Infect Dis Trop Med 2022; 8: e1044

Mycobacterium tuberculosis: mechanisms and interactions between drug resistance mutations with fitness costs and the drug resistance phenotypes

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ABSTRACT:

Multidrug-resistant and extensively drug-resistant *Mycobacterium tuberculosis* are no news and have contributed to the continued scourge of tuberculosis today. Acquisition of drug resistance by *M. tuberculosis* occurs due to mutations in genes, overexpression of some genes, and changes in the expression of specific genes. Thus, the development of resistance to first and second-line drugs. A fitness cost is also paid by drug-resistant *M. tuberculosis*. This is due to their weakened virulence, transmission, and reduced growth rate under normal growth conditions. Some studies show that "Persisters" or "Phenotypic drug-tolerant" populations occur in recovered patients. This results in a relapse of treated patients in immunosuppressive conditions. Findings suggest that drug-tolerant persister populations exist following antibiotic treatment, even if they are temporarily undetected by conventional microbiologic approaches. Drug resistance is a global issue that poses a danger to effective *M. tuberculosis* control. Even though drug resistance in *M. tuberculosis* is linked to changes in various genes, many resistant strains lack these common mutations. This review aims at delivering a comprehensive overview to global health authorities and prospective readers worldwide, thus improving the knowledge of the molecular basis of drug resistance in *M. tuberculosis*.

Keywords: Drug resistance, Mutation, Tuberculosis, Genes, Fitness Cost.

INTRODUCTION

Multiple-drug therapy, while helpful, does not guarantee the absence of drug-resistant infections. As a result, we cannotbe sure that multidrug therapy will not lead to drug-resistant tubercle bacilli[1].

According to the World Health Organization's (WHO) 2014 global tuberculosis report[2], there had been approximately 9.0 million new tuberculosis (TB) patients and 1.5 million deaths in 2013. In addition, 3.5% of newly diagnosed and 20.5% of previously treated patients had multidrug-resistant TB (MDR-TB, defined as bacillary resistance to at least rifampicin [RMP] and Isoniazid [INH]) in 2013. Eastern Europe and Central Asia had the highest prevalence of MDR-TB, with rates exceeding 20% and 50%, respectively. Furthermore, by the end of 2012, the WHO had received reports of at least one case of extensively drug-resistant tuberculosis (XDR-TB, defined as MDR-TB with additional resistance to fluoroquinolone(s) [FQs] and one or more of three second-line injectable drugs [SLIDs], namely capreomycin [CPM], kanamycin [KM], and amikacin

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[AMK]). XDR-TB was found in about 9% of MDR-TB patients. As a result, the global drug-resistant tuberculosis (DR-TB) epidemic continues to be a serious concern, which is exacerbated by co-infection with the human immunodeficiency virus (HIV)[3].

In *M. tuberculosis*, two forms of antibiotic resistance are known: genetic and phenotypic resistance. Mutations in chromosomal genes cause drug resistance in growing bacteria. In contrast, phenotypic resistance or drug tolerance is caused by epigenetic alterations in gene expression and protein modification in non-growing persister bacteria. The two types of resistance have generated a slew of issues in effective TB control, with genetic resistance, as seen in MDR-/XDR-TB, wreaking havoc worldwide. In contrast, phenotypic drug resistance, or tolerance as seen in persisters, necessitates prolonged treatment and increases the risk of post-treatment relapse[4,5]. *In vivo*, the situation appears to be more complicated, with the two types of resistance overlapping and interconverting. Prior stress or sub-inhibitory drug concentrations can induce efflux pump expression, resulting in phenotypic resistance and possibly facilitating the development of more stable genetic drug resistance[6].

