

## XDR TUBERCULOSIS: DANGER AHEAD

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**Abstract:** Tuberculosis contributes to two million deaths per year. WHO's fourth global report on anti-tuberculosis drug resistance in 2008 noted the highest levels of multidrug resistance ever recorded in a general population, with an estimated half a million cases occurring globally, including 50 000 cases of extensively drug-resistant tuberculosis. Early awareness and diagnosis of drug-resistant TB are essential. Aggressive treatment of multidrug-resistant-TB and XDR-TB is required as per patient tolerance, to achieve success. To win the battle against drug resistant tuberculosis, a much faster development of the pipeline of new drugs is necessary, on top of properly functioning national TB programs, to minimize and compete with the potential mutating capability of *Mycobacterium tuberculosis*.

### INTRODUCTION

Tuberculosis (TB) is among the most common infectious diseases and frequent causes of death worldwide<sup>1</sup>. Around two billion people are infected with *Mycobacterium tuberculosis*, the causal agent of this disease accounting for a third of the total world population. It is estimated that nine million people become infected each year. Tuberculosis also contributes to two million deaths per year<sup>2</sup>. Infected persons who do not have underlying medical problems have a 5%—10% lifetime risk for progressing to TB disease. However, the risk for disease progression increases substantially in the presence of immunosuppression, such as that caused by the human immunodeficiency virus (HIV) and immunosuppressive medications<sup>3</sup>. Persons with pulmonary TB can be cured with a 6-month course of antibiotics that includes isoniazid, rifampin, pyrazinamide, and ethambutol during the first 2 months. The emergence of multi-drug resistant tuberculosis (MDR-TB), particularly in the 1990s, has become an important health problem and threatened TB control worldwide<sup>4</sup>. The appearance of extremely drug resistant tuberculosis in 2006 has become a worrisome threat for tuberculosis control<sup>5</sup>.

### EXTENT OF THE PROBLEM

Based on surveillance and survey data, WHO estimates around 9.27 million new cases of TB occurred in 2007. Of these 9.27 million new cases, an estimated 44% or 4.1 million were new smear positive cases. India, China, Indonesia, Nigeria and South Africa occupy first five places in terms of the total number of incident cases. Asia (the South-East Asia and Western Pacific regions) accounts for 55% of global cases while the African Region accounts for 31%. Americas, European and Eastern Mediterranean regions account for small fractions of global cases<sup>2</sup>.

### EMERGENCE OF DRUG-RESISTANT TUBERCULOSIS

During 1985—1992, the United States experienced an unprecedented TB resurgence marked by a substantial number of patients with TB who did not respond to treatment and who eventually died. Physicians and epidemiologists quickly determined that these persons had multidrug-resistant tuberculosis (MDR TB), which is defined as resistance to both isoniazid and rifampicin<sup>6</sup>.

Although persons with MDR TB usually can be treated effectively by relying on second-line drugs (amikacin, kanamycin, or capreomycin), these have more side effects and are more expensive

and less effective than first-line drugs. They also require regimens lasting 18-24 months and, the cure rate for persons with MDR TB is 50%-60%, compared with 95%-97% for persons with drug-susceptible TB<sup>7</sup>.

XDR-TB was first widely publicized following the report of an outbreak in South Africa in 2006. 53 patients in a rural hospital in Kwa Zulu-Natal were found to have XDR-TB of whom 52 died<sup>8</sup>. CDC, the World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease (IUATLD) reported the results of a survey regarding drug-resistant TB conducted by 25 reference laboratories. The findings indicated that 20% of *M. tuberculosis* isolates were MDR, and 2% also were resistant to multiple second-line drugs. This highly resistant form of tuberculosis was identified in every region of the world, including the United States, where 4% of MDR TB isolates also were resistant to multiple second-line drugs. In a report published in 2006, this highly resistant form of TB was named extensively drug-resistant TB (XDR TB)<sup>8</sup>. Treatment failures and subsequent death are more common among patients with XDR TB, and the drugs available to treat XDR TB are associated with serious adverse effects<sup>9</sup>. In countries with high rates of HIV and limited health-care resources, substantial numbers of XDR TB cases are likely to be present. As of April 2007, South Africa (where HIV prevalence was estimated at 10.8% in 2005) had reported 352 cases of XDR TB, with the actual prevalence likely being much higher<sup>9</sup> because cultures and susceptibility testing are performed on only a small fraction of TB patients, and many drug-resistant cases will go undetected.

