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Drug Resistant *Mycobacterium tuberculosis* and New Drug Development

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Abstract

Mycobacterium tuberculosis is an extraordinarily successful human pathogen, infecting one-third of the world's population and causing nearly two million deaths each year. In this article, current trends in worldwide tuberculosis (TB) resistance are discussed along with pathogenesis of drug resistance, emergence of resistance, mechanism of resistance development, prevention of drug resistance and new drug development. The global TB emergency has been further exacerbated by multi drug-resistant (MDR) TB and extensively drug-resistant (XDR) TB strains that are resistant to our best antibiotics and very difficult to treat. Finally, this review briefly describes new anti-tuberculosis drugs and the impetus for discovering new antibacterial compounds to target drug resistant *M. tuberculosis* and improve tuberculosis therapy.

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Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* it is the world's second common cause of death from infectious diseases, after AIDS. From 1700 to 1900, it is estimated that TB was responsible for one billion deaths and killed more people than any other disease.[1] Tuberculosis (TB) is a global pandemic, with 9.4 million incident cases occurring in 2009 and 1.7 million deaths attributed to the disease. In addition to the worrisome reality that the total number of cases globally is still increasing (although incidence rates are decreasing slightly), disease due to strains of *Mycobacterium tuberculosis (MTB)* that are resistant to treatment by first-line drugs is a serious threat to global TB control.[2] TB is transmitted via the

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respiratory route as a highly infectious aerosol with varying outcomes occurring from this initial *Mycobacterium tuberculosis* exposure. These outcomes can range from immediate organism destruction by the host's immune system to infected individuals developing active primary TB disease within 1–3 years.[3]

The TB global emergency is further complicated by MDR- and XDR-TB strains that are resistant to our best antibiotics, very difficult to treat, and associated with greater morbidity and mortality than antibiotic-susceptible TB (Figure 1). An individual may develop the drug resistant form of TB via inadequate therapy that enables the selection of drug-resistance (acquired resistance) or infection with a drug-resistant TB strain (primary resistance).[4] While infection with an exogenous drug-resistant TB strain is related to infection control measures, the development of acquired *M. tuberculosis* resistance is multi-faceted and can be attributed to various social, political, economic, epidemiological, and pathophysiological factors.[5] Certainly, scientists investigate the cellular and molecular mechanisms to explain the development of drug-resistant TB strains, but other influences including, but not limited to, improper or poor health management practices or infrastructure, inadequate therapeutic regimens, antibiotic misuse, insufficient or unobtainable resources, poor socioeconomic conditions, individual immunocompetence, patient compliance, and complicated personal issues have also played roles in the evolution and progression of antibiotic resistance.[5–11]

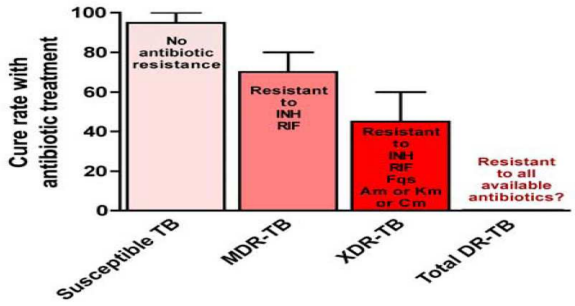

Figure 1

Table I

Drug	Average mutation rate
Isoniazid	2.56×10^{-8}
Rifampicin	2.25×10^{-10}
Ethambutol	1×10^{-7}
Streptomycin	2.95×10^{-8}
Pyrazinamide	1×10^{-3}

Antibiotic cure rates of TB based on drug resistance patterns. Estimated cure rates for antibiotic-susceptible TB, MDR-TB, and XDR-TB in HIV-negative patients are >95%, 60– 80%, and 30–60%, respectively. Although TB strains exhibiting resistance to all first-line and second-line antibiotics have not been identified, total drug-resistant (DR)-TB would be untreatable with existing chemotherapeutic agents. INH, isoniazid; RIF, rifampin; Fqs, fluoroquinolones; Am, amikacin; Km, kanamycin; Cm, capreomycin.