In contrast, genetic drug resistance in growing organisms can develop the persistence of phenotypic resistance. Understanding the biology of mycobacterial persisters and creating anti-tuberculosis medications that target them is becoming increasingly popular amongst researchers. The selection of genetic mutations predominantly develops drug-resistant strains of *M. tuberculosis*. This is almost entirely artificial, resulting from poor physician prescribing or patient compliance. However, recent evidence suggests that pharmacokinetic-pharmacodynamics situations involving the stimulation of the mycobacterial drug efflux pump may aid the establishment of genetic alterations in *M. tuberculosis*[7]. The development of drug resistance in *M. tuberculosis* due to mutations in drug resistance genes may cost the organism's fitness and virulence. Recent comprehensive analyses[8,9] have suggested a link between *M. tuberculosis* primary resistance and HIV co-infection, implying that transmitted DR-TB poses a severe barrier to managing this patient population. In addition, recent investigations[11] from China have found that a considerable number of MDR- and XDR-TB cases are due to active transmission of (mostly) the Beijing genotype ("W-Beijing")[10], and the same genotype is found in Europe and Africa[12]. This is a concerning development that necessitates additional research to understand how such virulent DRTB strains evolve and adapt in the host and the need for more effective transmission control methods.

Antibiotic resistance is typically linked to fitness losses, such as those caused by high energy consumption by resistance machinery or the onerous expression of resistance proteins[13-17]. These findings show that a bacterial population should be biased away from drug resistance without antibiotic pressure[18-20]. Compensatory mutations and precise regulation of costly protein production lower the effective cost in practice, substantially eliminating fitness disparities. For example, tetracycline-resistant bacteria that produce the expensive TetA efflux pump are likely to be outcompeted by susceptible strains in an antibiotic-free environment[21]. However, experiments have shown that the fitness cost of tetracycline resistance without antibiotic pressure is minimal[19,22]. This review aims at clarifying the mechanisms and interactions between drug resistance mutations with fitness costs and the drug resistance phenotypes of *Mycobacterium tuberculosis*.

GENES INVOLVED IN DRUG RESISTANCE, MECHANISMS, INTERACTIONS, MUTATIONS AND DRUG RESISTANCE ACQUISITION

A good number of genes naturally found in *Mycobacterium tuberculosis* contribute to its well-renowned drug resistance with the occurrence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. *M. tuberculosis,* like every other bacterium, possesses intrinsic mechanisms of drug resistance, including a thicker and more hydrophobic cell wall due to the presence of a variety of lipids, which include mycolic acids to prevent drug penetration. It also possesses various enzymes that hydrolyze various drugs, such as β-lactamases, which break down β-lactam antibiotics, and enzymatic drug target modification and drug efflux from the cytoplasm[23].

The genes of interest in mycobacterial drug resistance are numerous, and this work is focused on those relevant to resistance to first and second-line anti-tubercular drugs.

KatG

The katG gene is a 2223bp long gene with locus MG995340, which encodes the protein Catalase-Peroxidase in *Mycobacterium tuberculosis* (strain ATCC 25618/H37Rv)[24]. It is a bi-functional enzyme oxidizing numerous electron sources, including NADP (H), with catalase and broad-spectrum peroxidase activity[25]. It protects *M. tuberculosis* from damaging reactive oxygen species (ROS), such as hydrogen peroxide and organic peroxides, by dismutation and helps it surviving in host macrophages by preventing phagocyte oxidative burst[26,27].

The enzyme Catalase-Peroxidase has a function in acquiring drug resistance by *M. tuberculosis*, that is, its broad-spectrum catalase and peroxidase activity that leads to the oxidation of numerous electron sources, including NADP(H). Consequently, this leads to activating one of the essential first-line anti-tubercular drugs, i.e., Isoniazid, administered as a pro-drug. In its pro-drug form, INH penetrates the bacterial cell to the cytoplasm, where it is activated. Therefore, the enzyme encoded by katG mediates the susceptibility of *M. tuberculosis* to Isoniazid[28]. This phenomenon is of particular interest as it has been shown that in *Escherichia coli*, with a similar Catalase-Peroxidase enzyme called hydroperoxidase 1, the activity of this enzyme does not induce isoniazid susceptibility[28].

It has been noticed in most isoniazid-resistant strains of *M. tuberculosis* that resistance is linked to either simple base pair alterations that result in missense mutations or minor deletions in the katG gene[29-32]. Many isoniazid-resistant clinical isolates have mutations in katG, which cause catalase/peroxidase activity to be abolished or decreased, resulting in a lack of Isoniazid activation or a lower affinity for Isoniazid. Other Isoniazid resistance pathways include katG gene deletion and katG expression down-regulation caused by mutations in the furA-katG intergenic region[33-35]. The most critical mutation in the katG gene that contributes to isoniazid resistance results in a single change of the primary protein structure, with the replacement of the amino acid serine with threonine at position 315; thus, the generation of Isoniazid-NAD adducts is reduced by a factor of 20, and affinity for Isoniazid is greatly diminished[35,36].

