of mycolic acids in Mycobacterium tuberculosis. J Gen Microbiol. 1970;63:41–8.
33. Takayama K, Wang L, David HL. Effect of isoniazid on the in vivo mycolic acid synthesis, cell growth, and viability of Mycobacterium tuberculosis. Antimicrob Agents Chemother. 1972;2:29–35.
34. Takayama K, Schnoes HK, Armstrong EL, Boyle RW. Site of inhibitory action of isoniazid in the synthesis of mycolic acids in Mycobacterium tuberculosis. J Lipid Res. 1975;16:308–17.
35. Middlebrook G. Sterilization of tubercle bacilli by isonicotinic acid hydrazide and the incidence of variants resistant to the drug in vitro. Am Rev Tuberc. 1952;65:765–7.
36. Brennan PJ, Nikaido H. The envelope of mycobacteria. Annu Rev Biochem. 1995;64:29–63.
37. Draper P. The outer parts of the mycobacterial envelope as permeability barriers. Front Biosci. 1998;3:D1253–61.
38. Nikaido H. Preventing drug access to targets: cell surface permeability barriers and active efflux in bacteria. Semin Cell Dev Biol. 2001;12:215–23.
39. Karakousis PC, Williams EP, Bishai WR. Altered expression of isoniazid-regulated genes in drug-treated dormant Mycobacterium tuberculosis. J Antimicrob Chemother. 2008;61:323–31.
40. Barry III CE, Lee RE, Mdluli K, Sampson AE, Schroeder BG, Slayden RA, Yuan Y. Mycolic acids: structure, biosynthesis and physiological functions. Prog Lipid Res. 1998;37:143–79.
41. Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um KS, Wilson T, Collins D, de Lisle G, Jacobs Jr WR. inhA, a gene encoding a target for isoniazid and ethionamide in Mycobacterium tuberculosis. Science. 1994;263:227–30.
42. Quemard A, Sacchettini JC, Dessen A, Vilcheze C, Bittman R, Jacobs Jr WR, Blanchard JS. Enzymatic characterization of the target for isoniazid in Mycobacterium tuberculosis. Biochemistry. 1995;34:8235–41.
43. Larsen MH, Vilcheze C, Kremer L, Besra GS, Parsons L, Salfinger M, Heifets L, Hazbon MH, Alland D, Sacchettini JC, Jacobs Jr WR. Overexpression of inhA, but not kasA, confers resistance to isoniazid and ethionamide in Mycobacterium smegmatis, M. bovis BCG and M. tuberculosis. Mol Microbiol. 2002;46:453–66.
44. Wilson TM, de Lisle GW, Collins DM. Effect of inhA and katG on isoniazid resistance and virulence of Mycobacterium bovis. Mol Microbiol. 1995;15:1009–15.
45. Vilcheze C, Wang F, Arai M, Hazbon MH, Colangeli R, Kremer L, Weisbrod TR, Alland D, Sacchettini JC, Jacobs Jr WR. Transfer of a point mutation in Mycobacterium tuberculosis inhA resolves the target of isoniazid. Nat Med. 2006;12:1027–9.
46. McMurry LM, McDermott PF, Levy SB. Genetic evidence that InhA of Mycobacterium smegmatis is a target for triclosan. Antimicrob Agents Chemother. 1999;43:711–3.
47. Dessen A, Quemard A, Blanchard JS, Jacobs Jr WR, Sacchettini JC. Crystal structure and function of the isoniazid target of Mycobacterium tuberculosis. Science. 1995;267:1638–41.
48. Rozwarski DA, Grant GA, Barton DH, Jacobs Jr WR, Sacchettini JC. Modification of the NADH of the isoniazid target (InhA) from Mycobacterium tuberculosis. Science. 1998;279:98–102.
49. Argyrou A, Vetting MW, Aladegbami B, Blanchard JS. Mycobacterium tuberculosis dihydrofolate reductase is a target for isoniazid. Nat Struct Mol Biol. 2006;13:408–13.
50. Liu J, Nikaido H. A mutant of Mycobacterium smegmatis defective in the biosynthesis of mycolic acids accumulates meromycolates. Proc Natl Acad Sci U S A. 1999;96:4011–6.

51. Mdluli K, Swanson J, Fischer E, Lee RE, Barry III CE. Mechanisms involved in the intrinsic isoniazid resistance of Mycobacterium avium. Mol Microbiol. 1998;27:1223–33.
52. Winder F. The antibacterial action of streptomycin, isoniazid, and PAS. In: Barry V, editor. Chemotherapy of tuberculosis. London: Butterworth; 1964. p. 111–49.
53. Zhang Y. Isoniazid. In: Rom W, Garay S, editors. Tuberculosis. Philadelphia: Lippincott Williams & Wilkins; 2004. p. 739–58.
54. Cohn DL, Bustreo F, Raviglione MC. Drug-resistant tuberculosis: review of the worldwide situation and the WHO/IUATLD Global Surveillance Project. International Union Against Tuberculosis and Lung Disease. Clin Infect Dis. 1997;24 Suppl 1:S121–30.
55. Hobby GL, Lenert TF. Resistance to isonicotinic acid hydrazide. Am Rev Tuberc. 1952;65:771–4.
56. Muller B, Streicher EM, Hoek KG, Tait M, Trollip A, Bosman ME, Coetzee GJ, Chabula-Nxiweni EM, Hoosain E, Gey van Pittius NC, Victor TC, van Helden PD, Warren RM. inhA promoter mutations: a gateway to extensively drug-resistant tuberculosis in South Africa? Int J Tuberc Lung Dis. 2011;15:344–51.
57. Middlebrook G. Isoniazid-resistance and catalase activity of tubercle bacilli; a preliminary report. Am Rev Tuberc. 1954; 69:471–2.
58. Rouse DA, DeVito JA, Li Z, Byer H, Morris SL. Site-directed mutagenesis of the katG gene of Mycobacterium tuberculosis: effects on catalase-peroxidase activities and isoniazid resistance. Mol Microbiol. 1996;22:583–92.
59. Zhang Y, Garbe T, Young D. Transformation with katG restores isoniazid-sensitivity in Mycobacterium tuberculosis isolates resistant to a range of drug concentrations. Mol Microbiol. 1993;8:521–4.
60. Zhang Y, Young D. Strain variation in the katG region of Mycobacterium tuberculosis. Mol Microbiol. 1994;14:301–8.
61. Abate G, Hoffner SE, Thomsen VO, Miorner H. Characterization of isoniazid-resistant strains of Mycobacterium tuberculosis on the basis of phenotypic properties and mutations in katG. Eur J Clin Microbiol Infect Dis. 2001;20:329–33.
62. Marttila HJ, Soini H, Eerola E, Vyshnevskaya E, Vyshnevskiy BI, Otten TF, Vasilyef AV, Viljanen MK. A Ser315Thr substitution in KatG is predominant in genetically heterogeneous multidrug-resistant Mycobacterium tuberculosis isolates originating from the St. Petersburg area in Russia. Antimicrob Agents Chemother. 1998;42:2443–5.
63. Musser JM, Kapur V, Williams DL, Kreiswirth BN, van Soolingen D, van Embden JD. Characterization of the catalase-peroxidase gene (katG) and inhA locus in isoniazid-resistant and -susceptible strains of Mycobacterium tuberculosis by automated DNA sequencing: restricted array of mutations associated with drug resistance. J Infect Dis. 1996;173:196–202.
64. Saint-Joanis B, Souchon H, Wilming M, Johnsson K, Alzari PM, Cole ST. Use of site-directed mutagenesis to probe the structure, function and isoniazid activation of the catalase/peroxidase, KatG, from Mycobacterium tuberculosis. Biochem J. 1999;338(Pt 3): 753–60.
65. Bertrand T, Eady NA, Jones JN, Jesmin Nagy JM, Jamart-Gregoire B, Raven EL, Brown KA. Crystal structure of Mycobacterium tuberculosis catalase-peroxidase. J Biol Chem. 2004;279:38991–9.
66. Ando H, Kitao T, Miyoshi-Akiyama T, Kato S, Mori T, Kirikae T. Downregulation of katG expression is associated with isoniazid resistance in Mycobacterium tuberculosis. Mol Microbiol. 2011;79:1615–28.
67. Basso LA, Zheng R, Musser JM, Jacobs Jr WR, Blanchard JS. Mechanisms of isoniazid resistance in Mycobacterium tuberculosis: enzymatic characterization of enoyl reductase mutants identified in isoniazid-resistant clinical isolates. J Infect Dis. 1998;178:769–75.
68. Banerjee A, Sugantino M, Sacchettini JC, Jacobs Jr WR. The mabA gene from the inhA operon of Mycobacterium tuberculosis encodes a 3-ketoacyl reductase that fails to confer isoniazid resistance. Microbiology. 1998;144(Pt 10):2697–704.
69. Wade MM, Zhang Y. Mechanisms of drug resistance in Mycobacterium tuberculosis. Front Biosci. 2004;9:975–94.
70. Dalla Costa ER, Ribeiro MO, Silva MS, Arnold LS, Rostirolla DC, Cafrune PI, Espinoza RC, Palaci M, Telles MA, Ritacco V, Suffys PN, Lopes ML, Campelo CL, Miranda SS, Kremer K, da Silva PE, Fonseca Lde S, Ho JL, Kritski AL, Rossetti ML. Correlations of mutations in katG, oxyR-ahpC and inhA genes and in vitro susceptibility in Mycobacterium tuberculosis clinical strains segregated by spoligotype families from tuberculosis prevalent countries in South America. BMC Microbiol. 2009;9:39.
71. Mdluli K, Slayden RA, Zhu Y, Ramaswamy S, Pan X, Mead D, Crane DD, Musser JM, Barry III CE. Inhibition of a Mycobacterium tuberculosis beta-ketoacyl ACP synthase by isoniazid. Science. 1998;280:1607–10.
72. Lee AS, Teo AS, Wong SY. Novel mutations in ndh in isoniazid-resistant Mycobacterium tuberculosis isolates. Antimicrob Agents Chemother. 2001;45:2157–9.
73. Piatek AS, Telenti A, Murray MR, El-Hajj H, Jacobs Jr WR, Kramer FR, Alland D. Genotypic analysis of Mycobacterium tuberculosis in two distinct populations using molecular beacons: implications for rapid susceptibility testing. Antimicrob Agents Chemother. 2000;44:103–10.