The World Health Organization (WHO) has estimated that 17% of all newly diagnosed patients with active TB have disease caused by drug-resistant strains. Of all TB patients,14.1% have either mono- or poly-drug resistance, over half of whom (7.4% of the global total) are resistant to isoniazid alone. Such forms of mono- and poly-resistant strains are seen in higher proportions in countries with high rates of MDR-TB.[12] An estimated 2.9% of all new TB cases globally are multi-drug resistant (MDR-TB, defined as resistance to both isoniazid and rifampicin, the two most important first-line drugs). Less than 1% of new cases globally are reported to be extensively drug-resistant TB (XDR-TB), defined as MDR-TB plus resistance to a second-line injectable drug and a fluoroquinolone). In twelve countries, 6% or more of new cases have MDR-TB, while 58 countries to date have reported at least one case of XDR-TB.[13]

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Outcomes of treatment of drug-resistant TB are worse than drug-susceptible TB (DS-TB). The most extreme is XDR-TB – in the first reported outbreak of XDR-TB among HIV-infected individuals, all but one of 53 XDR-TB patients died of the disease, with a median survival of 16 days from the time the first sputum specimen was collected.[14] However, a subsequent study from Peru reported that 29 of 48 (60%) HIV-uninfected patients with XDR-TB were treated successfully.[15] Outcomes of treatment of MDR-TB are somewhat better. Three systematic reviews estimated pooled success treatment rates of only 60%–70%, with failure rates of 10%–11%, mortality of 10%–15%, and default rates of up to 20%.[16-18] Treatment of MDR-TB involves the use of second-line drugs that are less efficacious than first-line drugs, more expensive, and have more adverse effects, making tolerance and treatment adherence challenging. The cost of treatment from drugs alone is estimated to be 50–200 times higher for MDR-TB patients, compared with treatment of patients with drug-susceptible TB. An estimated 150,000 deaths were caused by MDR-TB in 2008, the majority because they were not treated with second-line drugs.[19]

The remaining and much more common forms of drug resistant TB have significantly worse outcomes than DS-TB, although cure rates with existing regimens are potentially higher than for MDR or XDR. A systematic review of studies of patients with strains that were isoniazid-resistant found significantly higher rates of failure, relapse, and acquired drug-resistance than in patients infected with strains that were susceptible to all drugs when treated with standardized regimens.3 Pooled failure and relapse rates were 10%–15% higher than among new cases with DS-TB.[20] Outcomes were even worse among previously treated patients with isoniazid mono-resistance, in whom the combined failure and relapse rates ranged from 29% to 70% when treated with the WHO recommended standardized re-treatment regimen.[20] A study of intermittent regimens among HIV-infected patients with TB found isoniazid mono-resistance to be the main risk factor for acquired rifamycin resistance.[21]

What do you mean by 'resistant'?

The term 'drug resistance' is ambiguously defined in many situations. What is drug resistance, especially in the context of *M. tuberculosis*? The WHO defines drug resistance as "the ability of certain microorganisms to withstand attack by antimicrobials." In the context of *M.tuberculosis*, this is defined as the ability of >1% proportion of a bacilli to grow in the presence of critical concentration of drug. The critical concentrations themselves are defined as the concentration of antibiotic that inhibit growth in 95% of wild type strains that have hitherto not been exposed to drug. Thus, these are essentially epidermiologic cut-off values.[22] Antibiotics have a long history, beginning in the 1930s and earlier, during which several distinct drug classes were discovered and numerous improved analogs were made available.[23] Because of these efforts, today's antibiotics satisfactorily address most clinical situations; the escalating multidrug resistance problem is a major exception. Therefore, resistance remains as a primary driver for antibacterial R&D. Indeed, there is little economic and medical justification for the development of new antibiotics that do not solve relevant resistance problems. Without resistance the future of antibacterial R&D would be limited.[24]

Pathogenesis of drug resistance

In every 10^6 to 10^8 replications, wild strains of *MTB* undergo spontaneous mutations that confer resistance to a single drug; the average number of such spontaneous mutations to anti-TB drugs is shown. (Table 1).[25,26]