InhA

The inhA gene is an 828bp DNA long gene with locus MG995265, which encodes the protein, Enoyl-[acyl-carrier-protein] reductase [NADH] in *M. tuberculosis* (strain ATCC 25618/H37Rv)[24]. Functionally, the type II fatty acid synthase (FAS-II) system enzyme enoyl-ACP reductase is involved in the production of mycolic acids, which are a key component of mycobacterial cell walls[37]. It catalyzes the NADH-dependent reduction of the double bond of 2-Trans-enoyl-[acyl-carrier protein], which is a significant step in the FAS-II pathway's fatty acid elongation[38]. Phosphorylation on Thr-266 lowers InhA activity (5-fold reduction) and presumably negatively controls mycolic acid production and bacterial growth[39,40].

InhA, much like katG, mediates resistance to Isoniazid and ethionamide (ETH), a second-line anti-tubercular drug[41]. The enzyme encoded by inhA, Enoyl-[acyl-carrier-protein] reductase, is NADH-dependent. Its function of interest in mediating drug resistance is the part it plays in mycolic acid synthesis as part of the bacterial FAS-II system. Isoniazid, in its active form, forms a covalent compound with Enoyl-[acyl-carrier-protein] reductase and kasA, a beta-ketoacyl carrier protein synthetase, which prevents mycolic acid synthesis and kills the cell. Isoniazid's action against InhA is mediated *via* covalent attachment of the drug's activated form to NAD's nicotinamide ring and binding of the INH-NAD adduct to inhA's active site[42,43]. Ethionamide, a drug similar in structure to Isoniazid, also inhibits mycolic acid synthesis in *M. tuberculosis*[44].

It has been observed[45] that the overexpression of inhA confers isoniazid and ethionamide resistance *to M. tuberculosis*, and there is a good amount of cross-resistance between both drugs. Many clinical isolates resistant to Isoniazid and ethionamide have mutations in the inhA gene. The single substitution of alanine for serine 94 confers resistance to Isoniazid and ethionamide; this drug resistance appears to be directly related to a disruption in the hydrogen-bonding network that reduces the binding of NADH and the INH-NAD adduct[43,46].

AhpC

The ahpC gene is a 255bp DNA long gene with locus MTU43812 that encodes the Alkyl hydroperoxide reductase C protein in *Mycobacterium tuberculosis* (strain ATCC 25618/H37Rv)[24]. Hydrogen peroxide and organic hydroperoxides are reduced to water and alcohol, respectively, by this thiol-specific peroxidase. Detoxifying peroxides aids in cell defence against oxidative stress. With AhpD, DlaT, and Lpd, it forms an NADH-dependent peroxidase that can degrade hydrogen and alkyl peroxides while also acting as a peroxynitrite reductase, shielding the bacterium against reactive nitrogen intermediates and oxidative stress caused by the host immune system[47-49].

AhpC encodes alkyl hydroperoxide reductase C, an enzyme that serves to protect *M. tuberculosis* from oxidative stress from reactive nitrogen intermediates and hydrogen peroxides produced by the host cell, as stated earlier. No mutations in the ahpC have been shown to mediate resistance to Isoniazid; however, in an extensive collection of Isoniazid (INH)-resistant clinical isolates of *Mycobacterium tuberculosis*, mutations in the regulatory region of the ahpC gene that result in overproduction of alkyl hydroperoxide reductase were found often, but not in INH-susceptible bacteria. The overexpression of ahpC does not appear to be harmful. However, because most of these strains were already catalase-peroxidase deficient, this is critical for INH resistance[50].