### DEFINITION OF XDR-TB

The term XDR-TB was used for the first time in March 2006, in a report jointly published by US CDC and World Health Organization<sup>8</sup>. According to this report, XDR-TB was defined as tuberculosis caused by *M. Tuberculosis* that was resistant not only to isoniazid and rifampicin (MDR-TB) but also to at least three of the six classes of second-line anti-TB drugs (aminoglycosides, polypeptides, fluoroquinolones, thioamides, cycloserine and para-aminosalicylic acid). This definition was dependent on difficult-to-perform drug susceptibility testing and some forms of drug resistance are less treatable than others. Hence this definition was difficult to accept. Eventually this definition was modified at a meeting of WHO-XDR-TB Task Force, held at Geneva in 2006.

Accordingly committee then gave a much-accepted definition of XDR-TB which defines it as **“resistance to at least Rifampicin and INH among the first line-anti tubercular drugs (MDR-TB) in**

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addition to resistance to any fluoroquinolones i.e. ofloxacin, ciprofloxacin and levofloxacin, and at least one of three injectable second line anti tubercular drugs i.e. amikacin, kanamycin and capreomycin<sup>9,10</sup>.

## CURRENT SITUATION OF XDR-TB

WHO fourth global report on resistance in M. tuberculosis reports data from thirty five countries and two special administrative regions. In total, data were reported on 4012 MDR-TB cases, among which 301 (7.0%) XDR-TB cases were detected. Twenty five countries that reported were European; however, three countries from the WHO Region of the Americas and seven settings from the WHO Western Pacific Region also reported data. Data recently released from South Africa showed that 996 (5.6%) of 17 615 MDR isolates collected from 2004 through to October of 2007 were XDR-TB.

Since 2002, a total of 45 countries have reported at least one case globally. Several other countries are in the process of completing drug susceptibility testing. Despite limitations in the quality assurance applied to laboratory testing, data from this report indicate that XDR-TB is widespread, with 45 countries having reported at least one case<sup>11</sup>.

## FACTORS RESPONSIBLE FOR RESISTANCE IN M. TUBERCULOSIS

Several well-documented factors, including high treatment interruption rates of drug-sensitive TB and consequent low cure rates, together with the HIV epidemic, have contributed to the emergence of MDR-TB and XDR-TB in South Africa and merit urgent remediation. The development of drug resistance may result from inappropriate treatment regimens (e.g., choice of drugs, dosage, duration of treatment), programme factors (e.g., irregular drug supply, incompetent health personnel), and patient factors (e.g., poor adherence, mal-absorption). In fact, it could be said that the emergence of MDR-TB itself is evidence of the systematic failure of the global community to tackle a curable disease<sup>12</sup>.

Both failure in assuring an uninterrupted supply of a wide range of second-line drugs and inadequate management of MDR-TB patients can fuel the development of 'difficult-to-treat' fluoroquinolone-resistant MDR-TB. The common pathway is that drug-susceptible TB evolves into MDR-TB due to inadequate initial management, and that suboptimal use of second-line drugs leads to the development of XDR-TB, and perhaps even beyond<sup>13</sup>.

The alternative pathway in the development of fluoroquinolone-resistant TB may be related to the exuberant use of antimicrobials in the treatment of lower respiratory tract and other infections. TB patients could be mistakenly diagnosed as having pneumonia and treated with a fluoroquinolone<sup>13</sup>.

## DIAGNOSIS OF XDR TB

Early awareness and diagnosis of drug-resistant TB are essential. Recognition of risk factors for drug resistance would provide useful clues. History of past treatment, especially if erratic, close contact with drug-resistant TB, drug addiction, alcoholism and migration from an area endemic for drug resistance can help in suspecting drug resistance. The evidence for an association between HIV co-infection and drug resistance is not conclusive<sup>14</sup>. Drug-susceptibility testing constitutes the gold standard for diagnosing bacillary resistance. In clinical cases with risk factors, molecular genetic tests using rifampicin resistance as a surrogate for MDR-TB would be highly beneficial<sup>2</sup>.