74. Slayden RA, Lee RE, Barry III CE. Isoniazid affects multiple components of the type II fatty acid synthase system of Mycobacterium tuberculosis. Mol Microbiol. 2000;38:514–25.
75. Kremer L, Dover LG, Morbidoni HR, Vilcheze C, Maughan WN, Baulard A, Tu SC, Honore N, Deretic V, Sacchettini JC, Locht C, Jacobs Jr WR, Besra GS. Inhibition of InhA activity, but not KasA activity, induces formation of a KasA-containing complex in mycobacteria. J Biol Chem. 2003;278:20547–54.
76. Miesel L, Weisbrod TR, Marcinkeviciene JA, Bittman R, Jacobs Jr WR. NADH dehydrogenase defects confer isoniazid resistance and conditional lethality in Mycobacterium smegmatis. J Bacteriol. 1998;180:2459–67.
77. Wilson TM, Collins DM. ahpC, a gene involved in isoniazid resistance of the Mycobacterium tuberculosis complex. Mol Microbiol. 1996;19:1025–34.
78. Telenti A, Honore N, Bernasconi C, March J, Ortega A, Heym B, Takiff HE, Cole ST. Genotypic assessment of isoniazid and rifampin resistance in Mycobacterium tuberculosis: a blind study at reference laboratory level. J Clin Microbiol. 1997;35:719–23.
79. Sherman DR, Mdluli K, Hickey MJ, Arain TM, Morris SL, Barry III CE, Stover CK. Compensatory ahpC gene expression in isoniazid-resistant Mycobacterium tuberculosis. Science. 1996;272:1641–3.
80. Kelley CL, Rouse DA, Morris SL. Analysis of ahpC gene mutations in isoniazid-resistant clinical isolates of Mycobacterium tuberculosis. Antimicrob Agents Chemother. 1997;41:2057–8.
81. Guimaraes BG, Souchon H, Honore N, Saint-Joanis B, Brosch R, Shepard W, Cole ST, Alzari PM. Structure and mechanism of the alkyl hydroperoxidase AhpC, a key element of the Mycobacterium tuberculosis defense system against oxidative stress. J Biol Chem. 2005;280:25735–42.
82. Colangeli R, Helb D, Sridharan S, Sun J, Varma-Basil M, Hazbon MH, Harbacheuski R, Megjugorac NJ, Jacobs Jr WR, Holzenburg A, Sacchettini JC, Alland D. The Mycobacterium tuberculosis iniA gene is essential for activity of an efflux pump that confers drug tolerance to both isoniazid and ethambutol. Mol Microbiol. 2005;55:1829–40.
83. Wilson M, DeRisi J, Kristensen HH, Imboden P, Rane S, Brown PO, Schoolnik GK. Exploring drug-induced alterations in gene

expression in Mycobacterium tuberculosis by microarray hybridization. Proc Natl Acad Sci U S A. 1999;96:12833–8.
84. Tessema B, Beer J, Emmrich F, Sack U, Rodloff AC. Analysis of gene mutations associated with isoniazid, rifampicin and ethambutol resistance among Mycobacterium tuberculosis isolates from Ethiopia. BMC Infect Dis. 2012;12:37.
85. Sensi P. History of the development of rifampin. Rev Infect Dis. 1983;5 Suppl 3:S402–6.
86. Lounis N, Roscigno G. In vitro and in vivo activities of new rifamycin derivatives against mycobacterial infections. Curr Pharm Des. 2004;10:3229–38.
87. Donald PR, Sirgel FA, Botha FJ, Seifart HI, Parkin DP, Vandenplas ML, Van de Wal BW, Maritz JS, Mitchison DA. The early bactericidal activity of isoniazid related to its dose size in pulmonary tuberculosis. Am J Respir Crit Care Med. 1997;156:895–900.
88. Sirgel FA, Donald PR, Odhiambo J, Githui W, Umapathy KC, Paramasivan CN, Tam CM, Kam KM, Lam CW, Sole KM, Mitchison DA. A multicentre study of the early bactericidal activity of anti-tuberculosis drugs. J Antimicrob Chemother. 2000;45:859–70.
89. Chan SL, Yew WW, Ma WK, Girling DJ, Aber VR, Felmingham D, Allen BW, Mitchison DA. The early bactericidal activity of rifabutin measured by sputum viable counts in Hong Kong patients with pulmonary tuberculosis. Tuber Lung Dis. 1992;73:33–8.
90. Grosset J, Lounis N, Truffot-Pernot C, O'Brien RJ, Raviglione MC, Ji B. Once-weekly rifapentine-containing regimens for treatment of tuberculosis in mice. Am J Respir Crit Care Med. 1998;157:1436–40.
91. Mitchison DA. Mechanisms of the action of drugs in the short-course chemotherapy. Bull Int Union Tuberc. 1985;60:36–40.
92. Nightingale SD, Cameron DW, Gordin FM, Sullam PM, Cohn DL, Chaisson RE, Eron LJ, Sparti PD, Bihari B, Kaufman DL, et al. Two controlled trials of rifabutin prophylaxis against Mycobacterium avium complex infection in AIDS. N Engl J Med. 1993;329:828–33.
93. Sullam PM, Gordin FM, Wynne BA, The Rifabutin Treatment Group. Efficacy of rifabutin in the treatment of disseminated infection due to Mycobacterium avium complex. Clin Infect Dis. 1994;19:84–6.
94. Dutta NK, Alsultan A, Peloquin CA, Karakousis PC. Preliminary pharmacokinetic study of repeated doses of rifampin and rifapentine in guinea pigs. Antimicrob Agents Chemother. 2013;57:1535–7.
95. Dutta NK, Illei PB, Peloquin CA, Pinn ML, Mdluli KE, Nuermberger EL, Grosset JH, Karakousis PC. Rifapentine is not more active than rifampin against chronic tuberculosis in guinea pigs. Antimicrob Agents Chemother. 2012;56:3726–31.
96. Benator D, Bhattacharya M, Bozeman L, Burman W, Cantazaro A, Chaisson R, Gordin F, Horsburgh CR, Horton J, Khan A, Lahart C, Metchock B, Pachucki C, Stanton L, Vernon A, Villarino ME, Wang YC, Weiner M, Weis S. Rifapentine and isoniazid once a week versus rifampicin and isoniazid twice a week for treatment of drug-susceptible pulmonary tuberculosis in HIV-negative patients: a randomised clinical trial. Lancet. 2002;360:528–34.
97. Vernon A. Rifamycin antibiotics, with a focus on newer agents. In: Rom W, Garay S, editors. Tuberculosis. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 2000. p. 759–71.
98. Hartmann G, Honikel KO, Knusel F, Nuesch J. The specific inhibition of the DNA-directed RNA synthesis by rifamycin. Biochim Biophys Acta. 1967;145:843–4.
99. Archambault J, Friesen JD. Genetics of eukaryotic RNA polymerases I, II, and III. Microbiol Rev. 1993;57:703–24.
100. Campbell EA, Korzheva N, Mustaev A, Murakami K, Nair S, Goldfarb A, Darst SA. Structural mechanism for rifampicin inhibition of bacterial rna polymerase. Cell. 2001;104:901–12.
101. Sat B, Hazan R, Fisher T, Khaner H, Glaser G, Engelberg-Kulka H. Programmed cell death in Escherichia coli: some antibiotics can trigger mazEF lethality. J Bacteriol. 2001;183:2041–5.
102. Gerdes K, Christensen SK, Lobner-Olesen A. Prokaryotic toxin-antitoxin stress response loci. Nat Rev Microbiol. 2005;3:371–82.
103. Zhu L, Zhang Y, Teh JS, Zhang J, Connell N, Rubin H, Inouye M. Characterization of mRNA interferases from Mycobacterium tuberculosis. J Biol Chem. 2006;281:18638–43.
104. Ramaswamy S, Musser JM. Molecular genetic basis of antimicrobial agent resistance in Mycobacterium tuberculosis: 1998 update. Tuber Lung Dis. 1998;79:3–29.
105. Zhang Y, Telenti A. Genetics of drug resistance in Mycobacterium tuberculosis. In: Hatfull G, Jacobs Jr WR, editors. Molecular genetics of mycobacteria. Washington, DC: ASM Press; 2000. p. 235–54.
106. Jin DJ, Gross CA. Mapping and sequencing of mutations in the Escherichia coli rpoB gene that lead to rifampicin resistance. J Mol Biol. 1988;202:45–58.
107. Lisitsyn NA, Sverdlov ED, Moiseyeva EP, Danilevskaya ON, Nikiforov VG. Mutation to rifampicin resistance at the beginning of the RNA polymerase beta subunit gene in Escherichia coli. Mol Gen Genet. 1984;196:173–4.
108. Ovchinnikov YA, Monastyrskaya GS, Guriev SO, Kalinina NF, Sverdlov ED, Gragerov AI, Bass IA, Kiver IF, Moiseyeva EP, Igumnov VN, Mindlin SZ, Nikiforov VG, Khesin RB. RNA polymerase rifampicin resistance mutations in Escherichia coli: sequence changes and dominance. Mol Gen Genet. 1983;190:344–8.
109. Telenti A, Imboden P, Marchesi F, Lowrie D, Cole S, Colston MJ, Matter L, Schopfer K, Bodmer T. Detection of rifampicin-resistance mutations in Mycobacterium tuberculosis. Lancet. 1993;341:647–50.
110. Musser JM. Antimicrobial agent resistance in mycobacteria: molecular genetic insights. Clin Microbiol Rev. 1995;8:496–514.
111. Kapur V, Li LL, Hamrick MR, Plikaytis BB, Shinnick TM, Telenti A, Jacobs Jr WR, Banerjee A, Cole S, Yuen KY, et al. Rapid Mycobacterium species assignment and unambiguous identification of mutations associated with antimicrobial resistance in Mycobacterium tuberculosis by automated DNA sequencing. Arch Pathol Lab Med. 1995;119:131–8.
112. Kapur V, Li LL, Iordanescu S, Hamrick MR, Wanger A, Kreiswirth BN, Musser JM. Characterization by automated DNA sequencing of mutations in the gene (rpoB) encoding the RNA polymerase beta subunit in rifampin-resistant Mycobacterium tuberculosis strains from New York City and Texas. J Clin Microbiol. 1994;32:1095–8.