KasA

The kasA gene is a 1251bp DNA long gene that encodes the protein, 3-oxoacyl-[acyl-carrier-protein] synthase 1 in *Mycobacterium tuberculosis* (strain ATCC 25618/H37Rv)[24]. It is a part of the mycobacterial fatty acid elongation system, FAS-II, which is important in mycolic acid synthesis. It specifically catalyzes long-chain acyl-ACP substrates' elongation by adding two malonyl-ACP carbons to an acyl group acceptor[51,52]. It is also involved in the mycolate chain's initial elongation and the formation of monounsaturated fatty acids with an average carbon length of forty[53].

kasA, much like inhA, encodes the enzyme, 3-oxoacyl-[acyl-carrier-protein] synthase 1. As stated earlier, this enzyme is also a part of the mycobacterial fatty acid elongation system, FAS-II, important in mycolic acid synthesis. The enzyme is a target of the activated form of Isoniazid, forming a part of the covalent complex along with inhA that inhibits mycolic acid synthesis and kills the bacteria. It is also worthy of note that kasA is a target for and is inhibited by the indazole JSF-3285[54]; unlike inhA, where its overexpression plays a significant part in drug resistance[45], kasA has undergone several mutations that contribute to drug resistance. The mutations include a change of Aspartate to Asparagine at position 66, Glycine to Serine at position 269, Glycine to Serine at position 312, and Phenylalanine to Leucine at position 413, with all four mutations contributing to increased resistance to Isoniazid[53].

RpoB

The rpoB gene is a 1577bp DNA long gene with locus MG995115 that encodes the protein, DNA-directed RNA polymerase subunit beta in *Mycobacterium tuberculosis* (strain ATCC 25618/H37Rv)[24]. It is a DNA-dependent RNA polymerase that uses the four ribonucleoside triphosphates as substrates to catalyze the transcription of DNA into RNA[55].

The gene rpoB encodes the protein DNA-directed RNA polymerase subunit beta, and this is the target of the first-line drug Rifampin, which is bacteriocidal for *M. tuberculosis*. Rifampin binds to the beta-subunit of RNA polymerase to inhibit its activity[56].

Point mutations in the ropB gene sequence lead to changes in the protein sequence of the polymerase that prevent rifampin binding and mediate resistance. The amino acid changes include a change of glutamate to arginine in position 138, isoleucine to alanine in position 147, lysine to alanine in position 148, and finally serine to alanine in position 149, with these mutations contributing to increasing resistance to Rifampin[57,58].

RRS

The RRS gene, also known as the 16S rRNA gene, is a 1550bp DNA long gene that encodes the ribosome's small subunit ribosomal RNA molecules, which are responsible for the critical step of turning genetic material into functional cell components *via* mRNA to protein translation in *Mycobacterium tuberculosis* (strain ATCC 25618/H37Rv)[59].

The 16S-rRNA subunit encoded by the RRS gene targets the first-line drugs (Streptomycin), and second-line injectable drugs (Kanamycin, Capreomycin, and Amikacin). Mutations in this gene have been shown to confer resistance to the above-listed medicines, and these mutations include: Alanine-Glycine substitution at position 1408, Threonine-Alanine at 1406, Cysteine-Threonine at 1409, and Glycine-Threonine at 1491[60,61].

RsmG/GidB

The rsmG/gidB gene is a 568bp DNA long gene with locus MK783876 that encodes the Ribosomal protein RNA small subunit methyltransferase G in *M. tuberculosis* (strain ATCC 25618/H37Rv). It functions to methylate the N7 position of guanine in position 518 of 16S rRNA precisely[24].

GidB is one of the most conserved genes in all bacterial species, being highly conserved in both gram-positive and gram-negative species. Mutations in this gene have been shown to cause a low level of resistance to Streptomycin in *M. tuberculosis* and possibly in all other bacteria[61]. The mutation responsible for this resistance is a deletion of alanine at position 488[61].

EmbB

The embB gene is an 860bp DNA long gene with locus MK526900 that encodes the protein arabinosyl-transferase in *M. tuberculosis* (strain ATCC 25618/H37Rv)[24]. Arabinosyl-transferase is a polymerization enzyme that converts arabinose to arabinan in arabinogalactan, an essential component of the mycobacterial cell wall of *M. tuberculosis*[62].