When treated with a single drug, the population of TB bacilli initially shrinks due to the killing of

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susceptible organisms in the population, often rendering a person smear-negative (as a result of fewer organisms being present). However, the organisms that survive the initial phase are the drug-resistant mutants, and the proliferation of these mutants eventually causes the entire population of bacilli to be replaced by drug-resistant forms that continue to proliferate until they are numerous enough to cause recurrence of symptoms, and smear positivity; this is termed "the fall and rise phenomenon".[27] If treated with a single drug, and the bacillary load of the organisms exceeds 106, then emergence of strains that are resistant to that drug is almost certain. If the bacillary load exceeds 108 then resistance is likely to develop if only two drugs are used. Bacillary loads exceed 106 with tuberculous infiltrates alone (when sputum direct smears are negative although cultures are positive), and exceed 108 when cavities are present in patients with TB, at which time sputum direct smears are usually positive.[28,29]

One of the aims of modern anti-TB therapy is to prevent drug resistant mutants from proliferating. This is best accomplished by including at least three likely effective anti-tuberculous agents in the initial treatment regimen, as this will reduce the probability of emergence of drug resistance to 10^{-18} or lower. During the initial phase of treatment the few mutants with spontaneous resistance to one drug will be killed more slowly than the "wild type" bacilli that are susceptible to all drugs. Hence during the first months of therapy these more resistant bacilli will survive longer. If therapy is interrupted early, through default, then these drug-resistant mutants will proliferate, increasing the proportion of drug-resistant forms, until this proportion becomes clinically significant. Low drug levels, either from malabsorption (as occurs in HIV-infected patients) or inadequate dosages of medications, will have the same effect.

Current beliefs of how *M. tuberculosis* resistance emerges

The understanding of the mechanism of anti-TB drug resistance has been shaped by the history of development of anti-TB drugs in the past 60 years, and was arrived at as part of inductive generalization. Unfortunately, this approach is prone to bias. Based on observations in regimens tested between 1952 and 1980, each drug in the regimen was assigned special roles in treatment of *M. tuberculosis*. Pyrazinamide, isoniazid, ethambutol, rifampin and streptomycin are each thought to target certain specific populations of the *M. tuberculosis* such as bacilli under acidic, aerobic and/or hypoxic conditions within caseous foci, at the edge of pulmonary cavities and inside macrophages, respectively.[30,31] Resistance suppression is defined as one drug preventing resistance to another, not one drug preventing resistance to itself. The resulting belief, almost universally accepted, is that if patients take these multi-drug regimens without defaulting, then MDR-TB and XDR-TB emergence would be ameliorated. Accordingly, it is believed that missing drug doses leads to 'effective monotherapy' for some bacillary populations because of different drug half-life's and differential drug penetrations into effective compartments. It has been theorized that resistance evolves independently for each drug one at any one time through 'unlinked processes', leading to the standard step-wise pick up of mutations that leads to sequential acquisition of resistance.[30,32] Finally, the belief has been that resistance arises from replicating bacilli, so that non-replicating persistent bacilli (NRP) do not mutate and cause drug resistance. Recently, each of these staple beliefs has been challenged in well designed experiments that applied both PK/PD and none PK/PD methodology.

PK/PD dose selection and clinical application to prevent drug resistance

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When a drug dose is administered to patients it becomes part of the non-deterministic process of pharmacokinetic variability. In other words, a particular dose does not lead to a specific concentration–time profile in all patients, but rather a distribution determined partly by alleles of genes encoding enzymes involved in xenobiotic metabolism, the particular physique of each patient as is the case of pyrazinamide, or even dietary considerations.[33] This means that in some patients, despite patients taking all their drug doses, low drug concentrations could still be encountered, which could lead to emergence of drug resistance. Thus, resistance emergence could occur partly owing to non-deterministic causes that have nothing to do with DOTS or default.