113. Williams DL, Waguespack C, Eisenach K, Crawford JT, Portaels F, Salfinger M, Nolan CM, Abe C, Sticht-Groh V, Gillis TP. Characterization of rifampin-resistance in pathogenic mycobacteria. Antimicrob Agents Chemother. 1994;38:2380–6.
114. Billington OJ, McHugh TD, Gillespie SH. Physiological cost of rifampin resistance induced in vitro in Mycobacterium tuberculosis. Antimicrob Agents Chemother. 1999;43:1866–9.
115. Chia BS, Lanzas F, Rifat D, Herrera A, Kim EY, Sailer C, Torres-Chavolla E, Narayanaswamy P, Einarsson V, Bravo J, Pascale JM, Ioerger TR, Sacchettini JC, Karakousis PC. Use of multiplex allele-specific polymerase chain reaction (MAS-PCR) to detect multidrug-resistant tuberculosis in Panama. PLoS One. 2012;7, e40456.
116. Bodmer T, Zurcher G, Imboden P, Telenti A. 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;35:345–8.
117. Moghazeh SL, Pan X, Arain T, Stover CK, Musser JM, Kreiswirth BN. Comparative antimycobacterial activities of rifampin,

rifapentine, and KRM-1648 against a collection of rifampin-resistant Mycobacterium tuberculosis isolates with known rpoB mutations. Antimicrob Agents Chemother. 1996;40:2655–7.
118. Williams DL, Spring L, Collins L, Miller LP, Heifets LB, Gangadharam PR, Gillis TP. Contribution of rpoB mutations to development of rifamycin cross-resistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother. 1998;42:1853–7.
119. Yoshida S, Suzuki K, Iwamoto T, Tsuyuguchi K, Tomita M, Okada M, Sakatani M. Comparison of rifabutin susceptibility and rpoB mutations in multi-drug-resistant Mycobacterium tuberculosis strains by DNA sequencing and the line probe assay. J Infect Chemother. 2010;16:360–3.
120. Heep M, Brandstatter B, Rieger U, Lehn N, Richter E, Rusch-Gerdes S, Niemann S. 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.
121. Heep M, Odenbreit S, Beck D, Decker J, Prohaska E, Rieger U, Lehn N. Mutations at four distinct regions of the rpoB gene can reduce the susceptibility of Helicobacter pylori to rifamycins. Antimicrob Agents Chemother. 2000;44:1713–5.
122. Yang B, Koga H, Ohno H, Ogawa K, Fukuda M, Hirakata Y, Maesaki S, Tomono K, Tashiro T, Kohno S. Relationship between antimycobacterial activities of rifampicin, rifabutin and KRM-1648 and rpoB mutations of Mycobacterium tuberculosis. J Antimicrob Chemother. 1998;42:621–8.
123. Cavusoglu C, Karaca-Derici Y, Bilgic A. In-vitro activity of rifabutin against rifampicin-resistant Mycobacterium tuberculosis isolates with known rpoB mutations. Clin Microbiol Infect. 2004;10:662–5.
124. Dabbs ER, Yazawa K, Mikami Y, Miyaji M, Morisaki N, Iwasaki S, Furihata K. Ribosylation by mycobacterial strains as a new mechanism of rifampin inactivation. Antimicrob Agents Chemother. 1995;39:1007–9.
125. Quan S, Venter H, Dabbs ER. Ribosylative inactivation of rifampin by Mycobacterium smegmatis is a principal contributor to its low susceptibility to this antibiotic. Antimicrob Agents Chemother. 1997;41:2456–60.
126. Traore H, Fissette K, Bastian I, Devleeschouwer M, Portaels F. 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;4:481–4.
127. Steele MA, Des Prez RM. The role of pyrazinamide in tuberculosis chemotherapy. Chest. 1988;94:845–50.
128. Wright A, Zignol M, Van Deun A, Falzon D, Gerdes SR, Feldman K, Hoffner S, Drobniewski F, Barrera L, van Soolingen D, Boulabhal F, Paramasivan CN, Kam KM, Mitarai S, Nunn P, Raviglione M. Epidemiology of antituberculosis drug resistance 2002–07: an updated analysis of the Global Project on Anti-Tuberculosis Drug Resistance Surveillance. Lancet. 2009;373:1861–73.
129. Simons SO, van Ingen J, van der Laan T, Mulder A, Dekhuijzen PN, Boeree MJ, van Soolingen D. Validation of pncA gene sequencing in combination with the mycobacterial growth indicator tube method to test susceptibility of Mycobacterium tuberculosis to pyrazinamide. J Clin Microbiol. 2012;50:428–34.
130. Jindani A, Aber VR, Edwards EA, Mitchison DA. The early bactericidal activity of drugs in patients with pulmonary tuberculosis. Am Rev Respir Dis. 1980;121:939–49.
131. Mitchison DA. The action of antituberculosis drugs in short-course chemotherapy. Tubercle. 1985;66:219–25.
132. Zhang Y, Permar S, Sun Z. Conditions that may affect the results of susceptibility testing of Mycobacterium tuberculosis to pyrazinamide. J Med Microbiol. 2002;51:42–9.
133. Yeager RL, Munroe WG, Dessau FI. Pyrazinamide (aldinamide) in the treatment of pulmonary tuberculosis. Am Rev Tuberc. 1952;65:523–46.
134. Results at 5 years of a controlled comparison of a 6-month and a standard 18-month regimen of chemotherapy for pulmonary tuberculosis. Am Rev Respir Dis. 1977;116:3–8.
135. Controlled clinical trial of four short-course regimens of chemotherapy for two durations in the treatment of pulmonary tuberculosis. Second report. Third East African/British Medical Research Council Study. Tubercle. 1980;61:59–69.
136. Santha T, Nazareth O, Krishnamurthy MS, Balasubramanian R, Vijayan VK, Janardhanam B, Venkataraman P, Tripathy SP, Prabhakar R. Treatment of pulmonary tuberculosis with short course chemotherapy in south India—5-year follow up. Tubercle. 1989;70:229–34.
137. Tarshis MS, Weed Jr WA. Lack of significant in vitro sensitivity of Mycobacterium tuberculosis to pyrazinamide on three different solid media. Am Rev Tuberc. 1953;67:391–5.
138. McDermott W, Tompsett R. Activation of pyrazinamide and nicotinamide in acidic environments in vitro. Am Rev Tuberc. 1954;70:748–54.
139. Kushner S, Dalalian H, Sanjurjo J, Bach Jr F, Safir S, Smith Jr V, Williams J. Experimental chemotherapy of tuberculosis. II. The synthesis of pyrazinamides and related compounds. J Am Chem Soc. 1952;74:3617–21.
140. Salfinger M, Crowle AJ, Reller LB. Pyrazinamide and pyrazinoic acid activity against tubercle bacilli in cultured human macrophages and in the BACTEC system. J Infect Dis. 1990;162:201–7.
141. Crowle AJ, Dahl R, Ross E, May MH. Evidence that vesicles containing living, virulent Mycobacterium tuberculosis or Mycobacterium avium in cultured human macrophages are not acidic. Infect Immun. 1991;59:1823–31.
142. Sturgill-Koszycki S, Schlesinger PH, Chakraborty P, Haddix PL, Collins HL, Fok AK, Allen RD, Gluck SL, Heuser J, Russell DG. Lack of acidification in Mycobacterium phagosomes produced by exclusion of the vesicular proton-ATPase. Science. 1994;263:678–81.
143. Heifets L, Lindholm-Levy P. Pyrazinamide sterilizing activity in vitro against semidormant Mycobacterium tuberculosis bacterial populations. Am Rev Respir Dis. 1992;145:1223–5.
144. Crowle AJ, Sbarbaro JA, May MH. Inhibition by pyrazinamide of tubercle bacilli within cultured human macrophages. Am Rev Respir Dis. 1986;134:1052–5.
145. Heifets L, Higgins M, Simon B. Pyrazinamide is not active against Mycobacterium tuberculosis residing in cultured human monocyte-derived macrophages. Int J Tuberc Lung Dis. 2000;4:491–5.
146. Controlled clinical trial of 4 short-course regimens of chemotherapy (three 6-month and one 8-month) for pulmonary tuberculosis: final report. East and Central African/British Medical Research Council Fifth Collaborative Study. Tubercle. 1986;67:5–15.
147. Long-term follow-up of a clinical trial of six-month and four-month regimens of chemotherapy in the treatment of pulmonary tuberculosis. Singapore Tuberculosis Service/British Medical Research Council. Am Rev Respir Dis. 1986;133:779–83.
148. 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. Results at 30 months. Hong Kong Chest Service/British Medical Research Council. Am Rev Respir Dis. 1991;143:700–6.
149. Wade MM, Zhang Y. Anaerobic incubation conditions enhance pyrazinamide activity against Mycobacterium tuberculosis. J Med Microbiol. 2004;53:769–73.
150. Raynaud C, Laneelle MA, Senaratne RH, Draper P, Laneelle G, Daffe M. Mechanisms of pyrazinamide resistance in mycobacteria: importance of lack of uptake in addition to lack of pyrazinamidase activity. Microbiology. 1999;145(Pt 6):1359–67.
151. Zhang Y, Scorpio A, Nikaido H, Sun Z. Role of acid pH and deficient efflux of pyrazinoic acid in unique susceptibility of

Mycobacterium tuberculosis to pyrazinamide. J Bacteriol. 1999;181:2044–9.
152. Konno K, Feldmann FM, McDermott W. Pyrazinamide susceptibility and amidase activity of tubercle bacilli. Am Rev Respir Dis. 1967;95:461–9.
153. 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.
154. Chang KC, Yew WW, Zhang Y. Pyrazinamide susceptibility testing in Mycobacterium tuberculosis: a systematic review with meta-analyses. Antimicrob Agents Chemother. 2011;55:4499–505.
155. Zhang JL, Zheng QC, Li ZQ, Zhang HX. Molecular dynamics simulations suggest ligand's binding to nicotinamidase/pyrazinamidase. PLoS One. 2012;7, e39546.
156. Zimhony O, Cox JS, Welch JT, Vilcheze C, Jacobs Jr WR. Pyrazinamide inhibits the eukaryotic-like fatty acid synthetase I (FASI) of Mycobacterium tuberculosis. Nat Med. 2000;6:1043–7.
