Tuberculosis Drug Resistance: A Global Threat

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Resistance to antituberculosis drugs has been a problem since the era of chemotherapy began. After dramatic outbreaks of multidrug-resistant tuberculosis (MDR-TB) in the early 1990s, resistance became recognized as a global problem. MDR-TB now threatens the inhabitants of countries in Europe, Asia, Africa, and the Americas. An understanding of the molecular basis of drug resistance may contribute to the development of new drugs. Management of MDR-TB relies on prompt recognition and treatment with at least 3 drugs to which an isolate is susceptible.

Tuberculosis (TB) remains a major cause of morbidity and mortality worldwide. The World Health Organization (WHO) estimates that one-third of the population of the world is infected with *Mycobacterium tuberculosis* and that more than 8 million new cases of active TB occur annually [1–3]. The estimated global annual mortality from TB is close to 2 million people. Although management of TB has faced many challenges in the past, today there are 2 monumental threats to global TB control: the HIV epidemic and the increasing prevalence of drug resistance. HIV-1 infection is contributing to large escalations in the incidence of TB in countries most heavily affected by AIDS, notably sub-Saharan Africa [4]. Resistance to anti-TB drugs, a problem recognized in the very early days of the chemotherapeutic era, has also emerged as a serious problem. TB drug resistance is characterized by both the types of drugs to which the bacteria lack susceptibility and the manner in which resistance was acquired. Resistance to single agents is the most common type; resistance to multiple agents is less frequent but of greater concern. By convention, “multidrug resistance” is defined as resistance to at least isoniazid and rifampin. As described below, drug-resistant *M. tuberculosis* can emerge during treatment of an individual with initially susceptible TB, or it can be acquired at the time of infection. In this article, we review the current global epidemiology, mechanisms, and management strategies of TB drug resistance.

HISTORICAL BACKGROUND

Drug-resistant TB was recognized shortly after the introduction of effective anti-TB chemotherapy, with the description of streptomycin resistance by Pyle in 1947 [5]. In 1948, the British Medical Research Council (MRC) published its groundbreaking report of streptomycin therapy for pulmonary TB and noted that mortality was similar in treated and untreated patients [6]. Among patients who had been treated with streptomycin, however, most who died had experienced relapse that was the result of streptomycin-resistant strains. The recognition of this phenomenon led to the principle of multiagent chemotherapy for TB, which was proved effective in a subsequent trial by the MRC [7]. Resistance to anti-TB drugs continued to be recognized as a sporadic clinical problem through the 1960s, 1970s, and 1980s, but little attention was paid to the problem by researchers or public health officials. The emergence of multidrug-resistant TB (MDR-TB) in the United States in the early 1990s led to renewed interest in this topic [8]. During that period, a number of MDR-TB cases, defined as disease caused by strains resistant to at least isoniazid and rifampin, were iden-
tified in epidemics in New York, New Jersey, and Florida. The majority of these cases were the result of microepidemics with direct transmission among persons in hospital, jails, and homeless shelters, particularly among people with HIV infection [8–10]. The mortality in MDR-TB has been reported to be high both in HIV-infected and uninfected individuals [11–15]. Aggressive public health interventions at a cost of tens of millions of dollars helped to quickly contain these outbreaks, but not before the loss of many lives [16].

In subsequent years, drug-resistant TB, especially MDR-TB, has been recognized as a potentially catastrophic challenge to global public health. Major outbreaks of MDR-TB have been reported in the former Soviet Union, and low levels of MDR-TB in countries with high rates of TB, such as Peru, have resulted in large numbers of patients with disease. As a consequence, drug-resistant TB now constitutes a global problem [17].

GLOBAL EPIDEMIOLOGY OF TB DRUG RESISTANCE

The global distribution of drug-resistant TB was poorly defined until recently. In 1994, the International Union Against Tuberculosis and Lung Disease (IUATLD) and WHO launched the Global Project on Antituberculosis Drug Resistance Surveillance. The initial results of this global survey of anti-TB drug resistance were published in 1998 [18]. The first report was based on data collected from 35 countries from 5 continents, representing >50,000 patients worldwide. Resistance to anti-TB drugs such as isoniazid and streptomycin was found in all 35 countries and regions surveyed. Overall, 9.9% of patients with TB had drug resistance, and a median of 1% had MDR-TB. But there were countries of concern, such as the former Soviet states, the Dominican Republic, and Argentina.

