Extensively Drug-resistant Tuberculosis – A Threat to Tuberculosis Control

a report by

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The emergence of multidrug-resistant tuberculosis (MDR-TB)[1–5] and, more recently, of extensively drug-resistant tuberculosis (XDR-TB)[6,7] is considered a real threat to achieving TB control and elimination. The aim of this article is first to define MDR-TB and XDR-TB; second, to give an overview of the available information on epidemiology and the spread of MDR-TB and XDR-TB and to discuss implications for TB control and elimination; and third, to summarise current standards and challenges related to the laboratory diagnosis of MDR-TB and XDR-TB.

Definitions

MDR-TB is caused by mycobacteria that are resistant to at least the two most potent first-line anti-TB drugs, isoniazid (INH) and rifampicin (RIF).[1–5] Although MDR-TB has been documented in the past, the term XDR-TB appeared in the literature for the first time only in March 2006 in a report jointly published by the US Centers for Disease Control (CDC) and the World Health Organization (WHO) to describe a severe form of disease caused by strains of *Mycobacterium tuberculosis* that were resistant to at least INH and RIF (i.e. MDR-TB), in addition to any fluoroquinolone, and to at least one of the three following injectable drugs used in anti-TB treatment: capreomycin, kanamycin and amikacin.[6–8]

Epidemiology and Spread

As a result of its recent description, information available on XDR-TB is much less comprehensive than that available for MDR-TB (see *Table 1*).

A retrospective, non-representative study performed on 17,690 isolates from 14 of 23 laboratories belonging to the Network of Supranational Reference Laboratories (48 countries surveyed between 2000 and 2004) demonstrated that 234 were XDR (6.6% of the MDR-TB strains collected): 6.5% in industrialised nations (Australia, Canada, Japan, US and western Europe), 5.9% in Latin America, 13.6% in Eastern Europe and Russia, 0.6% in Africa and the Middle East and 1.5% in Asia (excluding the Republic of Korea, where the proportion was 15.4%).[8]

Representative surveys revealed that in the US 4% of the MDR-TB strains isolated between 1993 and 2004 were XDR-TB,[6] whereas in a different study the proportion was 3%.[9] Data from Asia show that 15% of the MDR strains isolated in South Korea in 2004 were XDR,[8] as well as 11% of those isolated in Iran[10] and 12% of those isolated in Hong Kong.[11] In Europe, representative data on the proportion of MDR strains isolated as being XDR-TB are available only from Latvia (19%),[8] Estonia (5.9%),[12] Archangels Oblast, the Russian Federation (1.3)[13] and Norway, where 15 XDR-TB cases were treated for more than 10 years.[14] The proportion of XDR-TB strains isolated in reference centres in Germany and Italy between 2003 and 2006 was 0.4%.[15] In the same period, the proportion of XDR-TB strains in Estonia, Germany, Italy and Russia was 1.4%.[12] In Africa, non-representative data are available from South Africa only, where between 2005 and 2006 3.4% of the cases were XDR. Fifty-two of 53 of those cases died soon after diagnosis, the median survival time after sputum smear sample collection being only 16 days.[16]

The South African outbreak demonstrates the consequences of introducing XDR strains into the particularly vulnerable population of HIV-infected people. In this setting, a large proportion of the cases probably resulted from in-hospital infection. This view is supported by the results of spoligotyping studies demonstrating that 85% of the 46 XDR-TB strains tested belonged to the Kwa-Zulu-natal family. In addition, 55% of the XDR-TB patients had not been previously treated for TB, suggesting that many of the cases resulted from new infections. Finally, contact tracing revealed that patients with XDR-TB were from a remote geographical region and had no known contact with each other apart from receiving healthcare from the same district hospital.

In Europe and elsewhere, the majority of XDR-TB cases are not emerging among people living with HIV/AIDS, and 75% of them had previously been treated for TB.[12,15,17,18] XDR-TB strains are therefore likely to be selected in many different places and on multiple occasions, resulting from the following sequence of events: poor TB control generates MDR-TB, which results in the use of second-line drugs, the misuse of which generates XDR-TB.

