Policy guidance on drug-susceptibility testing (DST) of second-line antituberculosis drugs

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Contributors

L. Barrera, E. Cooreman, J. de Dieu Iragena, F. Drobniewski, P. Duda, M. Havelkova, S. Hoffner, K.M. Kam, S.J. Kim, S. Labelle, K. Lambregts, V. Leimane, P. Nunn, A. Ramsay, M. Raviglione, M. Rich, J. Ridderhof, F. Rodrigues, S. Rüsch-Gerdes, M. Salfinger, J. Scholten, N. Selvakumar, T. Shinnick, M. Shul'gina, G. Šķenders, A. Sloutsky, P. Small, A. Van Deun, F. Varaine, M. Yagui, V. Vincent, K. Weyer, A. Wright, M. Zignol.

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Preamble

Results from drug-resistance surveys and ongoing surveillance show that drug-resistant tuberculosis (TB) is widespread geographically (*1*). Drug-resistant TB is a man-made problem of global concern – the result of mismanagement of antituberculosis drugs through poor TB control, drug-prescription errors and non-adherence of patients to treatment. However, the extent of the problem remains underestimated or unknown in many settings owing to insufficient laboratory capacity and inadequate policies to detect drug-resistant TB patients accurately and in a timely manner. Multidrug-resistant TB (MDR-TB)[1] has become a serious threat to global TB control as a result of the difficulties in diagnosis and treatment and the associated high cost to TB control programmes. Documented transmission of MDR-TB to vulnerable populations and in high-burden HIV settings compounds this threat (*2*). The emergence of extensively drug-resistant TB (XDR-TB),[2] with poor treatment outcomes, very high mortality in XDR-TB patients with concomitant HIV infection (*3*), and the risk of XDR-TB spread across country borders, has heightened global concern over a potentially untreatable epidemic that may jeopardize recent advances in global TB control.

Guided by the Stop-TB Partnership Working Group on MDR-TB and the Green Light Committee (GLC), concurrent efforts by various private, nongovernmental and public organizations focus on confronting the challenges of drug-resistant TB, and sharing information and strategies in an unprecedented, collaborative way. However, estimates by the World Health Organization (WHO) highlight the need for diagnostic capacity as one of the most crucial aspects in mobilizing an effective response to the challenges of drug-resistant TB, with fewer than 5% of existing MDR-TB cases estimated to be diagnosed (*4*). The weakest link in TB control remains the need for appropriate, affordable and sustainable laboratory services, and this has been brought into stark relief by the pressing need for an accelerated and extensive scale-up of MDR-TB programmes.

GLC-assisted projects in different epidemiological and resource-constrained settings have shown that the management of MDR-TB is feasible and effective, even in resource-constrained settings (*5*). However, major challenges remain in the area of laboratory capacity to meet the demand for scale-up of MDR-TB programmes within the context of routine TB control. Laboratory constraints are centred on: programmatic requirements such as infrastructure development, acquisition and maintenance of equipment, quality assurance and biosafety; an urgent need for reliable and reproducible methodologies for second-line drug-susceptibility testing; and the need for rational use of second-line DST in programmes about to engage in MDR-TB treatment. In order to address these issues, WHO has taken the lead in developing interim laboratory policy guidance for countries establishing or expanding MDR-TB treatment programmes.

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[1] MDR-TB: *Mycobacterium tuberculosis* complex isolates with in vitro resistance against isoniazid and rifampicin, with or without resistance to additional first-line anti-TB drugs.

[2] XDR-TB: *Mycobacterium tuberculosis* complex isolates defined as multidrug-resistant, with additional in vitro resistance to a fluoroquinolone and one or more of the following injectable drugs: kanamycin, amikacin, capreomycin.

Aim

This document is intended to provide an interim policy framework for the laboratory component relevant to programmatic implementation of MDR-TB strategies. A detailed technical manual on laboratory methodology, laboratory biosafety and standard operating procedures related to second-line drug-susceptibility testing (DST) is also under preparation.

Process

When preparing this document, priority consideration was given to data from published studies; however, scientific literature is limited and extrapolation from expert opinion and experience within laboratories involved in second-line DST was very useful in developing current consensus on second-line DST procedures. To this end, a core group of international TB laboratory experts reviewed the available literature, shared experiences and provided consensus expert opinion on controversial technical issues.

Date of review

Given the paucity of scientific data on several aspects of second-line DST, the need for additional research and rapid translation of research findings into policy and practice is evident. This document therefore constitutes work in progress and will be complemented by ongoing and future research, guided by increased collaboration of partners involved in laboratory services, and subject to review by early 2010.

Conflict of interest

F. Drobniewski: grants from Becton Dickinson and Co. and association with Life Sciences for Diagnostics.

Introduction

One of the main aims of effective TB control is the prevention of drug resistance resulting from a variety of programmatic, health provider- and patient-related factors. Irregular drug supply, poor drug quality, clinical errors in drug prescription and a lack of patient adherence to treatment are known determinants of anti-TB drug resistance (*5*). Subsequent transmission of resistant bacilli is facilitated by inadequate infection control, especially in congregate settings. MDR-TB and XDR-TB outbreaks have almost invariably been linked with HIV infection (*2, 3*), resulting in exceptionally high patient mortality and highlighting the urgent need for rapid diagnosis and intervention in vulnerable populations.