A second report was published in April 2000 in which surveillance was extended to 72 countries and contained data on trends of TB drug resistance [19, 20]. In new TB cases, the prevalence of resistance to more than one drug ranged from 1.7% in Uruguay to 36.9% in Estonia, with a median of 10.7%. A number of regions with a high prevalence of drug resistance among new cases were identified, including Ivanovo Oblast in the Russian Federation (32.4%), Latvia (19.9%), and Henan Province, China (35%). Although the overall median prevalence of MDR-TB in new cases was only 1%, the level of MDR-TB among new cases in 9 regions was >3%. It is of note that no MDR-TB in new cases was reported in Cuba, Finland, and France. A high level of MDR-TB, defined as >3% overall prevalence, was found in 14 countries or regions. MDR-TB hot spots (>500 new MDR-TB cases per year) included Sierra Leone, Zimbabwe, Swaziland, Peru, Bolivia, Brazil, Nepal, Korea, and Romania.

Particular areas of concern with a high prevalence of MDR-TB in new cases were Estonia (14%), Latvia (9%), Ivanovo (9%), and Tomsk Oblasts (6.5%), in the Russian Federation, and Henan Province (11%) in China. A major problem was also identified in the Russian prison population, where 10% of 1 million prisoners have active TB [21, 22]. Portaels et al. [23] reported a rate of MDR-TB of 24.6% in Baku, Azerbaijan, and Mariinsk, Siberia. Kimerling et al. [24] found that 75% of new TB cases among prisoners in Colony 33, a Siberian prison hospital, were drug resistant, with ~40% MDR-TB.

The high rates of MDR-TB found in the 2 most populous countries of the world, China and India, is of significance because these countries account for 40% of all TB cases worldwide. In Henan Province, the most populous province in China, 11% of new cases had MDR-TB. In Tamil Nadu, India, 3.4% of new cases had MDR-TB, the prevalence of isoniazid resistance was 15%, and rifampin resistance was found in 4.4%.

The situation in the developing world is highly variable. According to the recent WHO/IUATLD survey, resistance is not yet a problem in most sub-Saharan African countries. This may be because rifampin-based regimens have been introduced only recently. In addition, rifampin-sparing regimens are often used in the continuation phase, and a growing number of countries use directly observed therapy (DOT). However, countries such as Mozambique, Cote D’Ivoire, Cameroon, Argentina, the Dominican Republic, and Mexico are a concern because the prevalence of MDR-TB in new cases is >3%. The full magnitude of the problem is still unknown in a number of countries with high TB incidence, such as Democratic Republic of the Congo, Ethiopia, Nigeria, Indonesia, Bangladesh, and Pakistan.

Both WHO/IUATLD reports showed that a well-functioning TB-control program was associated with a low prevalence of MDR-TB and that previous anti-TB therapy was a strong predictor of drug resistance. Botswana, for example, has managed to prevent the emergence of resistant TB with an effective DOT program [25]. Countries or regions with poor TB-control programs were found to have a prevalence of MDR-TB that was 2.5-fold greater than areas with good TB-control programs. Although HIV infection has been linked to primary MDR-TB in institutional outbreaks in the developed world, this association was not found in the geographic hot spots of resistance [8–10, 26]. There is no evidence that HIV infection is associated with the development of MDR-TB per se. In settings where MDR-TB is being transmitted, HIV-infected people who acquire infection with a resistant organism are at greatly increased risk of progressing to clinical illness with the resistant strain. In addition, because HIV-infected people are more likely than healthy individuals to visit hospitals and other health care facilities where MDR-TB is transmitted, they have an added risk for infection. On clinical grounds, risk factors for TB drug resistance include previous treatment for TB, high community
MECHANISMS OF TB DRUG RESISTANCE