157. Kikuchi S, Rainwater DL, Kolattukudy PE. Purification and characterization of an unusually large fatty acid synthase from Mycobacterium tuberculosis var. bovis BCG. Arch Biochem Biophys. 1992;295:318–26.
158. Zimhony O, Vilcheze C, Jacobs Jr WR. Characterization of Mycobacterium smegmatis expressing the Mycobacterium tuberculosis fatty acid synthase I (fas1) gene. J Bacteriol. 2004;186:4051–5.
159. Boshoff HI, Mizrahi V, Barry III CE. Effects of pyrazinamide on fatty acid synthesis by whole mycobacterial cells and purified fatty acid synthase I. J Bacteriol. 2002;184:2167–72.
160. Zhang Y, Wade MM, Scorpio A, Zhang H, Sun Z. Mode of action of pyrazinamide: disruption of Mycobacterium tuberculosis membrane transport and energetics by pyrazinoic acid. J Antimicrob Chemother. 2003;52:790–5.
161. Zhang Y, Zhang H, Sun Z. Susceptibility of Mycobacterium tuberculosis to weak acids. J Antimicrob Chemother. 2003;52:56–60.
162. Shi W, Zhang X, Jiang X, Yuan H, Lee JS, Barry III CE, Wang H, Zhang W, Zhang Y. Pyrazinamide inhibits trans-translation in Mycobacterium tuberculosis. Science. 2011;333:1630–2.
163. Boshoff HI, Mizrahi V. Expression of Mycobacterium smegmatis pyrazinamidase in Mycobacterium tuberculosis confers hypersensitivity to pyrazinamide and related amides. J Bacteriol. 2000;182:5479–85.
164. Cheng SJ, Thibert L, Sanchez T, Heifets L, Zhang Y. pncA mutations as a major mechanism of pyrazinamide resistance in Mycobacterium tuberculosis: spread of a monoresistant strain in Quebec, Canada. Antimicrob Agents Chemother. 2000;44:528–32.
165. Hirano K, Takahashi M, Kazumi Y, Fukasawa Y, Abe C. Mutation in pncA is a major mechanism of pyrazinamide resistance in Mycobacterium tuberculosis. Tuber Lung Dis. 1997;78:117–22.
166. Scorpio A, Lindholm-Levy P, Heifets L, Gilman R, Siddiqi S, Cynamon M, Zhang Y. Characterization of pncA mutations in pyrazinamide-resistant Mycobacterium tuberculosis. Antimicrob Agents Chemother. 1997;41:540–3.
167. Sreevatsan S, Pan X, Zhang Y, Kreiswirth BN, Musser JM. Mutations associated with pyrazinamide resistance in pncA of Mycobacterium tuberculosis complex organisms. Antimicrob Agents Chemother. 1997;41:636–40.
168. Rajendran V, Sethumadhavan R. Drug resistance mechanism of PncA in Mycobacterium tuberculosis. J Biomol Struct Dyn. 2013;32(2):209–21.
169. Cole ST. Microbiology. Pyrazinamide—old TB drug finds new target. Science. 2011;333:1583–4.
170. Zhang Y, Yew WW, Barer MR. Targeting persisters for tuberculosis control. Antimicrob Agents Chemother. 2012;56:2223–30.
171. Heifets L. Susceptibility testing of Mycobacterium tuberculosis to pyrazinamide. J Med Microbiol. 2002;51:11–2.
172. Maddry JA, Suling WJ, Reynolds RC. Glycosyltransferases as targets for inhibition of cell wall synthesis in M. tuberculosis and M. avium. Res Microbiol. 1996;147:106–12.
173. Thomas JP, Baughn CO, Wilkinson RG, Shepherd RG. A new synthetic compound with antituberculous activity in mice: ethambutol (dextro-2,2′-(ethylenediimino)-di-l-butanol). Am Rev Respir Dis. 1961;83:891–3.
174. Falkinham III JO. Epidemiology of infection by nontuberculous mycobacteria. Clin Microbiol Rev. 1996;9:177–215.
175. Forbes M, Kuck NA, Peets EA. Mode of action of ethambutol. J Bacteriol. 1962;84:1099–103.
176. Gangadharam PR, Pratt PF, Perumal VK, Iseman MD. The effects of exposure time, drug concentration, and temperature on the activity of ethambutol versus Mycobacterium tuberculosis. Am Rev Respir Dis. 1990;141:1478–82.
177. Liss RH. Bactericidal activity of ethambutol against extracellular Mycobacterium tuberculosis and bacilli phagocytized by human alveolar macrophages. S Afr Med J. 1982;Spec No.15–9.
178. Controlled trial of 6-month and 8-month regimens in the treatment of pulmonary tuberculosis: the results up to 24 months. Tubercle. 1979;60:201–10.
179. Five-year follow-up of a controlled trial of five 6-month regimens of chemotherapy for pulmonary tuberculosis. Hong Kong Chest Service/British Medical Research Council. Am Rev Respir Dis. 1987;136:1339–42.
180. Forbes M, Kuck NA, Peets EA. Effect of ethambutol on nucleic acid metabolism in Mycobacterium smegmatis and its reversal by polyamines and divalent cations. J Bacteriol. 1965;89:1299–305.
181. Takayama K, Armstrong EL, Kunugi KA, Kilburn JO. Inhibition by ethambutol of mycolic acid transfer into the cell wall of Mycobacterium smegmatis. Antimicrob Agents Chemother. 1979;16:240–2.
182. Cheema S, Khuller GK. Phospholipid composition and ethambutol sensitivity of Mycobacterium smegmatis ATCC 607. Indian J Exp Biol. 1985;23:511–3.
183. Cheema S, Khuller GK. Metabolism of phospholipids in Mycobacterium smegmatis ATCC 607 in the presence of ethambutol. Indian J Med Res. 1985;82:207–13.
184. Paulin LG, Brander EE, Poso HJ. Specific inhibition of spermidine synthesis in Mycobacteria spp. by the dextro isomer of ethambutol. Antimicrob Agents Chemother. 1985;28:157–9.
185. Takayama K, Kilburn JO. Inhibition of synthesis of arabinogalactan by ethambutol in Mycobacterium smegmatis. Antimicrob Agents Chemother. 1989;33:1493–9.
186. Mikusova K, Slayden RA, Besra GS, Brennan PJ. Biogenesis of the mycobacterial cell wall and the site of action of ethambutol. Antimicrob Agents Chemother. 1995;39:2484–9.
187. Kilburn JO, Greenberg J. Effect of ethambutol on the viable cell count in Mycobacterium smegmatis. Antimicrob Agents Chemother. 1977;11:534–40.
188. Kilburn JO, Takayama K. Effects of ethambutol on accumulation and secretion of trehalose mycolates and free mycolic acid in Mycobacterium smegmatis. Antimicrob Agents Chemother. 1981;20:401–4.
189. Deng L, Mikusova K, Robuck KG, Scherman M, Brennan PJ, McNeil MR. Recognition of multiple effects of ethambutol on metabolism of mycobacterial cell envelope. Antimicrob Agents Chemother. 1995;39:694–701.
190. Khoo KH, Douglas E, Azadi P, Inamine JM, Besra GS, Mikusova K, Brennan PJ, Chatterjee D. Truncated structural variants of lipoarabinomannan in ethambutol drug-resistant strains of Mycobacterium smegmatis. Inhibition of arabinan biosynthesis by ethambutol. J Biol Chem. 1996;271:28682–90.
191. Wolucka BA, McNeil MR, de Hoffmann E, Chojnacki T, Brennan PJ. 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;269:23328–35.

192. Belanger AE, Besra GS, Ford ME, Mikusova K, Belisle JT, Brennan PJ, Inamine JM. 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 U S A. 1996;93:11919–24.
193. Telenti A, Philipp WJ, Sreevatsan S, Bernasconi C, Stockbauer KE, Wieles B, Musser JM, Jacobs Jr WR. The emb operon, a gene cluster of Mycobacterium tuberculosis involved in resistance to ethambutol. Nat Med. 1997;3:567–70.
194. Lety MA, Nair S, Berche P, Escuyer V. A single point mutation in the embB gene is responsible for resistance to ethambutol in Mycobacterium smegmatis. Antimicrob Agents Chemother. 1997;41:2629–33.
195. Ramaswamy SV, Amin AG, Goksel S, Stager CE, Dou SJ, El Sahly H, Moghazeh SL, Kreiswirth BN, Musser JM. Molecular genetic analysis of nucleotide polymorphisms associated with ethambutol resistance in human isolates of Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2000;44:326–36.
196. Escuyer VE, Lety MA, Torrelles JB, Khoo KH, Tang JB, Rithner CD, Frehel C, McNeil MR, Brennan PJ, Chatterjee D. The role of the embA and embB gene products in the biosynthesis of the terminal hexaarabinofuranosyl motif of Mycobacterium smegmatis arabinogalactan. J Biol Chem. 2001;276:48854–62.
197. Zhang N, Torrelles JB, McNeil MR, Escuyer VE, Khoo KH, Brennan PJ, Chatterjee D. The Emb proteins of mycobacteria direct arabinosylation of lipoarabinomannan and arabinogalactan via an N-terminal recognition region and a C-terminal synthetic region. Mol Microbiol. 2003;50:69–76.
198. Sreevatsan S, Stockbauer KE, Pan X, Kreiswirth BN, Moghazeh SL, Jacobs Jr WR, Telenti A, Musser JM. Ethambutol resistance in Mycobacterium tuberculosis: critical role of embB mutations. Antimicrob Agents Chemother. 1997;41:1677–81.
199. Kiet VS, Lan NT, An DD, Dung NH, Hoa DV, van Vinh Chau N, Chinh NT, Farrar J, Caws M. Evaluation of the MTBDRsl test for detection of second-line-drug resistance in Mycobacterium tuberculosis. J Clin Microbiol. 2010;48:2934–9.
200. Zhang Y, Yew WW. Mechanisms of drug resistance in Mycobacterium tuberculosis. Int J Tuberc Lung Dis. 2009;13:1320–30.
201. Alcaide F, Pfyffer GE, Telenti A. Role of embB in natural and acquired resistance to ethambutol in mycobacteria. Antimicrob Agents Chemother. 1997;41:2270–3.