The response of the pathogen to a particular drug concentration– time profile is itself related to several PK/PD factors. For *M. tuberculosis*, PK/PD factors have been derived in monotherapy studies in the hollow fiber system (HFS).[34–39] First, the shape of the concentration– time curve has been related to resistance emergence for each of the first line anti-TB drugs. Studies with isoniazid and pyrazinamide revealed that the relationship between drug exposure and population of drug-resistant *M. tuberculosis* was a series of curves that changed with time, starting with a 'U' shaped curve, which then evolved over time to an inverted 'U' curve (Figure 1). In other words, the relationship is defined by a quadratic function, with time as part of the defining characteristics of the leading coefficient.[38] Therefore, in interpreting indices at which resistance can be suppressed, the duration of therapy should be taken into consideration. Rifampin resistance emergence and suppression are linked to the peak concentration (Cmax) to MIC, with optimal suppression of resistance at a free drug Cmax/MIC of 175.[37] Isoniazid resistance emergence was demonstrated to be closely linked to both Cmax/MIC and AUC/MIC.[40] On the contrary, both pyrazinamide and ethambutol resistance emergence were associated with the % time concentration persisted above MIC (%TMIC).[38,41] The lessons are obvious, resistance emergence to a drug depends on the drug exposure achieved, and in many situations the actual shape of the concentration– time curve, which often differ from the PK/PD parameter linked to microbial kill.

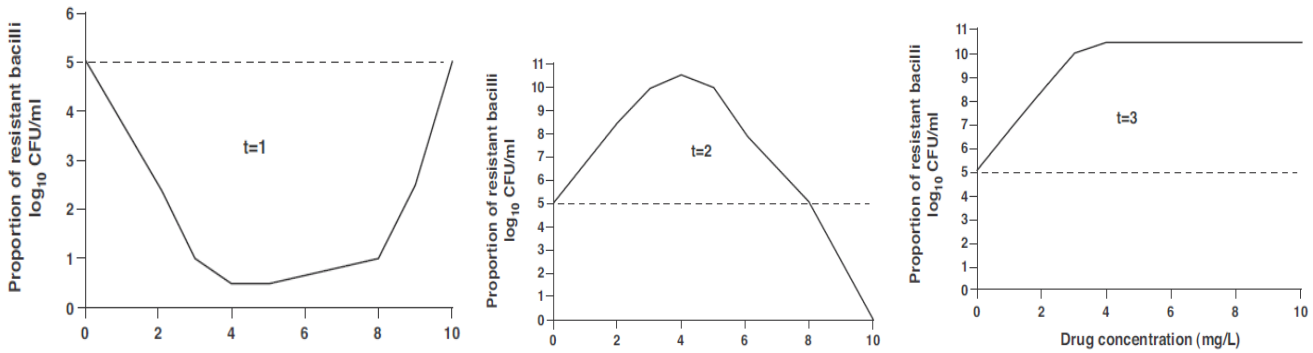

Figure 2. Change in size of drug-resistant *M. tuberculosis* population with exposure and time. Figure shows an upright 'U' at the beginning of therapy (t = 1), which evolves to an inverted 'U' curve at t = 2, and eventually reaches a time when no concentration of the antibiotic in question can suppress drug resistance at t = 3.

Mechanisms of resistance emergence

It is believed that during non-compliance, one of several mechanisms may lead to emergence of drug resistance. According to the pharmacokinetic mismatch hypothesis, during non-compliance the drug with the short half-life disappears quickly, leaving *M. tuberculosis* exposed to the drug with the longer half-life as monotherapy. In some scenarios, even without non-compliance, if the half-lives of two drugs are very mismatched (e.g. rifapentine and

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isoniazid), then the same situation can arise especially during intermittent phases of therapy. We recently expressed this as a falsifiable hypothesis and tested it in HFS for rifampin and isoniazid with and without pre-existed resistant subpopulations (Srivastava et al., in revision). The drugs were administered as well matched regimens, or isoniazid administered 6 h, 12 h, or 24 h after rifampin. Essentially the more mismatched regimens performed better and the pharmacokinetic mismatch hypothesis was rejected. Another theory on resistance emergence has been the time in mutant suppression window hypothesis. In our work on rifampin, isoniazid, pyrazinamide, and ethambutol in the HFS this hypothesis also failed to explain emergence of resistance to these agents.[37,38,40,41] However, one mouse study confirmed this theory for moxifloxacin.[42]