The arabinosyl-transferase encoded by the gene embB is the target for the first-line anti-tubercular drug, Ethambutol, whose mechanism of action involves inhibiting the enzyme's activity and thus preventing bacterial cell wall formation. Two main methods acquire resistance to Ethambutol. Firstly, there are many mutations in embB that confer ethambutol resistance, which include: Serine-Alanine at position 297, Methionine- Isoleucine/Leucine/Valine at position 306, Aspartate-Glycine/Tyrosine at position 328, Phenylalanine-Valine at position 330, Tyrosine-Histidine at position 336, Glycine-Alanine/Cysteine/Aspartate at position 406, Glutamine - Lysine/Arginine at position 497, Glycine-Aspartate at position 745, Aspartate-Alanine at position 959, Methionine-Arginine at position 1000, Aspartate-Asparagine at position 1024[63,64]. Secondly, overexpression of embB also results in resistance to Ethambutol[65].

GyrA and GyrB

These two genes, 2517bp and 2028bp DNA long genes, respectively with loci MG995190 and MG995415 respectively, encode the two portions of the DNA gyrase subunits A and B in *M. tuberculosis* (strain ATCC 25618/H37Rv)[24]. DNA gyrase A type II topoisomerase supercoils closed circular double-stranded (ds) DNA in an ATP-dependent way to keep chromosomes un-wound while relaxing supercoiled dsDNA in the absence of ATP[66,67]. Interconversion of other topological isomers of dsDNA rings, such as catenanes, is also catalyzed by this enzyme[68]. It is also worthy of note that, in comparison to *E. coli*, *M. tuberculosis*' gyrase shows a stronger decatenation than supercoiling activity; since *M. tuberculosis* only possesses one type II topoisomerase, its gyrase must also perform the decatenation function of topoisomerase IV[66,69].

These genes encode the two A and B subunits of the protein DNA gyrase, which targets the essential second-line anti-tubercular drug group,the fluoroquinolones. These drugs block mycobacterial DNA synthesis by inhibiting the activity of DNA gyrase (topoisomerase II) and topoisomerase IV. DNA gyrase inhibition prevents the relaxation of the positively supercoiled DNA, an essential step in bacterial transcription and, thus, replication[70]. One or more point mutations mediate a high level of resistance to fluoroquinolones in the gyrA and gyrB genes. The number of mutations elucidated in both genes is tremendous and cannot discussed here, and more information can be found in cited literature[70-75].

TlyA

The tlyA gene is a 696bp DNA long gene with locus MK783785 that encodes the protein 16S/23S rRNA (cytidine-2'-O)-methyltransferase TlyA in *M. tuberculosis* (strain ATCC 25618/H37Rv)[24]. It acts as a host evasion

factor that plays a crucial role in *M. tuberculosis* pathogenesis by modifying adaptive immune responses and suppressing host protective Th1 and Th17 cytokine responses and autophagy[76]. Also, 2'-O-methylation at nucleotides C1409 in 16S rRNA and C1920 in 23S rRNA is catalyzed by this enzyme, with the enzyme also exhibiting *in vitro* hemolytic activities[77,78].

The protein 16S/23S rRNA (cytidine-2'-O)-methyltransferase is the target for the second-line anti-tubercular drug capreomycin, an aminoglycoside. Capreomycin is an irreversible inhibitor of protein synthesis by binding to and preventing the activity of the above-stated protein[77]. In addition, TlyA appears to influence the ribosome, and capreomycin resistance is conferred by tlyA mutation[79,80].

PncA

The pncA gene is a 561bp DNA long gene with locus KY659393 that encodes the protein nicotinamidase/pyrazinamidase in *Mycobacterium tuberculosis* (strain ATCC 25618/H37Rv)[24]. Deamidation of nicotinamide (NAM) to nicotinate is catalyzed by this enzyme[81].

The enzyme encoded by the pncA gene, nicotinamidase/pyrazinamidase, converts the pro-drug pyrazinamide to its active form, pyrazinoic acid. Mutations have been detected in the pncA gene of pyrazinamide-resistant *M. tuberculosis*, most of which vary from a total loss of enzymatic activity to a decrease in enzymatic activity on pyrazinamide. However, the fold of decline differs for each mutation. These mutations include Aspartate-Alanine at position 8 (total loss of enzymatic activity), Aspartate-Alanine at position 49 (410-fold decrease in enzymatic activity), Histidine-Alanine at position 51(21-fold reduction in enzymatic activity), Histidine-Alanine at position 57 (164 fold decrease in enzymatic activity), Serine-Alanine at position 59 (2.4 fold decrease in enzymatic activity), Histidine-Alanine at position 71 (100 fold decrease in enzymatic activity), Lysine-Alanine at position 96 (total loss of enzymatic activity), Serine-Alanine at position 104 (3 fold decrease in enzymatic activity), and Cysteine-Alanine at position 138 (total loss of enzymatic activity)[81].