Drug resistance to M. tuberculosis results from spontaneous and random mutations in the bacterial chromosome that result in reduced susceptibility to specific agents [27]. There is no evidence that acquired genes or plasmids play a role in the emergence of antimicrobial resistance in mycobacteria. Although phenotypic resistance has been recognized for decades, the genotypic basis of drug resistance has only been examined in recent years. Current knowledge of the genetics of drug resistance in M. tuberculosis is summarized in table 1. Zhang et al. [29] were the first to report the molecular basis of TB drug resistance in 1992. These investigators found that deletions of or mutations in the katG gene associated with isoniazid resistance in clinical isolates of M. tuberculosis. katG encodes a catalase-peroxidase involved in converting isoniazid to its active form in the cell, and it had long been observed that isoniazid resistance was often associated with a lack of catalase activity. Subsequent surveys of resistant strains showed that katG mutations or deletions accounted for only 40%–50% of all isoniazid resistance, and other molecular markers of resistance were sought. Three additional genes have been identified as playing a role in isoniazid resistance: inhA and kasA code for cell wall mycolic acid biosynthetic enzymes, and mutations in these genes are found in some resistant isolates [30, 31]. ahpC has been associated with some isoniazid resistance, but the role of this gene in isoniazid susceptibility or resistance is still unclear [32].

Rifampin resistance is caused by point mutations or nucleotide deletions or insertions in an 81–base pair region of the rpoB gene, which codes for the β-subunit of DNA-dependent RNA polymerase [33]. Unlike the situation with isoniazid, where a number of unrelated mutations or deletions are responsible for substantial proportions of resistant isolates, >95% of all rifampin-resistant isolates have a single polymorphism in this 81-base region of rpoB. Consequently, molecular tests that identify mutations in this region of the rpoB gene may prove clinically feasible and useful for the diagnosis of rifampin-resistant TB. The molecular basis of resistance to other anti-TB drugs has been described, as outlined in table 1 [28]. Research into the genetic basis of TB drug resistance has led to an improved understanding of the mechanisms of drug activity and is contributing to the development of new agents to treat TB.

The prevalence of resistant clones in populations of wild-type M. tuberculosis is extremely low [27]. The average mutation rate in M. tuberculosis for all forms of resistance to isoniazid is 2–3 × 10⁻⁸ mutations per bacterium per generation, resulting in an estimated prevalence of mutation of 1 in 10⁵ bacilli. For rifampin, the rate of mutation is 2.25 × 10⁻¹⁰, resulting in resistance of 1 in 10⁸ bacilli in a drug-free environment. Rates of mutation are similarly infrequent for ethambutol and streptomycin. The probability that random mutations in a single bacillus will result in resistance to more than one drug—for example, isoniazid and rifampin—is obtained by multiplying the rates for each individual drug—for example, 1 in 10¹⁰ (10⁸ × 10²). Given that the number of bacilli estimated to be in a lung cavity of 2.5 cm in diameter is 10⁸, an organism resistant to both isoniazid and rifampin would be expected to be very unlikely to occur in nature.

The scientific basis of multidrug therapy in the treatment of TB is the need to prevent the emergence of resistant clones under selective drug pressure. In a drug-free environment, mutant organisms evolve in the presence of a majority of drug-susceptible organisms. It is only in the presence an antimicrobial agent that selective pressure exists that favors the multiplication of a mutant organism. A patient who begins therapy for pulmonary TB with isoniazid alone will experience an initial response to treatment as the drug kills those organisms that are susceptible to this agent. However, a small population of resistant clones (100–1000 bacilli) may continue to replicate in the presence of the drug and become predominant, leading to a recrudescence of disease that is then resistant to drugs [34]. Treatment with at least 2 active agents results in killing of the small populations of bacilli resistant to one drug by the other.

Clinically, drug resistance is divided into 2 types: primary resistance and acquired resistance. Primary resistance occurs in persons who have never been treated for TB and who were presumably infected with a resistant strain of M. tuberculosis. Acquired resistance develops during therapy for TB by the mechanisms described above. Drug-resistant TB is therefore the product of inappropriate use of anti-TB drugs, either by patients or by clinicians. Some of the common causes of ac-
Acquired drug resistance are prescription of inadequate treatment regimen, irregular drug supply, poor drug quality with low bioavailability, and poor compliance. Some authors use the term *initial resistance* to refer to resistant TB at the start of treatment because it may be difficult to verify whether a patient received anti-TB treatment in the past.