## METHODS OF DETECTION OF DRUG

Table1: Diagnosis of Drug Resistance

Diagnostic test available	Advantage	Limitation
<b>Conventional techniques</b>		
Conventional culture based methods	Inexpensive,easy to perform	Long time needed, 8-12 weeks
<b>Broth based automated culture systems</b>	Rapid	High cost
<b>Rapid phenotypic methods</b>		
<b>Colorimetric redox indicators</b>	Less expensive, rapid	Less evidence, FDA not approved
MODS assay	Easy, inexpensive	Inverted microscope required, data limited
Bacteriophage assay	Rapid, high sensitivity	Lower specificity, biosafety issues
Thin layer agar	Rapid, easy, inexpensive safer	Not approved
Nitrate reduction assay	Easy, rapid, inexpensive	Not approved
<b>Molecular assays</b>		
Genome sequencing	Highly specific	High cost, technologically demanding, time consuming
Line probe assay	High specificity	Medium sensitivity, less accurate on direct sample, limited data
Molecular beacon assay	High sensitivity and specificity	High cost, Not FDA approved, Not available commercially

## RESISTANT M. TUBERCULOSIS

WHO has recommended liquid culture and rapid species identification to address the needs for culture and drug susceptibility testing (DST). These are more sensitive and reduce the delays in obtaining positive cultures and DSTs. However these systems require biosafety facilities that are expensive to build and maintain and specially trained laboratory technicians to perform the procedure. Few developing countries have capacity for good-quality drug-susceptibility testing (DST) for first-line drugs and even fewer have the capacity to test for second-line drug resistance. Even where capacity exists, diagnosing TB with culture can take weeks because of the slow growth rate of TB bacilli. WHO endorsed diagnostic methods include commercial broth based drug susceptibility systems and line probe assays<sup>15</sup>. Broth based culture systems detect fluorescence in a liquid culture medium, enriched with oxygen, to indicate the presence of bacteria. DST takes on average 7-14 days (range 4-14 days) after the initial culture. Studies have shown that both automated and manual systems perform well in detection of isoniazid and rifampicin susceptibility, but are not as effective for ethambutol and streptomycin<sup>15</sup>. Molecular assays to detect gene mutations that signal drug resistance are widely recognized as being most suited for rapid diagnosis, especially since these assays can be directly used on clinical specimens, such as sputum. Among the molecular assays, line probe assays have shown great promise<sup>16</sup>. These are a family of novel DNA strip-based tests that use nucleic acid amplification techniques

Table 2: Antitubercular Drugs<sup>4</sup>

Grouping	Drugs
<b>Group-1 First line oral agents</b>	isoniazid ,rifampicin , ethambutol, pyrazinamide , rifabutin
<b>Group-2 Injectable agents</b>	kanamycin , amikacin, capreomycin, streptomycin
<b>Group-3 Fluoroquinolones</b>	Moxifloxacin, levofloxacin, ofloxacin
<b>Group-4 Oral Bacteriostatic second-line agents</b>	ethionamide , protonamide, cycloserine, terizidone, <i>p</i> -aminosalicylic acid
<b>Group-5 Agents with unclear efficacy (not recommended by WHO for routine use in MDR-TB)</b>	Clofazimine, linezolid, amoxicillin/clavulanate , thioacetazone, imipenem/cilastatin, high-dose isoniazid, clarithromycin

(e.g., PCR) and reverse hybridization methods for the rapid detection of mutations associated with drug resistance. Currently, two line probe assays are commercially available: the INNO-LiPA® Rif. TB kit (Innogenetics NV, Gent, Belgium) and the GenoType® MTBDRplus assay (Hain Lifescience GmbH, Nehren, Germany). Table 2 lists advantages and limitations of tests available or under development.

## DIAGNOSTIC TECHNIQUES UNDER DEVELOPMENT

### 1. Automated detection and MDR screening

Amplification and detection of *M. tuberculosis* DNA is one of the fastest and most sensitive ways to detect tuberculosis, and also may allow the detection of genetic mutations associated with drug resistance. Unfortunately, conventional DNA amplification methods are cumbersome, require specialized training, and can only be performed on material that has been subjected to processing and DNA extraction. This has limited their use to reference laboratories. The Xpert MTB (Cepheid GeneXpert device and Xpert MTB cartridge) device is a fully automated system that allows a relatively untrained operator to perform sample processing, DNA amplification, and detection of *M. tuberculosis* and screening for rifampin resistance in less than 2 hours and only minutes of hands-on time<sup>15</sup>.

### 2. Colorimetric redox indicators

Colorimetric methods are based on the reduction of an indicator solution added to a liquid culture medium after TB organisms have been exposed to different antibiotics. Isoniazid and rifampicin resistance is detected by a change in colour of the indicator<sup>15</sup>.