202. Mokrousov I, Otten T, Vyshnevskiy B, Narvskaya O. Detection of embB306 mutations in ethambutol-susceptible clinical isolates of Mycobacterium tuberculosis from Northwestern Russia: implications for genotypic resistance testing. J Clin Microbiol. 2002;40:3810–3.
203. Huang WL, Chi TL, Wu MH, Jou R. Performance assessment of the GenoType MTBDRsl test and DNA sequencing for detection of second-line and ethambutol drug resistance among patients infected with multidrug-resistant Mycobacterium tuberculosis. J Clin Microbiol. 2011;49:2502–8.
204. Lacoma A, Garcia-Sierra N, Prat C, Maldonado J, Ruiz-Manzano J, Haba L, Gavin P, Samper S, Ausina V, Dominguez J. GenoType MTBDRsl for molecular detection of second-line-drug and ethambutol resistance in Mycobacterium tuberculosis strains and clinical samples. J Clin Microbiol. 2012;50:30–6.
205. Alland D, Kramnik I, Weisbrod TR, Otsubo L, Cerny R, Miller LP, Jacobs Jr WR, Bloom BR. Identification of differentially expressed mRNA in prokaryotic organisms by customized amplification libraries (DECAL): the effect of isoniazid on gene expression in Mycobacterium tuberculosis. Proc Natl Acad Sci U S A. 1998;95:13227–32.
206. Brossier F, Veziris N, Aubry A, Jarlier V, Sougakoff W. Detection by GenoType MTBDRsl test of complex mechanisms of resistance to second-line drugs and ethambutol in multidrug-resistant Mycobacterium tuberculosis complex isolates. J Clin Microbiol. 2010;48:1683–9.
207. Schatz A, Bugie E, Waksman SA. Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. Proc Soc Exptl Biol Med. 1944;55:66–9.
208. Streptomycin treatment of pulmonary tuberculosis. BMJ. 1948;2:769–82.
209. Iseman MD. Tuberculosis chemotherapy, including directly observed therapy. In: A clinician's guide to tuberculosis. Philadelphia: Lippincott Williams & Wilkins; 2000. p. 271–321.
210. Chan E, Chatterjee D, Iseman M, Heifets L. Pyrazinamide, ethambutol, ethionamide, and aminoglycosides. In: Rom W, Garay S, editors. Tuberculosis. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 2004. p. 773–89.
211. Winder FG. Mode of action of the antimycobacterial agents and associated aspects of the molecular biology of mycobacteria. In: Ratledge C, Stanford J, editors. The biology of the mycobacteria, vol. 1. New York: Academic; 1982. p. 354–438.
212. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry III CE, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Krogh A, McLean J, Moule S, Murphy L, Oliver K, Osborne J, Quail MA, Rajandream MA, Rogers J, Rutter S, Seeger K, Skelton J, Squares R, Squares S, Sulston JE, Taylor K, Whitehead S, Barrell BG. Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. Nature. 1998;393:537–44.
213. Cole ST, Eiglmeier K, Parkhill J, James KD, Thomson NR, Wheeler PR, Honore N, Garnier T, Churcher C, Harris D, Mungall K, Basham D, Brown D, Chillingworth T, Connor R, Davies RM, Devlin K, Duthoy S, Feltwell T, Fraser A, Hamlin N, Holroyd S, Hornsby T, Jagels K, Lacroix C, Maclean J, Moule S, Murphy L, Oliver K, Quail MA, Rajandream MA, Rutherford KM, Rutter S, Seeger K, Simon S, Simmonds M, Skelton J, Squares R, Squares S, Stevens K, Taylor K, Whitehead S, Woodward JR, Barrell BG. Massive gene decay in the leprosy bacillus. Nature. 2001;409:1007–11.
214. Ainsa JA, Perez E, Pelicic V, Berthet FX, Gicquel B, Martin C. Aminoglycoside 2′-N-acetyltransferase genes are universally present in mycobacteria: characterization of the aac(2′)-Ic gene from Mycobacterium tuberculosis and the aac(2′)-Id gene from Mycobacterium smegmatis. Mol Microbiol. 1997;24:431–41.
215. Allen BW, Mitchison DA, Chan YC, Yew WW, Allan WG, Girling DJ. Amikacin in the treatment of pulmonary tuberculosis. Tubercle. 1983;64:111–8.
216. Tsukamura M, Mizuno S. Cross-resistant relationships among the aminoglucoside antibiotics in Mycobacterium tuberculosis. J Gen Microbiol. 1975;88:269–74.
217. Prammananan T, Sander P, Brown BA, Frischkorn K, Onyi GO, Zhang Y, Bottger EC, Wallace Jr RJ. A single 16S ribosomal RNA substitution is responsible for resistance to amikacin and other 2-deoxystreptamine aminoglycosides in Mycobacterium abscessus and Mycobacterium chelonae. J Infect Dis. 1998;177:1573–81.
218. Alangaden GJ, Kreiswirth BN, Aouad A, Khetarpal M, Igno FR, Moghazeh SL, Manavathu EK, Lerner SA. Mechanism of resistance to amikacin and kanamycin in Mycobacterium tuberculosis. Antimicrob Agents Chemother. 1998;42:1295–7.
219. 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.
220. Finken M, Kirschner P, Meier A, Wrede A, Bottger EC. 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;9:1239–46.
221. Honore N, Cole ST. Streptomycin resistance in mycobacteria. Antimicrob Agents Chemother. 1994;38:238–42.
222. Kenney TJ, Churchward G. Cloning and sequence analysis of the rpsL and rpsG genes of Mycobacterium smegmatis and characterization of mutations causing resistance to streptomycin. J Bacteriol. 1994;176:6153–6.
223. Nair J, Rouse DA, Bai GH, Morris SL. The rpsL gene and streptomycin resistance in single and multiple drug-resistant strains of Mycobacterium tuberculosis. Mol Microbiol. 1993;10:521–7.
224. Sreevatsan S, Pan X, Stockbauer KE, Williams DL, Kreiswirth BN, Musser JM. Characterization of rpsL and rrs mutations in streptomycin-resistant Mycobacterium tuberculosis isolates from diverse geographic localities. Antimicrob Agents Chemother. 1996;40:1024–6.
225. Douglass J, Steyn LM. A ribosomal gene mutation in streptomycin-resistant Mycobacterium tuberculosis isolates. J Infect Dis. 1993;167:1505–6.
226. Reeves AZ, Campbell PJ, Sultana R, Malik S, Murray M, Plikaytis BB, Shinnick TM, Posey JE. Aminoglycoside cross-resistance in Mycobacterium tuberculosis due to mutations in the 5′ untranslated region of whiB7. Antimicrob Agents Chemother. 2013;57:1857–65.
227. Okamoto S, Tamaru A, Nakajima C, Nishimura K, Tanaka Y, Tokuyama S, Suzuki Y, Ochi K. Loss of a conserved 7-methylguanosine modification in 16S rRNA confers low-level streptomycin resistance in bacteria. Mol Microbiol. 2007;63:1096–106.
228. Spies FS, da Silva PE, Ribeiro MO, Rossetti ML, Zaha A. Identification of mutations related to streptomycin resistance in clinical isolates of Mycobacterium tuberculosis and possible involvement of efflux mechanism. Antimicrob Agents Chemother. 2008;52:2947–9.
229. Via LE, Cho SN, Hwang S, Bang H, Park SK, Kang HS, Jeon D, Min SY, Oh T, Kim Y, Kim YM, Rajan V, Wong SY, Shamputa IC, Carroll M, Goldfeder L, Lee SA, Holland SM, Eum S, Lee H, Barry III CE. Polymorphisms associated with resistance and cross-resistance to aminoglycosides and capreomycin in Mycobacterium tuberculosis isolates from South Korean Patients with drug-resistant tuberculosis. J Clin Microbiol. 2010;48:402–11.
230. Wong SY, Lee JS, Kwak HK, Via LE, Boshoff HI, Barry III CE. Mutations in gidB confer low-level streptomycin resistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2011;55:2515–22.
231. Georghiou SB, Magana M, Garfein RS, Catanzaro DG, Catanzaro A, Rodwell TC. Evaluation of genetic mutations associated with Mycobacterium tuberculosis resistance to amikacin, kanamycin and capreomycin: a systematic review. PLoS One. 2012;7, e33275.
232. Johansen SK, Maus CE, Plikaytis BB, Douthwaite S. Capreomycin binds across the ribosomal subunit interface using tlyA-encoded 2′-O-methylations in 16S and 23S rRNAs. Mol Cell. 2006;23:173–82.
233. Caminero JA, Sotgiu G, Zumla A, Migliori GB. Best drug treatment for multidrug-resistant and extensively drug-resistant tuberculosis. Lancet Infect Dis. 2010;10:621–9.
234. Jacobs MR. Fluoroquinolones as chemotherapeutics against mycobacterial infections. Curr Pharm Des. 2004;10:3213–20.
235. Garcia-Rodriguez JA, Gomez Garcia AC. In-vitro activities of quinolones against mycobacteria. J Antimicrob Chemother. 1993;32:797–808.
236. Jacobs MR. Activity of quinolones against mycobacteria. Drugs. 1999;58 Suppl 2:19–22.
237. Klemens SP, Sharpe CA, Rogge MC, Cynamon MH. Activity of levofloxacin in a murine model of tuberculosis. Antimicrob Agents Chemother. 1994;38:1476–9.
238. Lounis N, Ji B, Truffot-Pernot C, Grosset J. Which aminoglycoside or fluoroquinolone is more active against Mycobacterium tuberculosis in mice? Antimicrob Agents Chemother. 1997;41:607–10.
239. Yoshimatsu T, Nuermberger E, Tyagi S, Chaisson R, Bishai W, Grosset J. Bactericidal activity of increasing daily and weekly doses of moxifloxacin in murine tuberculosis. Antimicrob Agents Chemother. 2002;46:1875–9.
240. Ginsburg AS, Grosset JH, Bishai WR. Fluoroquinolones, tuberculosis, and resistance. Lancet Infect Dis. 2003;3:432–42.
241. Ji B, Lounis N, Maslo C, Truffot-Pernot C, Bonnafous P, Grosset J. In vitro and in vivo activities of moxifloxacin and clinafloxacin against Mycobacterium tuberculosis. Antimicrob Agents Chemother. 1998;42:2066–9.