Implications for Tuberculosis Control and Elimination

Seven key recommendations were developed by the WHO to prevent and control XDR-TB:

- preventing XDR-TB through basic strengthening of TB and HIV control – the new Stop TB strategy[18] and the Global Plan to Stop TB[19] are the essential guiding documents;

- improving the management of patients suspected to be affected by XDR-TB, e.g. accelerating access to laboratory facilities with drug susceptibility testing (DST) – including rapid test for RIF resistance –

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Table 1: Countries that Notified of at Least One Case of Extensively Drug-resistant Tuberculosis as of 31 October 2007, by Continent

Continent	Countries
Africa	Mozambique
	South Africa
America	Argentina
	Brazil
	Canada
	Chile
	Ecuador
	Mexico
	Peru
	US
Asia	Armenia
	Azerbaijan
	Bangladesh
	China, Hong Kong SAR
	Georgia
	India
	Islamic Republic of Iran
	Israel
	Japan
	Republic of Korea
	Russian Federation
	Thailand
	Vietnam
Europe	Czech Republic
	Estonia
	France
	Germany
	Ireland
	Italy
	Latvia
	Lithuania
	The Netherlands
	Norway
	Poland
	Portugal
	Romania
	Slovenia
	Spain
	Sweden
	UK
Australia	Australia

and improving detection of cases suspected of harbouring MDR strains both in high and low HIV prevalence settings;

- strengthening XDR-TB management as well as treatment design in both HIV-negative and HIV-positive individuals, e.g. applying adequately the new WHO guidelines for programmatic management of drug-resistant TB,[20] using second-line drugs adequately and implementing a patient-centred approach to ensure support and supervision;

- standardising the definition of XDR-TB in order to improve the comparability of data obtained through ongoing surveillance in low-TB-incidence countries and *ad hoc* surveys in high-TB-incidence countries;

- increasing healthcare worker infection control and protection in order to reduce the ongoing transmission of MDR-TB, especially among HIV-positive individuals in congregate care settings;

- implementing immediate XDR-TB surveillance activities – reference laboratories will need to initiate rapid surveys to allow a complete assessment of XDR-TB prevalence worldwide; and

- initiating advocacy, communication and social mobilisation activities, as there is an urgent need to inform and raise awareness about TB and XDR-TB.

Laboratory Diagnosis of Multidrug-resistant Tuberculosis and Extensively Drug-resistant Tuberculosis

With the exception of a few developed countries, most of the national TB programmes worldwide are unable to provide diagnostic services based on culture and DST for all TB cases. Therefore, the vast majority of drug-resistant TB goes undetected and untreated. The laboratory is an essential component in TB control programmes and broader access to DST is a priority for most countries, as early prescription of adequate treatment is an essential determinant of a favourable outcome. Rapid determination of drug resistance is key in customising treatment early enough in the course of the disease to reduce morbidity, mortality and infectiousness. The diagnosis of both MDR-TB and XDR-TB is currently hampered by the absence of effective and affordable rapid diagnostic techniques for DST. Several approaches, both phenotypic and molecular, have been explored in order to develop fast, robust, sensitive and affordable methods allowing the rapid detection of drug resistance in *M. tuberculosis*.

Conventional Culture-based Methods

Using standardised DST procedures with conventional methods, eight to 12 weeks are necessary to identify drug-resistant micro-organisms on solid media (e.g. Lowenstein-Jensen). In general, such methods assess inhibition of *M. tuberculosis* growth in the presence of antibiotics to distinguish between susceptible and resistant strains.

The proportion method allows the precise determination of the proportion of resistant mutants to a certain drug. The resistance ratio method compares the resistance of an unknown strain with that of a standard laboratory strain. Although relatively inexpensive and undemanding in terms of sophisticated equipment, results usually take weeks to become available. This is challenging, particularly where inappropriate choice of treatment regimen may cause death within weeks of initiation (such as in the case of XDR-TB).[16] In addition, delayed

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identification of drug resistance may result in inadequate treatment, leading to the generation of 'super-resistance'.