### 3. Rapid culture systems: TK Medium

TK Medium® (Salubris, Inc., MA, USA) is a novel colorimetric system where metabolic activity of growing mycobacteria changes the color of the culture medium, and this enables an early positive identification before bacterial colonies appear. TK Medium's sensitivity for mycobacterial detection was found comparable to that of the Lowenstein-Jensen (LJ) medium reducing the average time to detection to 2 weeks, as compared with 4 weeks with the conventional LJ medium<sup>17</sup>.

### 4. Microscopic observation drug susceptibility (MODS)

Microscopic observation drug susceptibility (MODS) is a manual liquid culture technique in which microscopic colonies (micro-colonies) of *M. tuberculosis* are observed in the culture media using an inverted microscope, through the bottom of a sealed plastic container. Concurrent culture of sputum in drug-free and drug-containing media facilitates direct rifampicin and isoniazid DST<sup>15</sup>.

### 5. Nitrate reduction assay

It is a solid culture technique which measures nitrate reduction to indicate resistance to isoniazid and rifampicin. It does not need sophisticated equipment, is not complex to perform and could therefore be appropriate for laboratories with limited resources<sup>15</sup>.

### 6. Thin layer agar TLA

The technique uses a standard microscope to simultaneously detect TB bacteria and indicate isoniazid and rifampicin resistance on plates with a thin layer of agar medium. This is inexpensive as compared to other culture techniques. *M. tuberculosis* can be detected in as little as 7 days, with results for DST between 10-15 days. It uses solid media, which may be safer than liquid media. Also it is simpler to manage large numbers of samples than for manual liquid culture<sup>15</sup>.

### 7. Phage-based tests

The technology uses bacteriophages to infect live *M. tuberculosis*

and detect the bacilli using either phage-amplification method or detection of light. It detects rifampicin resistance directly from sputum smear positive samples or indirectly from culture. A total of 11 out of 19 (58%) studies included in the review reported sensitivity and specificity estimates of at least 95%. Specificity estimates were slightly lower and more variable than sensitivity; five out of 19 (26%) studies reported specificity under 90%<sup>19</sup>.

### 8. Anopore method

Ingham et al. reported rapid drug susceptibility of mycobacteria on a highly porous ceramic support using microscopy, image-capture with a charge-coupled device camera and digital processing to quantify the inhibition of growth by drugs. This Anopore method gave an accurate result in 3 days<sup>20</sup>.

### 9. Malachite green microtube (MGMT) susceptibility assay

The technique is based on the use of malachite green dye, which changes colour in response to *M. tuberculosis* growth. Farnia et al. have evaluated MGMT susceptibility assay for the detection of MDR- or XDR-TB directly on sputum specimens and indirectly on *M. tuberculosis* clinical isolate<sup>21</sup>.

### 10. Genetic methods

Fluoroquinolone resistance detection in *M. tuberculosis* using locked nucleic acid probe real time polymerase chain reaction (PCR), detected 71% of phenotypically fluoroquinolone resistant isolates. Specificity was 100% in 40 fluoroquinolone-susceptible isolates<sup>22</sup>.

Gegia et al. reported on the development of a QIAplex system for determining the prevalence and the molecular basis of anti-tuberculosis drug resistance in the Republic of Georgia. The technology uses a target-enriched multiplex PCR to simultaneously amplify and detect 24 mutations in the *M. tuberculosis* genome responsible for resistance to isoniazid, rifampicin streptomycin and ethambutol<sup>23</sup>.

Molecular beacons for the rapid detection of mutations associated with drug resistance have been evaluated. The studies suggest that these tests have high sensitivity (89–98%) and specificity (99–100%) for the detection of mutations associated with rifampicin resistance<sup>24</sup>.

## TREATMENT OF XDR TUBERCULOSIS

Aggressive treatment of multidrug-resistant-TB and XDR-TB is required as per patient tolerance, to achieve success. Directly observed treatment should be employed to enhance the treatment outcomes. Drugs available for treatment are shown in Table 2.

Fluoroquinolone therapy is independently associated with improved treatment outcomes, and the loss of this category of drugs contributes to increased risk of death and failure. Thus, fluoroquinolone should be used whenever possible, although potential cross-resistance among the class members may hamper the utility of these agents even including those of later generations, especially in XDR-TB. Higher rate of relapse and a longer time to sputum culture conversions have been seen with older fluoroquinolones ( ciprofloxacin), and are hence not recommended. The later generation fluoroquinolones, moxifloxacin and levofloxacin are more effective and may have efficacy against some ofloxacin-resistant strains<sup>25</sup>. Moxifloxacin and gatifloxacin have been studied more intensively in recent years. Linezolid, amoxicillin-clavulanate, imipenem/cilastatin and clofazimine also have some action against drug resistant tuberculosis. However these are not generally recommended by the WHO for routine use because their anti-TB roles are not totally certain and are more toxic<sup>4</sup>. However, they have potential utility in cases where no other options are possible.