**DIAGNOSIS OF TB DRUG RESISTANCE**

Identification and treatment of infected patients is the primary strategy for the control of TB. The nonspecificity of clinical features of TB and the technical demands in identifying and determining drug susceptibility of *M. tuberculosis* in clinical specimens make the diagnosis of resistant TB difficult and extremely challenging. It is estimated that only 50%–60% of all patients with TB worldwide are actually diagnosed, and only a small proportion of those with drug-resistant disease are recognized. The identification of acid-fast bacilli via Ziehl-Neelsen staining and direct microscopy is the primary modality for diagnosing TB throughout the world. Smear-based diagnosis, however, provides no information on drug susceptibility and has only moderate sensitivity.

The identification of *M. tuberculosis* by culture is required for confirmation of TB [35]. Mycobacterial culture on solid media takes 3–8 weeks and is not available in many areas. Radiometric culture systems, combined with DNA probe analysis, can reduce the time required to cultivate *M. tuberculosis* to 1–3 weeks, but the use of these methods throughout much of the developing world is both prohibitively expensive and technically unfeasible.

Several direct techniques for DNA amplification from sputum have received approval by the US Food and Drug Administration. The primers most commonly target IS6110, an insertion sequence that is usually present in multiple copies in *M. tuberculosis*. The lower limit of detection reported by PCR varies between 1 and 100 bacilli. Amplified direct tests have a sensitivity of 84%–92% for smear- and culture-positive cases and 41%–75% for smear-negative, culture-positive cases, with a specificity of 96%–99%. Such rapid tests, however, do not provide information on drug susceptibility.

Standard laboratory methods for drug susceptibility testing in mycobacteria are tedious and slow. The reference standard is the proportions method, in which critical concentrations of drugs are placed in solid media, and the number of *M. tuberculosis* colonies that grow is compared with those growing in drug-free media; if the proportion is >1%, the isolate is considered resistant [35]. Results are generally not available for several months after the patient first seeks care. The use of radiometric or fluorescence-based systems speeds the detection of resistance considerably, although results still require a number of weeks from presentation. As noted, however, the expense of this approach limits its applicability in many parts of the world.

A number of methods for more rapidly identifying drug-resistant *M. tuberculosis* isolates have been proposed. Direct microscopic observation of characteristic colonial morphology in both cultures was shown to have high sensitivity (>90%) and fast turnaround (<10 days) for both initial diagnosis and drug susceptibility testing [36]. Use of redox agents such as Alamar blue or tetrazolium also can identify resistant organisms rapidly and at lower cost than more conventional approaches [37].

Molecular techniques for identifying resistant isolates have largely focused on rifampin resistance because the genetic target is circumscribed and the presence of rifampin resistance is almost always synonymous with MDR-TB. Direct detection of mutations in the *rpoB* gene can reliably identify rifampin-resistant strains. Recent reports of rapid detection of rifampin resistance mutations in clinical specimens by use of molecular beacons, line-probe assays, and PCR techniques are encouraging, but implementation of molecular diagnostics in settings where MDR-TB is common remains an enormous challenge [38, 39].

**TREATMENT OF DRUG-RESISTANT TB**

The clinical implications of drug-resistant TB depend on the agents to which an infecting strain is resistant. Isoniazid resistance, for example, can be effectively treated with a standard 4-drug regimen for 2 months, followed by 4 months of rifampin and isoniazid; this regimen has very high rates of treatment success despite essential monotherapy in the continuation phase. However, an analysis of patients treated in British MRC trials who had initial resistance to isoniazid found that relapses after cure occurred significantly more often than in patients whose initial isolate was not resistant [40]. Current practice for treating isolates with lone isoniazid resistance is to use rifampin, pyrazinamide, and ethambutol for 6–9 months [41].