Liquid-culture-based Methods

The introduction of automated liquid culture systems has been proved to be more sensitive and has significantly reduced the turnaround time

required. Unfortunately, at least two to four weeks are still needed and their considerately higher cost is frequently unaffordable for resource-limited countries. The BACTEC® TB-460 radiometric system (Becton Dickinson, Sparks, MD, US) allowed significant advances but, despite having been considered in the past to be the 'gold standard' for DST to first-line antituberculosis drugs, it was hindered by the need to dispose of large volumes of radioactive materials to the point at which this method has been abandoned. Moreover, although turnaround time was significantly shorter than for conventional methods, it was still in the order of three weeks.

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More recently, many published studies have shown the high performance of the Mycobacteria Growth Indicator Tube (MGIT) (Becton Dickinson, Sparks, MD, US) for the rapid detection of resistance to first- and second-line anti-TB drugs.[21] Detection of drug resistance can be accomplished in days rather than weeks, although it is still constrained by high cost in terms of both equipment and consumables.

New Rapid Phenotypic Methods
Among novel rapid phenotypic methods, the microcolony method is relatively low-cost. It has been adapted for the rapid detection of drug resistance directly from sputum samples and has been shown in preliminary studies to be accurate for the detection of MDR-TB compared with the reference proportion method, with results available in one week.[22] Newly developed phenotypic tests such as TK Medium, MODS (microscopic observation drug susceptibility) assay and FASTPlaque-TB-Response phage assay are usually cheaper but not always simple to perform, with some requiring high standards of biosafety and quality control.[23]

TK Medium (Salubris Inc., Cambridge, Massachusetts, US) is a novel colorimetric system that indicates growth of mycobacteria by changing its colour. TK Medium also permits DST and can allow for differentiation between M. tuberculosis and non-tuberculous mycobacteria. Unfortunately, there is insufficient published evidence on the field performance of this test in developing countries.[23]

The MODS assay is based on the observation of the characteristic cord formation of M. tuberculosis that is visualised microscopically in liquid medium with the use of an inverted microscope.[24] MODS uses simple light microscopy to detect early growth of M. tuberculosis as 'strings and tangles' of bacterial cells in the broth medium, with or without antimicrobial drugs (for DST). The agreement between MODS and the reference standard for DST is 97% for INH, 100% for RIF and 99% for INH and RIF combined (MDR). Lower values of agreement were obtained for ethambutol (95%) and streptomycin (92%). One minor disadvantage of MODS is the requirement for an inverted microscope for observation of the mycobacterial growth.

FASTPlaque-TB-Response (Biotec Laboratories Ltd, Ipswich, Suffolk, UK) is a phage-amplification-based test developed for direct use on sputum specimens. Drug resistance is diagnosed when M. tuberculosis is detected in samples containing the drug (e.g. RIF). A recent meta-analysis of the accuracy of phage-based methods for detecting RIF resistance in M. tuberculosis concluded that these assays performed on M. tuberculosis culture isolates have high sensitivity but variable and slightly lower specificity.[25] Not enough evidence is available on the accuracy of these assays when performed directly on sputum samples. Safety and quality control issues related to the use of this technique should also be addressed carefully.

Several colorimetric methods have also been proposed in the last few years for the rapid detection of drug resistance in M. tuberculosis. A recent systematic review and meta-analysis of colorimetric redox indicator methods to detect MDR in M. tuberculosis found evidence of a high sensitivity and specificity for the rapid detection of MDR-TB.[26] Colorimetric methods represent a good alternative for the rapid detection of drug resistance in laboratories with limited resources. However, these methods cannot be directly used on clinical specimens.

Overall, large multicentric studies defining the accuracy of phenotypic DST methods are not yet available. Practical issues such as quality controls and training requirements have not been adequately addressed under field conditions. The application of these approaches to support individualised treatment through determination of second-

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line DST remains largely unexplored de facto, limiting the possibility of addressing effectively XDR-TB with individualised treatment.