FITNESS COSTS ASSOCIATED WITH DRUG RESISTANCE

Mycobacterium tuberculosis strains that are highly drug-resistant are a severe impediment to stopping the spread of tuberculosis in many contexts. According to recent World Health Organization estimates, approximately 450,000 (possible range, 300,000-600,000) incident cases of multidrug-resistant (MDR), *M. tuberculosis* (defined as a strain resistant to at least Isoniazid and rifampicin)[82] is already a well-known issue in areas where drug-resistant tuberculosis accounts for a significant proportion of tuberculosis cases or where the total burden of MDR tuberculosis is high, the threat that these highly resistant strains of *M. tuberculosis* pose to global containment is highly dependent on their evolutionary fitness[83,84].

Antibiotic resistance-causing mutations are frequently associated with fitness costs[85-91]. While early studies[85,90,92,93] of *in vitro*-generated resistance suggested that mutations associated with *M. tuberculosis* resistance impaired bacterial growth rates or virulence, recent evidence[94] shows that mutations observed among clinical drug-resistant *M. tuberculosis* strains differ from those observed among these laboratory-derived resistant mutants. These mutations are frequently not associated with a reduction in growth rate[88,95] and are often equally transmissible as their laboratory-derived resistant mutants. These drug-resistant mutants' lack of significant deficiencies could be due to low-cost resistance-conferring mutations or higher-cost resistance-conferring mutations that originated in so-called pre-adapted genetic backgrounds and were later compensated by additional mutations[88,96-101].

Evolutionary fitness, on the other hand, is a complicated feature that requires MDR *M. tuberculosis* to successfully infect, multiply, and transfer to a secondary host[102,103], while laboratory assays designed to evaluate fitness give valuable controlled and repeatable data, findings from *in vitro* techniques may not always correlate with evolutionary or epidemiological fitness[104,105] (i.e., transmissibility). On the other hand, epidemiological fitness is usually explored using cluster-based analysis, in which researchers compare the genetic similarities of sample isolates to discover possible transmission clusters.

Mutations have long conferred aminoglycoside resistance in the rpsL gene, which codes for the ribosomal protein S12 in *Escherichia coli* and *Salmonella species*. It has recently been linked[106,107] to the exact mechanism of *M. tuberculosis*. Lys43Arg is the only known rpsL mutation that allows non-restrictive ribosomal elongation and growth rates equivalent to wild-type *M. tuberculosis*[108]. Almost all experimental studies[104,109-112] The Lys43Arg substitution in *M. tuberculosis* and other organisms has been shown to be a low-cost resistance mutation that may remunerate in cis for higher-cost rpsL mutations and is potentially more virulent in vivo than other aminoglycoside resistance mutations.

PHENOTYPIC DRUG TOLERANCE

Bigger[113] coined the word "persisters" in 1944 to describe bacteria that resisted drugs without developing heritable resistance. Persistence was eventually dubbed "phenotypic drug resistance" or "phenotypic tolerance" after the quality that allowed persisters to live. These early experiments[112,113] significantly impact today's anti-infective finding approaches.

Hobby and Lenert[114] expanded the study of phenotypic tolerance to include a different pathogen, *M. tuberculosis*, and two additional medications, Isoniazid, and para-aminosalicylate, two decades later. Isoniazid inhibits mycolic acid production, para-aminosalicylate inhibits folate synthesis, and penicillin inhibits peptidoglycan synthesis. As a result, phenotypic tolerance was unaffected by the antibiotic's chemical class or inhibited mechanisms.