242. Miyazaki E, Miyazaki M, Chen JM, Chaisson RE, Bishai WR. Moxifloxacin (BAY12-8039), a new 8-methoxyquinolone, is active in a mouse model of tuberculosis. Antimicrob Agents Chemother. 1999;43:85–9.
243. Gosling RD, Uiso LO, Sam NE, Bongard E, Kanduma EG, Nyindo M, Morris RW, Gillespie SH. The bactericidal activity of moxifloxacin in patients with pulmonary tuberculosis. Am J Respir Crit Care Med. 2003;168:1342–5.
244. Johnson JL, Hadad DJ, Boom WH, Daley CL, Peloquin CA, Eisenach KD, Jankus DD, Debanne SM, Charlebois ED, Maciel E, Palaci M, Dietze R. Early and extended early bactericidal activity of levofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. Int J Tuberc Lung Dis. 2006;10:605–12.
245. Pletz MW, De Roux A, Roth A, Neumann KH, Mauch H, Lode H. Early bactericidal activity of moxifloxacin in treatment of pulmonary tuberculosis: a prospective, randomized study. Antimicrob Agents Chemother. 2004;48:780–2.
246. Paramasivan CN, Sulochana S, Kubendiran G, Venkatesan P, Mitchison DA. Bactericidal action of gatifloxacin, rifampin, and isoniazid on logarithmic- and stationary-phase cultures of Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2005;49:627–31.
247. Nuermberger EL, Yoshimatsu T, Tyagi S, O'Brien RJ, Vernon AN, Chaisson RE, Bishai WR, Grosset JH. Moxifloxacin-containing regimen greatly reduces time to culture conversion in murine tuberculosis. Am J Respir Crit Care Med. 2004;169:421–6.
248. Nuermberger EL, Yoshimatsu T, Tyagi S, Williams K, Rosenthal I, O'Brien RJ, Vernon AA, Chaisson RE, Bishai WR, Grosset JH. Moxifloxacin-containing regimens of reduced duration produce a stable cure in murine tuberculosis. Am J Respir Crit Care Med. 2004;170:1131–4.
249. Ginsburg AS, Hooper N, Parrish N, Dooley KE, Dorman SE, Booth J, Diener-West M, Merz WG, Bishai WR, Sterling TR. Fluoroquinolone resistance in patients with newly diagnosed tuberculosis. Clin Infect Dis. 2003;37:1448–52.
250. Ginsburg AS, Woolwine SC, Hooper N, Benjamin Jr WH, Bishai WR, Dorman SE, Sterling TR. The rapid development of fluoroquinolone resistance in M. tuberculosis. N Engl J Med. 2003;349:1977–8.
251. Bozeman L, Burman W, Metchock B, Welch L, Weiner M. Fluoroquinolone susceptibility among Mycobacterium tuberculosis isolates from the United States and Canada. Clin Infect Dis. 2005;40:386–91.
252. Drlica K, Malik M. Fluoroquinolones: action and resistance. Curr Top Med Chem. 2003;3:249–82.
253. Alangaden GJ, Manavathu EK, Vakulenko SB, Zvonok NM, Lerner SA. Characterization of fluoroquinolone-resistant mutant strains of Mycobacterium tuberculosis selected in the laboratory and isolated from patients. Antimicrob Agents Chemother. 1995;39:1700–3.
254. Takiff HE, Salazar L, Guerrero C, Philipp W, Huang WM, Kreiswirth B, Cole ST, Jacobs Jr WR, Telenti A. Cloning and

nucleotide sequence of Mycobacterium tuberculosis gyrA and gyrB genes and detection of quinolone resistance mutations. Antimicrob Agents Chemother. 1994;38:773–80.
255. Xu C, Kreiswirth BN, Sreevatsan S, Musser JM, Drlica K. Fluoroquinolone resistance associated with specific gyrase mutations in clinical isolates of multidrug-resistant Mycobacterium tuberculosis. J Infect Dis. 1996;174:1127–30.
256. Cambau E, Sougakoff W, Besson M, Truffot-Pernot C, Grosset J, Jarlier V. Selection of a gyrA mutant of Mycobacterium tuberculosis resistant to fluoroquinolones during treatment with ofloxacin. J Infect Dis. 1994;170:479–83.
257. Kocagoz T, Hackbarth CJ, Unsal I, Rosenberg EY, Nikaido H, Chambers HF. Gyrase mutations in laboratory-selected, fluoroquinolone-resistant mutants of Mycobacterium tuberculosis H37Ra. Antimicrob Agents Chemother. 1996;40:1768–74.
258. Zhou J, Dong Y, Zhao X, Lee S, Amin A, Ramaswamy S, Domagala J, Musser JM, Drlica K. Selection of antibiotic-resistant bacterial mutants: allelic diversity among fluoroquinolone-resistant mutations. J Infect Dis. 2000;182:517–25.
259. 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 Suppl 4:228–37.
260. Pantel A, Petrella S, Veziris N, Brossier F, Bastian S, Jarlier V, Mayer C, Aubry A. Extending the definition of the GyrB quinolone resistance-determining region in Mycobacterium tuberculosis DNA gyrase for assessing fluoroquinolone resistance in M. tuberculosis. Antimicrob Agents Chemother. 2012;56:1990–6.
261. Malik S, Willby M, Sikes D, Tsodikov OV, Posey JE. New insights into fluoroquinolone resistance in Mycobacterium tuberculosis: functional genetic analysis of gyrA and gyrB mutations. PLoS One. 2012;7, e39754.
262. Liu J, Takiff HE, Nikaido H. Active efflux of fluoroquinolones in Mycobacterium smegmatis mediated by LfrA, a multidrug efflux pump. J Bacteriol. 1996;178:3791–5.
263. Takiff HE, Cimino M, Musso MC, Weisbrod T, Martinez R, Delgado MB, Salazar L, Bloom BR, Jacobs Jr WR. Efflux pump of the proton antiporter family confers low-level fluoroquinolone resistance in Mycobacterium smegmatis. Proc Natl Acad Sci U S A. 1996;93:362–6.
264. Bateman A, Murzin AG, Teichmann SA. Structure and distribution of pentapeptide repeats in bacteria. Protein Sci. 1998;7:1477–80.
265. Montero C, Mateu G, Rodriguez R, Takiff H. Intrinsic resistance of Mycobacterium smegmatis to fluoroquinolones may be influenced by new pentapeptide protein MfpA. Antimicrob Agents Chemother. 2001;45:3387–92.
266. Hegde SS, Vetting MW, Roderick SL, Mitchenall LA, Maxwell A, Takiff HE, Blanchard JS. A fluoroquinolone resistance protein from Mycobacterium tuberculosis that mimics DNA. Science. 2005;308:1480–3.
267. Bhatt K, Banerjee SK, Chakraborti PK. Evidence that phosphate specific transporter is amplified in a fluoroquinolone resistant Mycobacterium smegmatis. Eur J Biochem. 2000;267:4028–32.
268. Dautzenberg B, Truffot C, Legris S, Meyohas MC, Berlie HC, Mercat A, Chevret S, Grosset J. Activity of clarithromycin against Mycobacterium avium infection in patients with the acquired immune deficiency syndrome. A controlled clinical trial. Am Rev Respir Dis. 1991;144:564–9.
269. Chaisson RE, Benson CA, Dube MP, Heifets LB, Korvick JA, Elkin S, Smith T, Craft JC, Sattler FR. Clarithromycin therapy for bacteremic Mycobacterium avium complex disease. A randomized, double-blind, dose-ranging study in patients with AIDS. AIDS Clinical Trials Group Protocol 157 Study Team. Ann Intern Med. 1994;121:905–11.
270. Young LS, Wiviott L, Wu M, Kolonoski P, Bolan R, Inderlied CB. Azithromycin for treatment of Mycobacterium avium-intracellulare complex infection in patients with AIDS. Lancet. 1991;338:1107–9.
271. Ward TT, Rimland D, Kauffman C, Huycke M, Evans TG, Heifets L. Randomized, open-label trial of azithromycin plus ethambutol vs. clarithromycin plus ethambutol as therapy for Mycobacterium avium complex bacteremia in patients with human immunodeficiency virus infection. Veterans Affairs HIV Research Consortium. Clin Infect Dis. 1998;27:1278–85.
272. Dunne M, Fessel J, Kumar P, Dickenson G, Keiser P, Boulos M, Mogyros M, White Jr AC, Cahn P, O'Connor M, Lewi D, Green S, Tilles J, Hicks C, Bissett J, Schneider MM, Benner R. A randomized, double-blind trial comparing azithromycin and clarithromycin in the treatment of disseminated Mycobacterium avium infection in patients with human immunodeficiency virus. Clin Infect Dis. 2000;31:1245–52.
273. Chaisson RE, Keiser P, Pierce M, Fessel WJ, Ruskin J, Lahart C, Benson CA, Meek K, Siepman N, Craft JC. Clarithromycin and ethambutol with or without clofazimine for the treatment of bacteremic Mycobacterium avium complex disease in patients with HIV infection. Aids. 1997;11:311–7.
274. Piscitelli SC, Danziger LH, Rodvold KA. Clarithromycin and azithromycin: new macrolide antibiotics. Clin Pharm. 1992;11:137–52.
275. Bermudez LE, Yamazaki Y. Effects of macrolides and ketolides on mycobacterial infections. Curr Pharm Des. 2004;10:3221–8.
276. Buriankova K, Doucet-Populaire F, Dorson O, Gondran A, Ghnassia JC, Weiser J, Pernodet JL. Molecular basis of intrinsic macrolide resistance in the Mycobacterium tuberculosis complex. Antimicrob Agents Chemother. 2004;48:143–50.
277. Madsen CT, Jakobsen L, Buriankova K, Doucet-Populaire F, Pernodet JL, Douthwaite S. Methyltransferase Erm(37) slips on rRNA to confer atypical resistance in Mycobacterium tuberculosis. J Biol Chem. 2005;280:38942–7.
278. Andini N, Nash KA. Intrinsic macrolide resistance of the Mycobacterium tuberculosis complex is inducible. Antimicrob Agents Chemother. 2006;50:2560–2.