Rifampin resistance is associated with poorer clinical outcomes and requires an increase in the duration of therapy from 6 months to at least 9 months (and many experts prefer 12 months) [42, 43]. Isolated rifampin resistance can be treated with a regimen of isoniazid, pyrazinamide, streptomycin, and ethambutol for 9 months [44]. Unfortunately, the presence of rifampin resistance is often a marker for MDR-TB, with the majority of such isolates also having resistance to isoniazid—and often to other agents as well. Moreover, isolates that are resistant to rifampin are usually resistant to other rifamycins, such as rifabutin and rifapentine. The treatment of patients with MDR-TB is much more difficult and relies extensively on second-line drugs, which include fluoroquinolones (moxifloxacin, gatifloxacin, levofloxacin, and ofloxacin), ethionamide,
the aminoglycosides kanamycin and amikacin, capreomycin, cycloserine, para-aminosalicylic acid, and clofazimine (table 2). Use of these agents, with the possible exception of the fluoroquinolones, is made more difficult by their poorer activity than the first-line drugs and their greater propensity to cause adverse reactions. Fluoroquinolones such as moxifloxacin and levofloxacin have considerable activity against M. tuberculosis and are preferred in the treatment of all MDR-TB cases, unless resistance to this class is also demonstrated.

Management of MDR-TB relies on strong laboratory support and qualified, dedicated personnel for treatment oversight and supervision. Treatment should be individualized for each patient on the basis of in vitro susceptibility data. Earlier reports suggested a very poor outcome of treatment for MDR-TB, with up to 45% of HIV-negative patients whose infections failed to respond to treatment and 85% of HIV-positive patients dying within 2 years of diagnosis [8, 10, 14]. However, recently published series show a better outcome when MDR-TB is recognized and treated promptly with the appropriate regimen [45, 46]. Iseman [47] reports that cure rates for patients with MDR-TB increased from 56% in 1973–1983 to 84% in 1983–2000, with the improvement attributed to the use of fluoroquinolones and surgery. As discussed below, in developing countries, improved outcomes are also reported for patients with MDR-TB treated under an intensively supervised program. Treatment regimens for specific patterns of drug resistance are listed in table 3.

Surgical treatment of MDR-TB has sometimes proved useful for patients with adequate pulmonary reserve and disease localized to a segment or lobe. Recently, Pomerantz et al. [48] reported the results of 180 resections in 172 patients with MDR-TB, 50% of whom were sputum culture positive at the time of surgery. They noted an operative mortality of 3% and late mortality of 7%. Only 2% of patients remained culture positive during follow-up. Although these results were achieved at a specialty center in highly selected patients, they indicate the potential role that surgery can play in the management of MDR-TB.

Treatment of MDR-TB in developing countries is a particular dilemma because the susceptibility testing and second-line agents are usually insufficient [49]. Most national TB-control programs endorse algorithms for treating patients whose infections fail to respond to treatment that rely on the addition of 1–2 second-line agents to the standard first-line regimen. In settings with limited amounts of MDR-TB, these algorithms may be appropriate [50]. However, in settings where MDR-TB is prevalent, such an approach may actually increase levels of resistance by selecting for additional mutants during therapy [24]. A new strategy that uses a standardized regimen of second-line drugs (kanamycin, a fluoroquinolone, pyrazinamide, ethambutol, and ethionamide) after the infection fails to respond to the standard retreatment regimen has been recommended recently by the WHO. Conversely, Farmer et al. [51] and Kim et al. [52] have advocated tailoring individual treatment regimens through drug susceptibility tests to first- and second-line TB drugs (DOTS-Plus). Using this approach, they have demonstrated treatment response rates of >70% in Peruvian patients with MDR-TB and are expanding the program to other regions, including Russia [51]. There is now considerable discussion and research underway regarding the most effective methods for managing MDR-TB in developing coun-