The issue of persisters is crucial to tuberculosis treatment. It is thought to be one of the reasons why, in formal studies, the current WHO-approved treatment regimen for drug-sensitive tuberculosis takes six months to cure 95% of participants; in standard practice, the cure rate is around 86%. Drug-resistant tuberculosis usually requires more than two years of treatment, and a cure is rarely attained[115]. In the Cornell model[116-118], mice with drug-sensitive tuberculosis treated with Isoniazid and pyrazinamide for two months harbour no detectable colony-forming units of *M. tuberculosis* when their organ homogenates are distributed on bacteriologic agar. However, one-third of the remaining mice in the same cohort relapse spontaneously after a few months, and virtually all relapse if immunosuppressed with corticosteroids, anti-IFN, anti-TNF, or inhibitors of inducible nitric oxide synthase. The *M. tuberculosis* recovered during relapse is just as susceptible to Isoniazid and pyrazinamide as the inoculated population. These findings suggest that drug-tolerant persister populations exist following antibiotic treatment, even if they are temporarily undetected by conventional microbiologic approaches. Similarly, *M. tuberculosis* was found[119,120] in sputa from roughly 80% of treatment-naive tuberculosis patients, although it was not measurable by CFU analysis.

The difficulty of turning the previous knowledge into a faster and more efficient tuberculosis treatment is illustrated by the experience with metronidazole. In several animal models, *M. tuberculosis* experiences hypoxia in necrotic granulomas. Hypoxia causes mycobacteria to stop reproducing *in vitro* and develop phenotypic tolerance to most treatments. On the other hand, metronidazole is an antibacterial and anti-parasitic medication that kills hypoxic mycobacteria *in vitro*. As a result, metronidazole appeared to be a good choice for killing non-replicating *M. tuberculosis*. However, metronidazole action in tuberculosis animal models did not always correspond with hypoxia in granulomas[121-128]. Other than contributing to peripheral neuropathy, metronidazole improved the proportion of patients whose sputum became smear- or culture-negative after one month of treatment but had no effect on treatment outcome at six months[129]. In retrospect, metronidazole's capacity to kill hypoxic *M. tuberculosis in vitro* was investigated without an alternate electron acceptor, putting the organism at a more significant disadvantage than it would experience *in vivo*. *M. tuberculosis* can receive electrons from various sources, including nitrate and fumarate[130-132]. Nitrate is a naturally occurring component of human bodily fluid. The *in vitro* efficacy of pyrazinamide was significantly reduced when nitrate was added[128].

The experience with metronidazole implies that finding medicines with the exceptional quality of killing bacteria that are phenotypically tolerant to most other antibiotics may not be enough. It is also essential to understand how bacteria become phenotypically tolerant. It concerns how the bacteria are prevented from replicating if phenotypic tolerance is created by using settings that prevent them from reproducing. The more closely the conditions match those in the host, the more probable medications that operate under those settings will also work in the host.

The above statements constitute a hypothesis that is currently being tested. It took another 40 years after Bigger's publication[113] for Coates to propose large-scale screening to target non-replicating *M. tuberculosis*. His idea seemed timely when many pharmaceutical companies cut back or abandoned anti-infective research. Other companies followed the industry's traditional practice of looking for broad-spectrum drugs that could heal widespread infections in economically prosperous areas. Only after 1999 a new financing landscape[133] emerged, favouring academic-collaborations for drug discovery for infectious diseases that primarily affected financially disadvantaged regions. Pharmaceutical corporations and their academic partners only started large-scale screenings for medications targeting phenotypically tolerant mycobacteria approximately ten years ago[134].

CONCLUSIONS

Drug resistance is a global issue that poses a danger to effective *Mycobacterium tuberculosis* control. Even though drug resistance in *M. tuberculosis* is linked to changes in various genes, many resistant strains lack these common mutations. Therefore, from a clinical standpoint, having diagnostic techniques that are simple to use, affordable, and deliver quick results on a strain's medication sensitivity or resistance is probably more crucial. However, given the dynamics of tuberculosis transmission and the need to create new anti-TB medications, it is critical to expand our understanding of drug resistance's molecular basis in all its complexities. It is essential to understand the link between specific mutations and the development of MDR-TB, as well as the link between drug resistance and fitness costs. This would allow for a more accurate forecast of future illness scenarios and a better evaluation of the transmission dynamics of resistant strains. Furthermore, understanding the molecular basis of drug resistance would aid in the more rational development of new medications, which is now critical, given the rising prevalence of MDR- and XDR-TB around the world.

Funding:
None.

Conflict of Interest:
The authors declare that they have no competing interests.

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