279. Philalay JS, Palermo CO, Hauge KA, Rustad TR, Cangelosi GA. Genes required for intrinsic multidrug resistance in Mycobacterium avium. Antimicrob Agents Chemother. 2004;48:3412–8.
280. Ji B, Lounis N, Truffot-Pernot C, Grosset J. Selection of resistant mutants of Mycobacterium avium in beige mice by clarithromycin monotherapy. Antimicrob Agents Chemother. 1992;36:2839–40.
281. Doucet-Populaire F, Truffot-Pernot C, Grosset J, Jarlier V. Acquired resistance in Mycobacterium avium complex strains isolated from AIDS patients and beige mice during treatment with clarithromycin. J Antimicrob Chemother. 1995;36:129–36.
282. Meier A, Kirschner P, Springer B, Steingrube VA, Brown BA, Wallace Jr RJ, Bottger EC. Identification of mutations in 23S rRNA gene of clarithromycin-resistant Mycobacterium intracellulare. Antimicrob Agents Chemother. 1994;38:381–4.
283. Skinner R, Cundliffe E, Schmidt FJ. Site of action of a ribosomal RNA methylase responsible for resistance to erythromycin and other antibiotics. J Biol Chem. 1983;258:12702–6.
284. Nash KA, Inderlied CB. Genetic basis of macrolide resistance in Mycobacterium avium isolated from patients with disseminated disease. Antimicrob Agents Chemother. 1995;39:2625–30.
285. Thiermann S, Munzinger J, Bodmer T. Comparison of phenotypic and genotypic methods for the detection of clarithromycin resistance in Mycobacterium avium. J Antimicrob Chemother. 2002;49:679–81.
286. Wallace Jr RJ, Meier A, Brown BA, Zhang Y, Sander P, Onyi GO, Bottger EC. Genetic basis for clarithromycin resistance among isolates of Mycobacterium chelonae and Mycobacterium abscessus. Antimicrob Agents Chemother. 1996;40:1676–81.

287. Inagaki T, Yagi T, Ichikawa K, Nakagawa T, Moriyama M, Uchiya K, Nikai T, Ogawa K. Evaluation of a rapid detection method of clarithromycin resistance genes in Mycobacterium avium complex isolates. J Antimicrob Chemother. 2011;66:722–9.
288. Jamal MA, Maeda S, Nakata N, Kai M, Fukuchi K, Kashiwabara Y. Molecular basis of clarithromycin-resistance in Mycobacterium avium intracellulare complex. Tuber Lung Dis. 2000;80:1–4.
289. 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.
290. DeBarber AE, Mdluli K, Bosman M, Bekker LG, Barry III CE. Ethionamide activation and sensitivity in multidrug-resistant Mycobacterium tuberculosis. Proc Natl Acad Sci U S A. 2000;97:9677–82.
291. Saribas Z, Kocagoz T, Alp A, Gunalp A. Rapid detection of rifampin resistance in Mycobacterium tuberculosis isolates by heteroduplex analysis and determination of rifamycin cross-resistance in rifampin-resistant isolates. J Clin Microbiol. 2003;41:816–8.
292. Uzun M, Erturan Z, Ang O. Investigation of cross-resistance between rifampin and rifabutin in Mycobacterium tuberculosis complex strains. Int J Tuberc Lung Dis. 2002;6:164–5.
293. Senol G, Erbaycu A, Ozsoz A. Incidence of cross resistance between rifampicin and rifabutin in Mycobacterium tuberculosis strains in Izmir, Turkey. J Chemother. 2005;17:380–4.
294. Yamada T, Masuda K, Mizuguchi Y, Suga K. Altered ribosomes in antibiotic-resistant mutants of Mycobacterium smegmatis. Antimicrob Agents Chemother. 1976;9:817–23.
295. Yamada T, Mizugichi Y, Nierhaus KH, Wittmann HG. Resistance to viomycin conferred by RNA of either ribosomal subunit. Nature. 1978;275:460–1.
296. Ng VH, Cox JS, Sousa AO, MacMicking JD, McKinney JD. Role of KatG catalase-peroxidase in mycobacterial pathogenesis: countering the phagocyte oxidative burst. Mol Microbiol. 2004;52:1291–302.
297. Cohen T, Becerra MC, Murray MB. Isoniazid resistance and the future of drug-resistant tuberculosis. Microb Drug Resist. 2004;10:280–5.
298. Agerton TB, Valway SE, Blinkhorn RJ, Shilkret KL, Reves R, Schluter WW, Gore B, Pozsik CJ, Plikaytis BB, Woodley C, Onorato IM. Spread of strain W, a highly drug-resistant strain of Mycobacterium tuberculosis, across the United States. Clin Infect Dis. 1999;29:85–92. discussion 93-5.
299. Mukherjee JS, Rich ML, Socci AR, Joseph JK, Viru FA, Shin SS, Furin JJ, Becerra MC, Barry DJ, Kim JY, Bayona J, Farmer P, Smith Fawzi MC, Seung KJ. Programmes and principles in treatment of multidrug-resistant tuberculosis. Lancet. 2004;363:474–81.
300. Sharma SK, Mohan A. Multidrug-resistant tuberculosis: a menace that threatens to destabilize tuberculosis control. Chest. 2006;130:261–72.
301. Borrell S, Gagneux S. Infectiousness, reproductive fitness and evolution of drug-resistant Mycobacterium tuberculosis. Int J Tuberc Lung Dis. 2009;13:1456–66.
302. Dutta NK, Karakousis PC. Tuberculosis chemotherapy: present situation, possible solutions, and progress towards a TB-free world. Indian J Med Microbiol. 2012;30:261–3.
303. Grosset JH, Singer TG, Bishai WR. New drugs for the treatment of tuberculosis: hope and reality. Int J Tuberc Lung Dis. 2012;16:1005–14.
304. Diacon AH, Dawson R, Hanekom M, Narunsky K, Venter A, Hittel N, Geiter LJ, Wells CD, Paccaly AJ, Donald PR. Early bactericidal activity of delamanid (OPC-67683) in smear-positive pulmonary tuberculosis patients. Int J Tuberc Lung Dis. 2011;15:949–54.
305. Diacon AH, Dawson R, von Groote-Bidlingmaier F, Symons G, Venter A, Donald PR, van Niekerk C, Everitt D, Winter H, Becker P, Mendel CM, Spigelman MK. 14-day bactericidal activity of PA-824, bedaquiline, pyrazinamide, and moxifloxacin combinations: a randomised trial. Lancet. 2012;380:986–93.
306. Diacon AH, Donald PR, Pym A, Grobusch M, Patientia RF, Mahanyele R, Bantubani N, Narasimooloo R, De Marez T, van Heeswijk R, Lounis N, Meyvisch P, Andries K, McNeeley DF. Randomized pilot trial of eight weeks of bedaquiline (TMC207) treatment for multidrug-resistant tuberculosis: long-term outcome, tolerability, and effect on emergence of drug resistance. Antimicrob Agents Chemother. 2012;56:3271–6.
307. Sq109. Tuberculosis (Edinb). 2008;88:159–61.
308. Cattaneo D, Orlando G, Cordier L. Linezolid for extensively drug-resistant tuberculosis. N Engl J Med. 2013;368:291.
309. Williams K, Minkowski A, Amoabeng O, Peloquin CA, Taylor D, Andries K, Wallis RS, Mdluli KE, Nuermberger EL. Sterilizing activities of novel combinations lacking first- and second-line drugs in a murine model of tuberculosis. Antimicrob Agents Chemother. 2012;56:3114–20.
310. Makarov V, Manina G, Mikusova K, Mollmann U, Ryabova O, Saint-Joanis B, Dhar N, Pasca MR, Buroni S, Lucarelli AP, Milano A, De Rossi E, Belanova M, Bobovska A, Dianiskova P, Kordulakova J, Sala C, Fullam E, Schneider P, McKinney JD, Brodin P, Christophe T, Waddell S, Butcher P, Albrethsen J, Rosenkrands I, Brosch R, Nandi V, Bharath S, Gaonkar S, Shandil RK, Balasubramanian V, Balganesh T, Tyagi S, Grosset J, Riccardi G, Cole ST. Benzothiazinones kill Mycobacterium tuberculosis by blocking arabinan synthesis. Science. 2009;324:801–4.
311. Amaral L, Udwadia Z, Abbate E, van Soolingen D. The added effect of thioridazine in the treatment of drug-resistant tuberculosis. Int J Tuberc Lung Dis. 2012;16:1706–8. author reply 1708-9.
312. Dey T, Brigden G, Cox H, Shubber Z, Cooke G, Ford N. Outcomes of clofazimine for the treatment of drug-resistant tuberculosis: a systematic review and meta-analysis. J Antimicrob Chemother. 2013;68:284–93.
313. Dutta NK, Pinn ML, Zhao M, Rudek MA, Karakousis PC. Thioridazine lacks bactericidal activity in an animal model of extracellular tuberculosis. J Antimicrob Chemother. 2013;68(6):1327–30.
314. Dutta NK, Alsultan A, Gniadek TJ, Belchis DA, Pinn ML, Mdluli KE, Nuermberger EL, Peloquin CA, Karakousis PC. Potent rifamycin-sparing regimen cures guinea pig tuberculosis as rapidly as the standard regimen. Antimicrob Agents Chemother. 2013;57:3910–6.
315. Rist N, Grumbach F, Libermann D. Experiments on the antituberculous activity of alpha-ethylthioisonicotinamide. Am Rev Tuberc. 1959;79:1–5.
316. Quemard A, Laneelle G, Lacave C. Mycolic acid synthesis: a target for ethionamide in mycobacteria? Antimicrob Agents Chemother. 1992;36:1316–21.
317. Baulard AR, Betts JC, Engohang-Ndong J, Quan S, McAdam RA, Brennan PJ, Locht C, Besra GS. Activation of the pro-drug ethionamide is regulated in mycobacteria. J Biol Chem. 2000;275:28326–31.
318. Vannelli TA, Dykman A, Ortiz de Montellano PR. The antituberculosis drug ethionamide is activated by a flavoprotein monooxygenase. J Biol Chem. 2002;277:12824–9.
319. Wang F, Langley R, Gulten G, Dover LG, Besra GS, Jacobs Jr WR, Sacchettini JC. Mechanism of thioamide drug action against tuberculosis and leprosy. J Exp Med. 2007;204:73–8.