New Rapid Molecular Methods
New weapons are represented by the molecular techniques allowing fast detection of mutations responsible for drug resistance. The detection of RIF resistance is proposed as a predictor of MDR-TB. Its positive predictive value is a function of the sensitivity and specificity of RIF resistance testing and the prevalence of MDR and non-MDR RIF resistance, which is highest among previously treated cases in settings with high MDR prevalence and low non-MDR RIF resistance. The techniques are based on PCR in conjunction with electrophoresis, sequencing or hybridisation.

Although most of the techniques were initially developed and evaluated to detect drug resistance in TB complex isolates (M. tuberculosis complex (MTBC)), they are being explored for direct detection of MTBC and identification of alleles related to drug resistance in clinical specimens. Their potential advantage is that there is no need for growth of the organism, and DST results can be

determined in days rather than weeks. Preliminary evidence suggests that they can be highly reliable.

Direct sequencing and restriction fragment length polymorphism remain expensive and time-consuming approaches. Techniques that are based on realtime polymerase chain reaction and make use of specific probes for the mutations and wildtype sequences for the amplified genes are also expensive and complicated, even if highly sensitive and specific. Reverse-hybridisation-based assays, referred to

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as line probe assays, represent a useful tool for their superior cost-effectiveness. These tests are based on the hybridisation of specific probes for wild-type and mutated sequences of genes involved in drug resistance and showed high specificity and medium/high sensitivity.

Commercially available line probe assays include the INNO-LiPA® Rif. TB kit (Innogenetics, Gent, Belgium) and the GenoType® MTBDR assay (Hain Lifescience, Nehren, Germany). A recent meta-analysis summarised the results obtained for the INNO-LiPA Rif. TB test.[27] This meta-analysis showed that LiPA is highly sensitive and specific when culture isolates are used. The majority of studies had sensitivity of 95% or greater, and nearly all were 100% specific. The results, however, are less accurate when the test is directly applied on clinical specimens (e.g. sputum). There is a paucity of data on application of this test directly to clinical specimens.

The Genotype MTBDR test is able to detect mutations in the hotspot region of rpoB for RIF resistance and the most frequent mutation at the codon 315 of the katG gene for INH resistance in either isolates or clinical specimens. The specificity and sensitivity of the assay for the RIF resistance were near to 100%. For isoniazid resistance, despite a high specificity (around 100%), the sensitivity of the test ranged from 70 to 90% depending on the prevalence of the particular mutation at the

katG locus.[28,29] Now commercially available is an implemented version of the assay, the Genotype MTBDRplus. The new assay includes probes for the identification of other mutations in the hotspot region of the rpoB gene for RIF resistance and probes to detect mutations in the promoter region of the inhA gene involved in INH resistance. Those improvements allow the identification of another 10–20% of INH-resistant cases, with an enhancement in rapid MDR-TB diagnosis.

Line probe assays are cost-effective and useful for the rapid detection of drug resistance directly in clinical specimens, but the number of genes that can be analysed remains limited and the test fails to distinguish insertion mutations. In addition, they retain a lower sensitivity among sputum-smear-negative samples. In general, line probe assays are expensive and require sophisticated laboratory infrastructure. Their role and utility in low-income, high-burden countries will be limited unless cost and technical issues are addressed.

Conclusions

The long-term goal of control of MDR-TB and XDR-TB requires the scaling up of culture and DST capacity (which is currently very limited in resource-limited countries) and the expanded use of high-technology assays for rapid determination of drug resistance. Overall, molecular approaches are still insensitive for many of the drugs as a result of our limited understanding of the mechanism responsible for the resistant phenotype. In addition, all genotype techniques are expensive and sophisticated, requiring DNA extraction, gene amplification and detection of mutation. They need resources and skills that are not usually available in most countries where the prevalence of MDR-TB and XDR-TB is high. The challenge, then, is to make sure that the benefits of promising new tools actually reach the populations that need them most but can least afford them. Agencies such as the Stop TB Partnership, the Foundation for Innovative New Diagnostics (FIND) and the WHO are making an enormous effort aimed at addressing these challenges. Funding and international support for the new Global Plan to Stop TB, 2006–2015[20] will also enhance the necessary development and implementation of new tools for MDR-TB and XDR-TB control. ■

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