### Table 2. Second-line drugs for the treatment of tuberculosis.

<table>
<thead>
<tr>
<th>Drug</th>
<th>How supplied</th>
<th>Adult dose</th>
<th>Maximum dose</th>
<th>Major toxicities</th>
<th>Monitoring during therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capreomycin</td>
<td>Vials, 1 g</td>
<td>15–30 mg/kg im</td>
<td>1 g</td>
<td>Auditory, vestibular, and renal toxicity</td>
<td>Vestibular function, audiometry, blood urea nitrogen, and creatinine</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>Vials, 75, 500, and 1 mg</td>
<td>15–30 mg/kg im</td>
<td>1 g</td>
<td>Auditory and renal toxicity, rare vestibular toxicity</td>
<td>Vestibular function, audiometry, blood urea nitrogen, and creatinine</td>
</tr>
<tr>
<td>Ethionamide</td>
<td>Tablets, 250 mg</td>
<td>15–20 mg/kg po</td>
<td>1 g</td>
<td>Gastrointestinal disturbance, hepatotoxicity, hypersensitivity</td>
<td></td>
</tr>
<tr>
<td>Para-aminoisalicic acid</td>
<td>Tablets, 500 mg, 1 g; bulk powder delayed-release granules</td>
<td>150 mg/kg po</td>
<td>12 g</td>
<td>Gastrointestinal disturbance, hypersensitivity, hepatotoxicity, sodium load</td>
<td>Hepatic enzymes</td>
</tr>
<tr>
<td>Cycloserine</td>
<td>Capsules, 250 mg</td>
<td>15–20 mg/kg po</td>
<td>1 g</td>
<td>Psychosis, convulsions, rash</td>
<td>Assessment of mental status</td>
</tr>
</tbody>
</table>

### Table 3. Potential regimens for patients with tuberculosis with various patterns of drug resistance.

<table>
<thead>
<tr>
<th>Resistance pattern</th>
<th>Treatment options</th>
<th>Duration of treatment, months</th>
</tr>
</thead>
<tbody>
<tr>
<td>INH</td>
<td>RIF, PZA, EMB (± FQ)</td>
<td>6–9</td>
</tr>
<tr>
<td>RIF</td>
<td>INH, PZA, EMB, STR or INH, PZA, EMB, FQ (± STR)</td>
<td>9</td>
</tr>
<tr>
<td>INH, RIF</td>
<td>PZA, EMB, FQ, INJ (± second-line therapy)</td>
<td>18–24</td>
</tr>
<tr>
<td>INH, RIF, PZA</td>
<td>FQ, INJ, second-line therapy</td>
<td>24</td>
</tr>
<tr>
<td>PZA</td>
<td>INH, RIF, EMB</td>
<td>9</td>
</tr>
</tbody>
</table>

**NOTE.** EMB, ethambutol; FQ, fluoroquinolone; INH, isoniazid; INJ, injectable agent (capreomycin, kanamycin, amikacin); PZA, pyrazinamide; RIF, rifampin; STR, streptomycin.
tries. Whatever the approach, data concerning efficacy, feasibility, and long-term cost-effectiveness are badly needed.

CONCLUSION

Drug resistance is a worldwide problem that threatens to undermine effective control of TB. As shown by the recent report of WHO/IUATLD, hot spots of MDR-TB have appeared in regions with weak TB-control programs and misuse of anti-TB drugs. General strategies to prevent and manage drug-resistant TB are listed in table 4. Prevention of drug resistance depends on appropriate treatment of all patients with TB with combination drug regimens and early detection of resistance followed by tailored treatment with second-line agents. In countries with low levels of MDR-TB, efforts should be concentrated on preventing acquired MDR-TB by endorsing and widely implementing the WHO DOT strategy. In regions with high levels of MDR-TB, although concentration on detecting and treating new susceptible TB cases remains critically important, MDR-TB management efforts should tailor treatment by performing drug susceptibility testing. In countries with limited resources, more operational research is needed to define the best cost-effective strategies for individual versus standardized patient management of MDR-TB under national program conditions. The development of better and more rapid diagnostic assays and new classes of anti-TB drugs are urgent priorities for the containment of MDR-TB.

TB Drug Resistance • CID 2003:36 (Suppl 1) • S29

Table 4. Principles for the management of multidrug-resistant tuberculosis.

Start with the standard 4-drug regimen while awaiting the results of drug susceptibility tests.

Use directly observed therapy.

If resistance is strongly suspected, add at least 2 agents to which the isolate is likely to be susceptible.

Single agents should never be added to a failing regimen.

Perform drug susceptibility testing on all initial isolates, and on subsequent isolates when circumstances suggest the emergence of resistance.

When resistance is confirmed, use at least 3 drugs known to be active against the isolate.

Therapy should be taken for at least 24 months and should be continued for at least 18 months after bacteriologic conversion.

Drug susceptibility testing should be repeated if cultures remain positive after 3 months of therapy.

References