The treatment regimens used should be reviewed periodically according to the laboratory data on susceptibility tests along with the clinical, radiological and bacteriological progress and response. Extended regimens

consisting of multiple second-line and third-line anti-TB drugs, given for prolonged periods are needed. A single drug should never be added to a failing regimen, for fear of selecting organisms in the bacterial population that are resistant to the newly added drug<sup>4</sup>.

## EMERGING PIPELINE OF NEW ANTI-TB DRUGS

In recent years there has been much renewed interest in anti-TB drug development, especially targeting bacillary genetic resistance and phenotypic persistence. Agents with potent sterilizing activity can potentially shorten duration of therapy, thus enhancing adherence, with resultant improved treatment success and curtailed development of drug resistance. There are now new classes of compounds with potential usefulness in the treatment of TB in the pipeline<sup>26</sup>.

## ROLE OF SURGERY

Adjunctive surgical resection is considered to play a useful role in selected cases of drug resistant tuberculosis especially under the following conditions: high chance of failure or relapse with drug treatment alone due to a rather extensive pattern of bacillary resistance; disease is sufficiently localized, alongside adequate reserve of lung function, and the residual disease after resection is amenable to medical therapy; adequate anti-TB agents still available to reduce the bacillary burden to allow healing of the bronchial stump<sup>27</sup>.

## ROLE OF IMMUNOTHERAPY

Macrophages, different types of T cells, alongside their elaborated cytokines are key factors in defense against tubercle bacilli. There has been active exploration for effective immunotherapy as a form of complementary treatment to speed up disease recovery and reduce relapse. New potential immunotherapeutic modalities explored include cytokines IFN- $\gamma$ , IFN- $\alpha$ , IL-2 and IL-12 and other agents<sup>28</sup>. It is clear that much more work needs to be done in the area of immunotherapy for MDR-TB and XDR-TB.

## FUTURE VIEW

It is estimated (using a mathematical model) that the chance of developing at least one new drug by the year 2010 was less than 5% and approximately 73% by 2019. Doubling the number of drug candidates would increase the chances to 93% by 2019<sup>29</sup>. To win the battle against drug resistant tuberculosis, a much faster development of the pipeline of new drugs is necessary, on top of properly functioning national TB programs, to minimize and compete with the potential mutating capability of MTB. Also, research in identifying novel drug targets is important, so that new candidates can join and feed into the earlier phases of the drug pipeline. Moreover, with better methodologies, the conduction of drug-evaluation clinical trials can be facilitated and expedited.

A new vaccine for TB is also needed. Today's TB vaccine, Bacille Calmette-Guérin (BCG), provides some protection against severe forms of TB in children but is unreliable against pulmonary TB, which accounts for most of the worldwide disease burden. In recent years, members of the Working Group on New TB vaccines, including non-profit organizations such as Aeras Global TB Vaccine Foundation ([www.aeras.org](http://www.aeras.org)) and the Tuberculosis Vaccine Initiative ([www.tbvi.eu](http://www.tbvi.eu)), and other researchers from the public, private and academic sectors have developed multiple new TB vaccine candidates. Some of these candidates would replace BCG, while others would improve or "boost" BCG, thereby extending the vaccine's protective effects. TB vaccines under development could work in several ways, including by preventing infection, disease, or reactivation of latent TB infection, or by improving the response to chemotherapy. Several new vaccine candidates are in various stages of clinical trials, with more in preclinical development.

TB experts hope that at least one new vaccine will be ready for global distribution by 2015. A list of TB vaccines under development is available

at [www.stoptb.org/retooling](http://www.stoptb.org/retooling)<sup>30</sup>.

## CONCLUSIONS

Drug-resistant TB results from inappropriate regimen, poor drug quality, erratic drug supply and poor adherence to treatment, reflecting failure in the implementation of an effective TB control program. Established multidrug-resistant (MDR)-TB requires a longer duration of treatment (generally 18-24 months) and necessitates the use of alternative chemotherapy regimens, which are more costly and toxic. Programmatic delivery of MDR-TB as Directly observed treatment, short-course (DOTS) treatment is mandatory for the optimal management of drug-resistance scenarios.

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