Genetic Basis of *M. tuberculosis* Antibiotic Resistance

Efforts to understand the molecular basis of *M. tuberculosis* antibiotic resistance have advanced significantly and investigations of potentially unique genetic traits in MDR- and XDR-TB strains are ongoing. Unlike other bacterial pathogens, there is no evidence that gene acquisition contributes to antibiotic resistance in *M. tuberculosis*.[43] The mutated genes and gene products associated with *M. tuberculosis* drug resistance are included inTables 2 and 3. Isoniazid resistance is linked to alterations in the catalase-peroxidase gene (*katG*), the *inhA* gene, which encodes an enzyme involved in mycolic acid biosynthesis, or the NADH dehydrogenase II gene (*ndh*).[44–47] Genetic mutations in *rpoB*, which encodes the RNA polymerase β-subunit, result in rifampin resistance.[48–50] Mutations in *pncA* that eliminate pyrazinamidase/nicotinamidase activity confer pyrazinamide resistance.[51,52] Numerous genetic mutations present within the *embCAB* operon, which facilitates production of arabinosyl transferase, have been linked to ethambutol resistance, but other genes may also be involved.[53-55]

Similar to the first-line TB antibiotics, genetic mutations account for all known mechanisms of resistance for the second-line TB drugs (Table 3). Streptomycin resistance is associated with mutations in the *rpsL*, ribosomal S12 protein, and *rrs*, 16S rRNA, gene.[56–59] Similar to streptomycin, kanamycin and amikacin resistance are linked to genetic mutations that occur within the *rrs* gene with evidence of cross-resistance occurring between kanamycin and capreomycin or viomycin in mycobacteria.[60–65] Mutagenesis of the *tlyA* gene, which has homology to rRNA methyltransferases, also confers capreomycin resistance.[66] Clinical resistance to the quinolone family antibiotics, levofloxacin, moxifloxacin, and gatifloxacin, is attributed to mutations occurring within the *gyrA* gene encoding DNA gyrase.[67,68] Ethionamide resistance is linked to *inhA* mutations, whereby cross-resistance occurs between isoniazid and ethionamide and to mutations in the *etaA* (*ethA*) gene, which codes for flavin monooxygenase responsible for activation of ethionamide.[45,69.70,71] Recent evidence links para-aminosalicylic acid resistance to mutations within the *thyA* gene, which produces thymidylate synthase A, but mechanisms or targets independent of thymine nucleotide biosynthesis are also likely involved.[72,73] While inactivation of the *alrA* gene, encoding D-alanine racemase, causes increased sensitivity to cycloserine in *Mycobacterium smegmatis* and over expression of *alrA* confers mycobacterial resistance to cycloserine, the genetic mechanism of cycloserine resistance in *M. tuberculosis* is currently unknown.[74,75]

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Table 2

First-line anti-TB drugs

First-line antibiotics	Antibiotic class/structure	Delivery route	Activity	Mechanism of action	Genes and gene products associated with resistance
Isoniazid	Pyridine hydrazide	Oral	Bactericidal	Inhibits mycolic acid (cell wall) synthesis	*katG*; catalase-peroxidase *inhA*; enoyl-ACP reductase *ndh*; NADH dehydrogenase II
Rifampin	Rifamycin	Oral	Bactericidal	Inhibits RNA synthesis	*rpoB*; β-subunit of RNA polymerase
Pyrazinamide	Nicotinamide analog	Oral	Bacteriostatic/bactericidal	Disrupts cell membrane energetics and inhibits membrane transport	*pncA*; nicotinamidase/pyrazinamidase
Ethambutol	Ethylenediamine derivative	Oral	Bacteriostatic	Inhibits arabinogalactan (cell wall) synthesis	*embCAB*; arabinosyl transferase