320. Vilcheze C, Av-Gay Y, Attarian R, Liu Z, Hazbon MH, Colangeli R, Chen B, Liu W, Alland D, Sacchettini JC, Jacobs Jr WR. Mycothiol biosynthesis is essential for ethionamide susceptibility in Mycobacterium tuberculosis. Mol Microbiol. 2008;69:1316–29.

321. Akbergenov R, Shcherbakov D, Matt T, Duscha S, Meyer M, Wilson DN, Bottger EC. Molecular basis for the selectivity of antituberculosis compounds capreomycin and viomycin. Antimicrob Agents Chemother. 2011;55:4712–7.
322. Taniguchi H, Chang B, Abe C, Nikaido Y, Mizuguchi Y, Yoshida SI. Molecular analysis of kanamycin and viomycin resistance in Mycobacterium smegmatis by use of the conjugation system. J Bacteriol. 1997;179:4795–801.
323. Engstrom A, Perskvist N, Werngren J, Hoffner SE, Jureen P. Comparison of clinical isolates and in vitro selected mutants reveals that tlyA is not a sensitive genetic marker for capreomycin resistance in Mycobacterium tuberculosis. J Antimicrob Chemother. 2011;66:1247–54.
324. Jugheli L, Bzekalava N, de Rijk P, Fissette K, Portaels F, Rigouts L. High level of cross-resistance between kanamycin, amikacin, and capreomycin among Mycobacterium tuberculosis isolates from Georgia and a close relation with mutations in the rrs gene. Antimicrob Agents Chemother. 2009;53:5064–8.
325. Shcherbakov D, Akbergenov R, Matt T, Sander P, Andersson DI, Bottger EC. Directed mutagenesis of Mycobacterium smegmatis 16S rRNA to reconstruct the in-vivo evolution of aminoglycoside resistance in Mycobacterium tuberculosis. Mol Microbiol. 2010;77(4):830–40.
326. Sandgren A, Strong M, Muthukrishnan P, Weiner BK, Church GM, Murray MB. Tuberculosis drug resistance mutation database. PLoS Med. 2009;6, e2.
327. Caceres NE, Harris NB, Wellehan JF, Feng Z, Kapur V, Barletta RG. Overexpression of the D-alanine racemase gene confers resistance to D-cycloserine in Mycobacterium smegmatis. J Bacteriol. 1997;179:5046–55.
328. Feng Z, Barletta RG. Roles of Mycobacterium smegmatis D-alanine: D-alanine ligase and D-alanine racemase in the mechanisms of action of and resistance to the peptidoglycan inhibitor D-cycloserine. Antimicrob Agents Chemother. 2003;47:283–91.
329. Chacon O, Feng Z, Harris NB, Caceres NE, Adams LG, Barletta RG. Mycobacterium smegmatis D-alanine racemase mutants are not dependent on D-alanine for growth. Antimicrob Agents Chemother. 2002;46:47–54.
330. Russell RR. Mapping of a D-cycloserine resistance locus in escherichia coli K-12. J Bacteriol. 1972;111:622–4.
331. Peteroy M, Severin A, Zhao F, Rosner D, Lopatin U, Scherman H, Belanger A, Harvey B, Hatfull GF, Brennan PJ, Connell ND. Characterization of a Mycobacterium smegmatis mutant that is simultaneously resistant to D-cycloserine and vancomycin. Antimicrob Agents Chemother. 2000;44:1701–4.
332. Lehmann J. para-Aminosalicylic acid in the treatment of tuberculosis. Lancet. 1946;i:15–6.
333. Lehmann J. Twenty years afterward historical notes on the discovery of the antituberculosis effect of para-aminosalicylic acid (Pas) and the first clinical trials. Am Rev Respir Dis. 1964;90:953–6.
334. TREATMENT of pulmonary tuberculosis with streptomycin and para-aminosalicylic acid; a Medical Research Council investigation. Br Med J. 1950;2:1073–85.
335. Rengarajan J, Sassetti CM, Naroditskaya V, Sloutsky A, Bloom BR, Rubin EJ. The folate pathway is a target for resistance to the drug para-aminosalicylic acid (PAS) in mycobacteria. Mol Microbiol. 2004;53:275–82.
336. Leung KL, Yip CW, Yeung YL, Wong KL, Chan WY, Chan MY, Kam KM. Usefulness of resistant gene markers for predicting treatment outcome on second-line anti-tuberculosis drugs. J Appl Microbiol. 2010;109:2087–94.
337. Stover CK, Warrener P, VanDevanter DR, Sherman DR, Arain TM, Langhorne MH, Anderson SW, Towell JA, Yuan Y, McMurray DN, Kreiswirth BN, Barry CE, Baker WR. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. Nature. 2000;405:962–6.
338. Lenaerts AJ, Gruppo V, Marietta KS, Johnson CM, Driscoll DK, Tompkins NM, Rose JD, Reynolds RC, Orme IM. Preclinical testing of the nitroimidazopyran PA-824 for activity against Mycobacterium tuberculosis in a series of in vitro and in vivo models. Antimicrob Agents Chemother. 2005;49:2294–301.
339. Tyagi S, Nuermberger E, Yoshimatsu T, Williams K, Rosenthal I, Lounis N, Bishai W, Grosset J. Bactericidal activity of the nitroimidazopyran PA-824 in a murine model of tuberculosis. Antimicrob Agents Chemother. 2005;49:2289–93.
340. Manjunatha UH, Boshoff H, Dowd CS, Zhang L, Albert TJ, Norton JE, Daniels L, Dick T, Pang SS, Barry III CE. Identification of a nitroimidazo-oxazine-specific protein involved in PA-824 resistance in Mycobacterium tuberculosis. Proc Natl Acad Sci U S A. 2006;103:431–6.
341. Singh R, Manjunatha U, Boshoff HI, Ha YH, Niyomrattanakit P, Ledwidge R, Dowd CS, Lee IY, Kim P, Zhang L, Kang S, Keller TH, Jiricek J, Barry III CE. PA-824 kills nonreplicating Mycobacterium tuberculosis by intracellular NO release. Science. 2008;322:1392–5.
342. Matsumoto M, Hashizume H, Tomishige T, Kawasaki M, Tsubouchi H, Sasaki H, Shimokawa Y, Komatsu M. OPC-67683, a nitro-dihydro-imidazooxazole derivative with promising action against tuberculosis in vitro and in mice. PLoS Med. 2006;3, e466.
343. Petrella S, Cambau E, Chauffour A, Andries K, Jarlier V, Sougakoff W. Genetic basis for natural and acquired resistance to the diarylquinoline R207910 in mycobacteria. Antimicrob Agents Chemother. 2006;50:2853–6.
344. Huitric E, Verhasselt P, Koul A, Andries K, Hoffner S, Andersson DI. Rates and mechanisms of resistance development in Mycobacterium tuberculosis to a novel diarylquinoline ATP synthase inhibitor. Antimicrob Agents Chemother. 2010;54:1022–8.
345. Jia L, Coward L, Gorman GS, Noker PE, Tomaszewski JE. Pharmacoproteomic effects of isoniazid, ethambutol, and N-geranyl-N′-(2-adamantyl)ethane-1,2-diamine (SQ109) on Mycobacterium tuberculosis H37Rv. J Pharmacol Exp Ther. 2005;315:905–11.
346. Hillemann D, Rusch-Gerdes S, Richter E. In vitro-selected linezolid-resistant Mycobacterium tuberculosis mutants. Antimicrob Agents Chemother. 2008;52:800–1.
347. Escribano I, Rodriguez JC, Llorca B, Garcia-Pachon E, Ruiz M, Royo G. Importance of the efflux pump systems in the resistance of Mycobacterium tuberculosis to fluoroquinolones and linezolid. Chemotherapy. 2007;53:397–401.
348. Sander P, Belova L, Kidan YG, Pfister P, Mankin AS, Bottger EC. Ribosomal and non-ribosomal resistance to oxazolidinones: species-specific idiosyncrasy of ribosomal alterations. Mol Microbiol. 2002;46:1295–304.
349. Wallis RS, Jakubiec W, Kumar V, Bedarida G, Silvia A, Paige D, Zhu T, Mitton-Fry M, Ladutko L, Campbell S, Miller PF. Biomarker-assisted dose selection for safety and efficacy in early development of PNU-100480 for tuberculosis. Antimicrob Agents Chemother. 2011;55:567–74.
350. Pasca MR, Degiacomi G, Ribeiro AL, Zara F, De Mori P, Heym B, Mirrione M, Brerra R, Pagani L, Pucillo L, Troupioti P, Makarov V, Cole ST, Riccardi G. Clinical isolates of Mycobacterium tuberculosis in four European hospitals are uniformly susceptible to benzothiazinones. Antimicrob Agents Chemother. 2010;54:1616–8.
351. Yano T, Kassovska-Bratinova S, Teh JS, Winkler J, Sullivan K, Isaacs A, Schechter NM, Rubin H. Reduction of clofazimine by mycobacterial type 2 NADH:quinone oxidoreductase: a pathway for the generation of bactericidal levels of reactive oxygen species. J Biol Chem. 2011;286:10276–87.
352. Xu J, Lu Y, Fu L, Zhu H, Wang B, Mdluli K, Upton AM, Jin H, Zheng M, Zhao W, Li P. In vitro and in vivo activity of clofazimine

against Mycobacterium tuberculosis persisters. Int J Tuberc Lung Dis. 2012;16:1119–25.
353. Dutta NK, Mazumdar K, Dastidar SG, Karakousis PC, Amaral L. New patentable use of an old neuroleptic compound thioridazine to combat tuberculosis: a gene regulation perspective. Recent Pat Antiinfect Drug Discov. 2011;6:128–38.
354. Dutta NK, Mehra S, Kaushal D. A Mycobacterium tuberculosis sigma factor network responds to cell-envelope damage by the promising anti-mycobacterial thioridazine. PLoS One. 2010;5, e10069.
355. Weinstein EA, Yano T, Li LS, Avarbock D, Avarbock A, Helm D, McColm AA, Duncan K, Lonsdale JT, Rubin H. Inhibitors of type II NADH:menaquinone oxidoreductase represent a class of antitubercular drugs. Proc Natl Acad Sci U S A. 2005;102: 4548–53.