Definitive diagnosis of MDR-TB and XDR-TB requires that *Mycobacterium tuberculosis* be isolated and identified, and drug-susceptibility testing (DST) completed. Using conventional methodologies, growth detection, identification of *M. tuberculosis* and DST may take weeks or even months. In addition, the interpretation of DST results for TB bacilli is complicated by the fact that organisms may be intra- or extracellular, may have a long generation time, may be dormant or active, and may be present in different tissues with variable drug-penetration ability. DST results may therefore not accurately reflect the bacterial population by the time the results become available, and cannot be exclusively relied upon to guide the design of treatment modalities.

Newer rapid phenotypic DST methods (e.g. direct tests, colorimetric methods, phage-based methods) and genotypic DST techniques (e.g. nucleic acid amplification assays, resistance mutation detection and sequence-based assays) are very promising but are either still in development, at early validation stage or in early field demonstration phase, and only aimed at first-line anti-TB drugs. While presenting an opportunity for rapid detection of MDR-TB, no tests for rapid identification of second-line drug resistance are yet available.

Conventional DST for first-line anti-TB drugs has been thoroughly studied and consensus has been reached on appropriate methodologies, critical drug concentrations, and reliability and reproducibility of testing. On the other hand, surveys of current practices for second-line DST in the global Supranational Reference Laboratory (SRL) Network as well as a few multicentre laboratory studies have revealed important differences with regard to methods, the critical concentrations of drugs, and the critical proportions of resistance (*6–8*). The reliability of drug-susceptibility testing for second-line drugs (SLDs) has therefore been questioned (*9–10*) and the urgent need to standardize methodologies, establish criteria for defining resistance and carry out proficiency testing is obvious. Recent studies have compared newer methodologies with conventional DST for selected SLDs and have suggested tentative critical concentrations for these drugs (*6–8*).

It should be noted however that ***no studies*** have systematically evaluated all available DST methods for all available SLDs, established critical concentrations for all available SLDs, or evaluated a large number of clinical isolates for microbiological and clinical end-points.

Countries embarking on diagnostic and treatment programmes for drug-resistant TB need policy guidelines on the rational use of DST, particularly for second-line drugs. Policy formulation has, however, been hampered by the following:

- Second-line DST has not been standardized internationally, owing to technical difficulties related to in vitro drug instability, drug loss caused by protein binding, heat inactivation, filter sterilization, incomplete dissolution and/or varying drug potency. Laboratory technique, medium pH, incubation temperature and incubation time also influence DST results. In addition, the drug critical concentration defining resistance is often very close to the minimal inhibitory concentration (MIC) required to achieve antimycobacterial activity, increasing the probability of misclassification of susceptibility or resistance, and leading to poor reproducibility of DST results.

- Only a few laboratories internationally have the required capacity and expertise to reliably test for all classes of available anti-TB drugs. These laboratories are largely limited to resource-rich settings. Many of the newer techniques are difficult to implement in the countries where they are most needed owing to high cost, technical complexity and lack of appropriately trained laboratory staff. As a result, conventional culture and DST methods using egg-based or agar-based media are still the most widely used in resource-limited settings, leading to long diagnostic delays. Even in sophisticated and well-resourced environments, wide variations in second-line DST systems and methods have been reported, reflecting the difficulties in securing reproducibility and optimizing the clinical relevance of DST results. In addition, the majority of newer techniques still need proper evaluation to verify their efficiency in different epidemiological settings.

- Many high-burden TB countries do not have access to the full range of second-line drugs because of financial, regulatory or other constraints. Fluoroquinolones, aminoglycosides and (to a much lesser extent) polypeptides are readily available in many countries, although specific drugs in these classes may not be. Cross-resistance between drugs in the same group further limits the selection of available drugs. In settings with limited access to SLDs, development of resistance to the most potent groups of SLDs (aminoglycosides, fluoroquinolones, polypeptides) therefore creates a situation where TB is virtually untreatable.

Current knowledge

Drug efficacy

The WHO *Guidelines for the programmatic management of drug-resistant tuberculosis* (*5*) categorize available anti-TB drugs in five groups, based on known efficacy (*Table 1*). The backbone of regimens for the treatment of MDR-TB consists of an injectable drug (aminoglycoside or polypeptide) and a fluoroquinolone, supported by at least two additional SLDs in order to ensure that the regimen includes at least four drugs confirmed or expected to be effective (*5*).

Table 1 Alternative method of grouping antituberculosis drugs

Grouping	Drugs
Group 1 First-line oral agents	Isoniazid (H); rifampicin (R); ethambutol (E); pyrazinamide (Z); rifabutin (Rfb)[a]
Group 2 Injectable agents	Kanamycin (Km); amikacin (Am); capreomycin (Cm); viomycin (Vm); streptomycin (S)
Group 3 Fluoroquinolones	Moxifloxacin (Mfx); levofloxacin (Lfx); ofloxacin (Ofx)
Group 4 Oral bacteriostatic second-line agents	Ethionamide (Eto); protionamide (Pto); cycloserine (Cs); terizidone (Trd); *p*-aminosalicylic acid (PAS)
Group 5 Agents with unclear role in DR-TB treatment (not recommended by WHO for routine use in DR-TB patients)	Clofazimine (Cfz); linezolid (Lzd); amoxicillin/clavulanate (Amx/Clv); thioacetazone (Thz); imipenem/cilastatin (Ipm/Cln); high-dose isoniazid (high-dose H);[b] clarithromycin (Clr)

[a] Rifabutin is not on the WHO List of Essential Medicines. It has been added here as it is used routinely in patients on protease inhibitors in many settings.
[b] High-dose H is defined as 16–20 mg/kg/day.