Table 3

Second-line anti-TB drugs

Second-line antibiotics	Antibiotic class/structure	Delivery route[a]	Activity	Mechanism of action	Genes and gene products associated with resistance
Streptomycin	Aminoglycoside	IM injection	Bactericidal	Inhibits protein synthesis	*rpsL*; S12 ribosomal protein *rrs*; 16S rRNA
Kanamycin/Amikacin	Aminoglycoside	IM injection	Bactericidal	Inhibits protein synthesis	*rrs*; 16S rRNA
Capreomycin	Polypeptide	IM injection	Bactericidal	Inhibits protein synthesis	*rrs*; 16S rRNA *tlyA*; putative rRNA methyltransferase
Levofloxacin	Fluoroquinolone	Oral or IV	Bactericidal	Inhibits DNA replication	*gyrA*; DNA gyrase subunit A
Moxifloxacin	Fluoroquinolone	Oral or IV	Bactericidal	Inhibits DNA replication	*gyrA*; DNA gyrase subunit A
Gatifloxacin	Fluoroquinolone	Oral or IV	Bactericidal	Inhibits DNA replication	*gyrA*; DNA gyrase subunit A
Ethionamide	Thioamide	Oral	Bacteriostatic	Inhibits mycolic acid (cell wall) synthesis	*inhA*; enoyl-ACP reductase *ethA/ethA*; flavin monooxygenase
Cycloserine	Isoxazolidinone	Oral	Bacteriostatic	Inhibits peptidoglycan (cell wall) synthesis	unknown (*alrA*; D-alanine racemase in *Mycobacterium smegmatis*)
Para-aminosalicylic acid	Salicylic acid	Oral	Bacteriostatic	Inhibits folic acid synthesis	*thyA*; thymidylate synthase

[a]IM, intramuscular; IV, intravenous.

Diagnosis of Drug resistant TB

The algorithm for the diagnosis of drug-resistant TB is founded on an understanding of the profile of TB patients who have a higher probability of harbouring drug-resistant strains. This profile holds true for all forms of the disease, including paucibacillary (smear negative) forms. Patients at risk of drug-resistant TB fall into three major groups: contacts of patients with drug-resistant TB, previously treated, and those who are failing therapy. Close contacts of patients with drug-resistant TB are more likely to have been infected with a resistant strain, and if they have active TB should be presumed to harbour a drug-resistant strain.[76] Patients who have received TB treatment in the past include those who relapsed (have TB disease again after being declared "cured" in the past) and those who defaulted (did not complete a course of TB treatment as recommended in the past). Close to one-third of all patients who default or relapse will have MDR-TB strains.[77] It is therefore vital to obtain an accurate history of prior TB treatment as this is a very strong risk factor for drug resistant TB.[77] Up to 90% of all patients who fail treatment, defined as a positive AFB sputum smear or culture after 5 months of therapy, will have MDR-TB strains.[77] Given this, patients who remain smear positive after 3 months of treatment should be investigated for possible drug-resistant TB.[78] The diagnosis of drug-resistant TB is made by performing drug-susceptibility testing (DST) of the strain of TB obtained from the patient. Patients who are at risk of drug-resistant TB based on the profiles mentioned above should be subjected to DST testing. Testing can be performed on traditional solid media such as Lowenstein-Jensen media, which is relatively inexpensive but requires 4–8 weeks for a result. DST using liquid media such as MGIT (Mycobacteria Growth Indicator Tube) provides results in 2–4 weeks, but is more expensive. Molecular methods,

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such as Xpert-Rif, can provide results within hours, but are the most expensive of all the currently available tests. A detailed description of these methods is beyond the scope of this article, but can be found at http:// www.tbevidence.org.

How to prevent Drug Resistance?

The broad objectives of anti-TB treatment are: (1) rapid reduction in bacillary load to reduce morbidity and mortality, and stop transmission, (2) prevent the emergence of drug resistant mutant strains, and (3) prevent relapse of disease. To achieve objective 1, potent bactericidal drugs such as isoniazid, especially in the first week and rifampicin are the most useful. To achieve the second objective, multiple drugs with proven (by DST) or likely (never previously used) efficacy are used, to prevent the selection of drug-resistant mutants as explained earlier. To achieve the third objective, treatment is prescribed for a sufficiently long duration, with monitoring of adherence to treatment, to eliminate residual surviving organisms that are responsible for disease relapse. The length of treatment with rifampicin plays an important role in achieving this third objective.[79] Recommendations for the dosages, duration, and Combinations of drugs for treatment of drug-susceptible TB are based on sound evidence-based principles derived from multiple randomized trials.[79] Adherence to authoritative guidelines for treatment and ensuring that all doses are taken correctly is unarguably the most effective means of preventing drug resistance.[80]

The Inherent Need for New Anti-TB Drugs

For the most part, TB therapy has remained unchanged for nearly four decades and often consists of taking more than 10 pills per day for a minimum of six months for antibiotic susceptible disease.[81] The diagnosis of MDR-TB or XDR-TB further subjects the patients to as many as 20 pills per day, as well as antibiotic intramuscular injections, for 18 to 24 months. This lengthy treatment is not only more expensive than first-line antibiotics, but also comes with devastating, toxic side effects, emotional and social anxieties, and pyschological stresses.[82–85] With XDR-TB treatment success rates ranging from 30–60% in HIV-negative patients (Figure 1,Table 4), XDR-TB strains threaten to return TB treatment to the pre-antibiotic era, when more than 50% of TB patients succumbed to the disease.[81] The spread of XDR-TB and the poor treatment outcomes in both developing and developed countries clearly indicate that XDR-TB knows no boundaries. Therefore, its emergence and spread potentially jeopardizes our abilities to fight the disease in all people, irrespective of geographic location, thus posing an incredible global threat to public health. While preserving the effectiveness of the existing first-line and second-line antibiotics is ideal, the mere existence of XDR-TB strains suggests that we are embarking upon an ominous era whereby totally drug-resistant TB strains could evolve. In order to combat MDR- and XDRTB and the overall spread of antibiotic resistant TB strains, the need for new anti-TB drugs is imminent.

Development of Novel Chemotherapeutic Anti-TB Compounds

After discovery and development of new anti-TB drugs flourished in the mid-1900s, the TB drug pipeline was reduced to a mere leaky faucet with the discovery of new classes of antibiotics being virtually nonexistent. It has been more than 40 years since the last novel TB-specific antibiotic was introduced into clinical practice. Given the challenge of treating XDR-TB, fortunately, the existing pipeline for new classes of anti-TB drugs shows promise. While there are a number of propitious candidates currently in various stages of discovery and clinical development, the new anti-TB compounds described here in represent drugs with novel structures and/or mechanisms of action currently in Phase II clinical evaluations. (Table 4) [86–88]

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Table 4

Novel anti-TB drugs currently in Phase II clinical trials.

Drug	Class	Mechanism of action
TMC207	Diarylquinoline	Inhibits ATP synthase and energy metabolism
PA-824	Nitroimidazo-oxazine	Inhibits mycolic acid synthesis
OPC-67683	Nitrodihydro-imidazooxazole	Inhibits mycolic acid synthesis

A novel diarylquinoline, TMC207 (also known as R207910), exhibits potent *in vitro* bactericidal activity against aerobically-replicating, drug-sensitive and MDR *M. tuberculosis* as well as dormant, antibiotic-susceptible *M. tuberculosis*.[89,90] Importantly, TMC207 also demonstrates rapid mycobactericidal activity in experimentally-infected animals and in patients with drug-susceptible or MDR pulmonary TB.[89,91,92] The diarylquinoline, TMC207, offers a new mechanism of anti-TB action by specifically inhibiting the mycobacterial ATP synthase, thus diminishing bacterial energy production in the form of ATP molecules.[89]

Another class of promising compounds with anti-TB activity is the nitro-imidazoles, including PA-824 and OPC-67683. Although the exact mechanism of their action is not completely understood, the PA-824 prodrug requires activation by bacterial dehydrogenase and nitroreductase to inhibit mycolic acid synthesis.[93–95] Mycolic acids are important constituents of the mycobacterial cell wall, are involved in pathogenicity and exhibit diverse immunological functions.[96] PA-824 displays strong bactericidal activity against replicating and non-replicating *M. tuberculosis* and exhibits bactericidal activity when administered orally to experimentally-infected animals.[93,97,98] Of particular significance, a regimen of PA-824, moxifloxacin and pyrazinamide demonstrated bactericidal and sterilizing activity against TB in experimentally-infected mice, suggesting efficacy against MDR-TB.[99] Additional safety, tolerability, and pharmacokinetic studies with PA-824 in healthy human subjects are ongoing.[100,101] The OPC-67683 dihydroimidazo-oxazole also demonstrates very potent *in vitro* and *in vivo* bactericidal activity against antibiotic-sensitive and MDR *M. tuberculosis*.[102–104] While the specific bacterial target of OPC-67683 is not yet known, the compound inhibits production of cell wall mycolic acids, suggesting a similar mechanism of action as PA-824.[102] Unlike metronidazole (another nitroimidazole compound), which kills dormant *M. tuberculosis* under anaerobic conditions, OPC-67683 and PA-824 are bactericidal against *M. tuberculosis* grown in either aerobic or anaerobic states.[105] The ability to target both actively replicating and dormant bacteria allows OPC-67683 and PA-824 to function as double edged swords and could potentially shorten the duration of TB treatment.[94]

Future Perspectives Regarding the Discovery of New Anti-TB Compounds

Fortunately, there has been resurgence in TB drug research over the past decade, and numerous compounds are progressing through the various stages of the pharmaceutical discovery and development pipeline. Efforts by the Stop TB Partnership and the TB Alliance have been instrumental in stimulating and advancing TB research and accelerating the discovery and development of new drug therapies for treating TB. For a comprehensive list of compounds and the current phase of discovery or development, see the Stop TB Partnership Working Group on New TB Drugs (http://www.stoptb.org/wg/new_drug) and the TB Alliance (http://www.tballiance.org). Although several new anti-TB drugs have advanced to preclinical development and/or clinical trials, the timeline for their potential introduction into clinical practice and the impact on TB treatment are difficult to predict.

Drug discovery and development is a very complex and expensive process with the estimated costs between $800 million and $1 billion to bring a new drug to market.[106,107] Despite this daunting process, a continuation of the basic research and discovery processes is critical to identify new families of anti-TB agents targeting novel enzymes and/or cell processes associated with viability and/or bacterial virulence.[86,88] Exploration of novel targets and mechanisms of action are necessary to discover candidate compounds with efficacy against drug-sensitive and drug-resistant TB, with activity against active and latent TB, and with the potential to shorten chemotherapeutic treatment. Ongoing and new drug discovery research initiatives, along with

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improvements to known classes of compounds, will ensure that the pipeline of new anti-TB compounds continues.

Concluding Remarks and Perspectives

Pathogenic organisms, such as *M. tuberculosis*, that significantly contribute to worldwide human infectious disease are also the most common antibiotic-resistant bacteria.[108] Our arsenal of antimicrobials is currently under attack by the microorganisms themselves as clinically-significant, antibiotic-resistant bacteria evolve at alarming rates.[109] The fight against antibiotic resistance is formidable, but must be endeavoured in the face of treatment failures, prolonged illnesses, increased deaths, and escalated risks of infections. With increases in worldwide cases of MDR- and XDR-TB occurring on a yearly basis, the grim progression from antibiotic effectiveness to antibiotic resistance drives this global crisis.

The comfort zone of antimicrobial discovery represents a path of proven bacterial targets, such as cell wall biosynthesis, protein synthesis, and nucleic acid metabolism and replication. The development of completely new classes of drugs necessitates new avenues of basic research which incorporate identifying new antimicrobial targets and discovering unique mechanisms of action using novel approaches. However, ensuring TB drug compliance and susceptibility testing is critical, since the introduction of new antibiotics could, ironically and unfortunately, generate additional antibiotic resistance and further intensify the existing problem. Nevertheless, aggressive strategies and innovative approaches are desperately needed to fight XDR-TB or we are likely to lose our grip on TB control and witness the emergence of completely drug-resistant TB.

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